Quality parameters, pollen and volatile profiles of honey from North and Central Mozambique

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Abstract

Honey from different provinces of North and Central Mozambique was characterised considering their physicochemical quality parameters, colour, sugars, total antioxidants, pollen analysis and volatile profile. Flora that surrounds the hives, and the apicultural practices also influence in their characteristics. According to a similar pollen spectrum, eight types of honey were found. In these, the predominant pollens were: I-Astragalus type; II-*Acanthus* sp; III-Celastraceae; IV-Brassicaceae; V-Anacardiaceae and Astragalus type; VI-Astragalus type and Myrtaceae; VII-Asteraceae family and VIII-unknown. Group I (from Nampula), especially distanced itself from the others mainly due to the special abundance of certain compounds (alcohols, aldehydes, esters, acids and terpenes). The presence of furan compounds largely identified in Sofala and Manica honeys could be due to inadequate beekeeping practices or storage conditions. A discriminant analysis correctly classified 96.7% of the groups, being electrical conductivity and moisture followed by the volatile-compound 3-Methylbutan-1-ol and the free acidity, the variables that most contributed.

Keywords: Mozambique, honey, pollen, physicochemical quality parameters, volatile-compounds.

1. Introduction

Mozambique, located in the Southeast coast of Africa is one of the poorest countries in the world. Here, apiculture does not play an important social, economic or environmental role but it has potential

to increase the sustainability of poor rural communities (Bradbear, 2005; Serem & Bester, 2012). North and Central regions of Mozambique enjoy a favourable climate and have sufficient natural resources, particularly vast forest areas rich in melliferous flora ideal for beekeeping. At present, honey production in Mozambique is very low, about 600 tons/year (FAOSTAT, 2016), with a growing trend in the last 5 years. However, due to the availability of agro-ecological resources, the production capacity could reach 3,600 tons/ year (Jooste & Smith, 2004).

In Mozambique, there are several possible benefits to beekeeping. It could be an attractive income generating activity for smallholder farmers and be exploited by women of rural populations. There is no doubt that beekeeping can help generate social change and play an important role in society while creating sustainable livelihoods. In addition, it can favour the development of many different sectors within society: vendors, carpenters who manufacture beehives, garment makers, protective clothes, and packaging processors. All this keeping in mind the contribution of apiculture for the development of agriculture and the environment associated with the increase of pollination.

In the last years, the world market demands differentiated agro-alimentary products with specific characteristics based on the following criteria: botanical or geographical origin, quality and safety, specific organoleptic or nutritional characteristics, among others (Borrás, Domenech, Hellebrandova, & Escriche, 2014). For this reason, there is a large number of global research studies about the characteristics of honey; however, there are very few focused on properties of African honeys. Among these, it is worth mentioning those papers of different African countries: Ethiopia (Sime, Atlabachew, Redi-Abshiroand, & Zewde, 2015); Burkina Faso (Meda, Lamien, Romito, Millogo, & Nacoulma, 2005; Nombré, Schweitzer, Boussim, & Rasolodimby, 2010; Paul, Issa, Kwamé, & Joseph, 2013; Escriche, Oroian, Visquert, Gras, & Vidal, 2016); South Africa (Serem & Bester, 2012); Sudan (Makawi, Gadkariem, & Ayoub, 2009); Tunisia (Martos, Cossentini, Ferreres, & Tomas-Barbera, 1997); Morroco (Malika, Mohamed, & Chakib, 2005) and Mozambique (Escriche, Tanleque-Alberto, Visquert, & Oroian, 2017). Generally, those papers are focused on physicochemical and rheological

properties. However, they do not deal with other important characteristics appreciated by the consumers such as; aroma and colour, or even the origin (botanical or geographical) that also provide added value in the marketplace. With respect to origin, pollen present in honey is "the witness" of the flowers that the bee has visited, since its entrainment by adhesion occurred when they collect the nectar. Therefore, the pollen analysis (consisting in the recognition of the pollen grains morphology of the different botanical species) is a powerful tool that allows knowing the botanical and geographical origin of honey (Oddo et al., 2004; Juan-Borrás et al., 2014).

Considering Mozambiquean honey, there is an almost total lack of scientific data; therefore, it would be interesting to expand its knowledge, especially related to quality indicators. Advertising could attract the attention of local authorities in charge of the national regulation of this product. This will facilitate its quality control, thus promoting the commercialization in local and international markets and to support and further develop the apiculture. All this in the context of the mandatory fulfilment of the international requirements with regards to specific quality criteria parameters (Council Directive 2001/110 relating to honey, 2002) and intrinsic characteristic that also provide added value in the marketplace.

Taking this into consideration, the aim of this work was to characterise honey from different provinces of North and Central Mozambique in terms of their physicochemical quality parameters, colour, sugars, pollen analysis and volatile profile. This will serve as useful information for the future regulation of honey from this region.

2. Materials and Methods

2.1. Collection samples

Seventy honey samples from northern and central Mozambique were analysed in the present study: 20 from Nampula (districts of Moma, Angoche and Ribáuè) in the north and 15 from Zambezia, 15 from Manica, and 20 from Sofala, in the centre. Each sample consisted of 750 g and collected in 2014

and 2015. Honey from Nampula and Zambezia was obtained using traditional beehives (made with local resources such as twigs, trunks and barks) and harvesting methods. However, samples from Sofala (packaged by Mozambique honey Company) and the majority of Manica samples were obtained and processed using more modern procedures.

2.2. Physicochemical quality parameters: colour and sugar analyses

The methods of the International Honey Commission (Bogdanov, 2009) were applied to determine the physicochemical quality parameters: Moisture by refractrometry (Abbe-type T1 Atago, Washington, USA, and the Chataway table); hydroxymethylfurfural (HMF) by HPLC-UV with a Compact LC 1120 (Agilent Technologies California, USA) using a column ZORBAX Eclipse Plus C18 (4.6 x 150 mm, 5 μm particle size, Agilent Technologies, USA) USA), with isocratic mode, water: acetonitrile (25:75); electrical conductivity by a conductimeter C830 (Crison Instrument, Barcelona, Spain,); pH, free and lactonic acidity by potentiometric titration with a 905 Titrando (Metrohm, Herisau, Switzerland). Furthermore, colour was measured using a millimetre Pfund scale Honey Colour Analyser C221 (Hanna Instruments, Barcelona, Spain) (Escriche et al., 2016). Water activity (a_w) was determined at 25 ° C (± 0.2 ° C) with an electronic dew point water activity meter, Aqualab Series 4 TE (Decagon Devices, Washington, USA), fitted with a temperature-controlled system (Escriche et al., 2016). The content of sugar samples (glucose, fructose, and sucrose) was determined with a HPLC Compact LC 1120 (Agilent Technologies, California, USA), coupled to an Evaporative Light Scattering detector 1200 (Agilent Technologies, Ratingen, Germany), equipped with a chromatographic Carbohydrate column (4.6 x 250 mm, 4 μm particle size, Waters, Ireland). The total antioxidant activity was measured based on the scavenging activities of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Madrid, Spain), in a UV-Vis spectrophotometer Helios alpha (Thermo Scientific, England) (Juan-Borrás, Soto, Gil-Sánchez, Pascual-Maté, & Escriche, 2017). All determinations were carried out in triplicate.

100 The laboratory where this study was carried out (Laboratory of Quality Control of Honey and
101 apiculture products of Universitat Politecnica de Valencia) participated with the described methods in
102 the last edition (June-August 2018) of "FAPAS® Proficiency Test specifically designed for Quality
103 indicators of Honey" (accredited by UKAS as complying with the requirement of ISO/IEC
104 17043:2010). The z-score values resulted from this last edition for all the parameters analyzed in the
105 present study ranged from −0.2 to 1.8. Considering that acceptable range must be $-2 \leq z \leq 2$, the validity
106 of the analytical methods is proven. Other authors also reported the Proficiency Test procedure as the
107 best way to achieve this goal (Camino-Sánchez, et al., 2012; Anagnostopoulos & Miliadis, 2013; De
108 Girolamo, et al., 2017).
109 The total antioxidant activity in honey is the only parameter, which was not a target of the FAPAS
110 Proficiency Test. For this reason, this parameter had to be validated by an internal and separate
111 procedure [linearity=0.9982; repeatability estimated as RSD=5.3% and reproducibility=8.0 (n=5) and
112 accuracy calculated as recovery=84%-110%].

113 *2.3.Pollen analysis*

114 The melissopalynologycal analyses were carried out as reported by Juan-Borrás et al., (2014). In
115 brief, 10 g of honey was dissolved in 20 mL of acidulated water (sulphuric acid, 5%) and then
116 centrifuged. After the supernatant was discarded, and the sediment was re-dissolved with distilled
117 water and centrifuged again. The residue obtained was then observed under ×40 magnification using
118 a Light optical microscope (Zeiss Axiolab, Göttingen, Germany). Pollen grains were identified
119 considering general palynological databases (Gosling, Miller, & Livingstone, 2013; Schüler & Hemp,
120 2016*;* Palynological Database on line, 2018; Hyde, Wursten, Ballings, & Coates Palgrave, 2018); and
121 the existing information about the flora present in the areas of honey collection (Crane, 1973;
122 Johannsmeier, 2016).

123 In the same manner to what was performed for ensuring the quality physicochemical parameters,
124 colour and sugar analyses results, this laboratory has participated in one of the few Proficiency Tests

specifically designed for pollen analysis in honey, organized by the "*Laboratorio Arbitral Agroalimentario-Ministerio de Agricultura Pesca y Alimentación (LAA-MAPAMA) (Spain)*" in cooperation with "*Gabinete de Servicios para la Calidad (SGCLA*)". In this case our Z-score was −0.07 (within the before mentioned acceptable range), which indicates that the validity of the pollen analysis is demonstrated.

2.4.Volatile compound analysis

The extraction of volatile compounds was done by the purge and trap methodology (45 °C for 20 min) using purified nitrogen (100 mL min^{-1}) (Juan-Borrás et al., 2014). The compounds were trapped in a glass tube packed with Tenax TA (20–35 mesh), thermally desorbed (220°C for 10 min at 10 mL min^{-1} helium flow), cryofocused in a cold trap at −30°C, and then transferred into a capillary column by heating the cold trap to 250 °C (rate of 99°C/s) using a TurboMatrix TD (Perkin Elmer TM, CT-USA). Thereafter, the extracted volatile compounds were separated and identified in a GC–MS (Finnigan TRACETM MS, Thermo Quest, Austin, USA) equipped with a DB-WAX capillary column (SGE, Australia) (60 m length, 0.32 mm i.d.,1.0 μm film thickness), using helium as a carrier gas (flow rate of 1 mL min^{-1}). The temperature oven programme was: from 40°C (2-minute hold time) to 190°C at 4°C min^{-1} (11-minute hold time) and finally to 220°C at 8°C min^{-1} (8-minute hold time). Electron ionization mass spectra were recorded in impact ionization mode at 70 eV (mass range of m/z 33-433). 2-Pentanol was used as an internal standard. Three extracts were obtained for each sample.

The identification of isolated volatile compounds was performed by comparing their mass spectra, retention times and linear retention indices against those obtained from authentic standards. When authentic standards were not available, the compounds were tentatively identified by comparing their mass spectra (m/z values of the most important ions) with those from the NIST library (National Institute of Standards and Technology) as well as the linear retention indices of all the compounds. These indices were obtained by injecting a range of C_8 to C_{20} alkanes into the Tenax applying the same temperature-programme as the samples. The variables used in the statistical analysis for differentiation

between honeys were the semi-quantified compounds data since authentic commercial standards for all identified compounds were not always possible. This data was obtained considering the relative area between the peak areas of each compound and the peak area of the internal standard, assuming a response factor equal to one and expressed as μg of compound/100 g of honey (Juan-Borrás et al., 2014).

2.5. Statistical analysis

An analysis of variance (ANOVA) (using Statgraphics Centurion for Windows) was carried out to study the influence of the province were the harvesting took place on the physicochemical quality parameters, colour, sugars and volatile compounds. The method used for multiple comparisons was the LSD test (least significant difference) with a significance level α=0.05. Furthermore, a Principal Component Analysis (PCA) data was applied by means of the software Unscrambler X.10. Stepwise linear discriminant analyses were carried out using the 'forward' procedure, which begins with no variables in the model and adds the variables with the greatest discriminating power (SPSS 16.0).

3. Results and discussion

3.1. Physicochemical quality parameters, colour and sugar content

With the aim of facilitating the comparison of variability patterns between "province" where the harvesting took place, Figure1 shows the box and whisker plots for all the physicochemical quality parameters (moisture, a_w, HMF, electrical conductivity, total antioxidant, free acidity, lactonic acidity and pH) as well as fructose, glucose, fructose/glucose ratio (F/G) and colour Pfund. According to the ANOVA analysis performed (data not shown), most parameters among provinces showed statistically significant differences, with electrical conductivity, colour, and F/G ratio being the only exception. However, the main cause of these exceptions is primarily due to the large range of variability observed for these parameters. For example, these values in Nampula samples ranged from 0.30 to 1.54 mS/cm;

54 to 152 mm Pfund scale; and 0.90 to 1.52 (F/G ratio), respectively. Considering the F-ratio values from ANOVA, moisture and a_w are the parameters most affected by the factor province.

In general, Manica and Sofala samples had the lowest moisture contents, fulfilling the international criteria for moisture (less than 20 g/100 g) (Council Directive 2001/110 relating to honey, 2002). This condition has a positive influence on the quality of the honey harvested in both provinces because low moisture levels slow down the probability of fermentation. The high stability of the honey from both provinces, especially from Manica, also remains patent due to the low levels of a_w. However, in Nampula, some samples exceeded the recommended limit of moisture and because of their high a_w, are at risk of presenting different problems of quality alteration. All these alterations, although not harmful for human health, may be the cause of negative appearance and consequently having an adverse effect on its market value. The highest moisture levels found in honey samples from specific areas of Mozambique as is the case of Nampula, could be associated with pluviosity conditions such In fact, Nampula located in the north, is humid with an average annual rainfall around 2,000 mm, in Manica and Sofala, in the centre, only 1,000 mm (Zandamela, 2008). Different authors reported that the fermentation processes of honey could be accelerated in humid areas and rainy seasons, resulting in a more vulnerable honey (Tornuk et al., 2013; Silva, Gauche, Gonzaga, Costa, & Fett, 2016). In addition to the climate conditions, inadequate beekeeping practices is another important factor that can affect the final moisture content; for instance, if it is extracted before the bees are able to dry it with their wings or if the honey is stored (after harvesting) in a very humid environment (due to the honey´s higroscopicity). Keeping in line with good beekeeping practices, another important parameter to consider is HMF, since it can increase during handling, extraction, conditioning, or storage operations and mostly as a result of thermal treatments (time and temperature) (Silva et al., 2016). In the present work HMF is ranged by province among the following minimum and maximum values: 1.1-65.0 mg/kg in Nampula; 25.3-60.6 mg/kg in Zambezia; 11.2-114.5 mg/kg in Manica and 2.2-47.19 mg/kg in Sofala. Although, in many cases these honeys exceeded the generally admitted maximum of 40

198 mg/kg, it must be considered that Mozambique has a tropical climate and therefore this parameter up
199 to 80 mg/kg is permitted (Council Directive 2001/110 relating to honey, 2002). When this value is
200 exceeded, honey is deemed unacceptable to be commercialised. The great dispersion observed for
201 HMF within the same province, highlights the importance of the beekeepers role. If some beekeepers
202 obtain honey with very low HMF values, others in the same region should do the same. All this
203 suggests that training in good practices is the first step in guaranteeing good quality honey that has the
204 potential of being sold on international markets.

205 In contrast to the above mentioned parameters, the dispersion of colour in most cases (54-152 mm
206 Pfund in Nampula; 140-150 mm Pfund in Zambezia; 88-150 mm Pfund in Manica and 84-137 mm
207 Pfund in Sofala) and electrical conductivity values (0.30-1.54 mS/cm in Nampula; 1.21-1.39 mS/cm
208 in Zambezia; 0.46-1.33 mS/cm in Manica and 0.39-1.36 mS/cm in Sofala) could be logical as a
209 consequence of the flora present in the different geographic areas. This is because both parameters
210 directly depend on the nectar or secretions of plants visited by bees. Since all Zambezia samples had
211 electrical conductivity values above 0.80 mS/cm, they could be considered as honeydew honeys.
212 However, in the other provinces, the electrical conductivity was indistinctly above and below this
213 value, for this reason, these honeys could come from both nectar of flowers or secretions of plants
214 (Council Directive 2001/110 relating to honey, 2002). The wide dispersion in the values of electrical
215 conductivity observed in the present work is in line with those reported for honey samples from Manica
216 and Sofala (Zandamela, 2008), although these authors found values of up to 2.62 mS/cm. Colour and
217 electrical conductivity parameters are related to each other, because the darker the colour, the higher
218 the mineral content resulting in higher conductivity (Juan-Borrás et al., 2014). Moreover, this trend
219 could be generally correlated to the antioxidant activity levels to the point that, in general, the greater
220 the intensity of colour and conductivity, the higher the phenolic total content of a honey (Juan-Borrás
221 et al., 2017). In fact, in the present work, one sample from Nampula simultaneously had the highest

222 total antioxidant activity (40.0 mg Trolox equivalent/100 g), colour (152 mm Pfund) and electrical
223 conductivity (1.54 mS/cm).

224 As usual in honey, fructose in all samples was the most abundant sugar, followed by glucose. The
225 average values for fructose was 39.1, 41.3, 45.1 and 39.2 g/100 g, and for glucose was 32.5, 32.4, 35.7
226 and 30.3 g/100 g, respectively for Nampula, Zambezia, Manica and Sofala. The observed differences
227 are attributed to the variable vegetation existing in provinces. The level of sucrose was less than 1g/100
228 g in all cases (LOQ, Limit of Quantification of the method)

229 The fructose and glucose ratio (F/G) provides an indication of the capability of crystallisation of
230 honey (Tosi, Lucero, & Bulacio, 2004). Although this characteristic does not affect the healthiness of
231 honey, the consumer tends to reject crystallized honey, hence its importance as a criterion of quality
232 in terms of its commercialisation. Crystallisation presents the additional problem of increasing the a_w,
233 on the upper layer, which could lead to enzymatic or microbial spoilage through the growth of
234 osmophilic yeasts (Silva, et al., 2016). In the present work, the average values of F/G ratio, around 1.2,
235 were very similar in the four provinces, the most extreme values being in Nampula province which
236 ranged from 0.9 to 1.5. Considering that the lower the ratio, the quicker the crystallisation, this
237 province could have samples that are more susceptible to granulation (Juan-Borrás et al., 2014).

238 Free acidity is considered as a quality parameter since its high levels is attributable to fermentation
239 by microorganisms. However, recent studies showed that there is no correlation between yeasts and
240 moulds count and the acidity of honey, but rather this parameter could be more related to the origin of
241 honey such as bee species, flowering, climate or

242 harvest time (Ananias, Melo, & Moura, 2013). In reality, honey is characterized by the presence of
243 organic acids in equilibrium with lactone, internal esters and some inorganic ions such as phosphates,
244 sulphates and chlorides (Silva et al., 2016). In any case, European legislation establishes 50 meq/kg as
245 the maximum limit permitted for marketing (Council Directive 2001/110 relating to honey, 2002). In

246 the present study, significant differences between provinces with respect to this parameter were found,
247 with average total values of: 15.5, 21.5, 14.7 and 10.9 meq/kg in Nampula, Zambezia, Manica and
248 Sofala, respectively, and a maximum of 22.3 meq/kg value in a Zambezia sample. It is worth
249 mentioning that the samples studied in this work were far from this maximum level established as the
250 quality criterion. However, in previous studies about honey from Mozambique (in Sofala and Manica
251 provinces) this value sometimes reached to 49.6 meq/kg (Zandamela, 2008*).* In other African countries
252 the ranges of variability of this parameter were similar to those of the present study. Terrab, Díez, &
253 Heredia (2002) reported between 10.3 to 102.0 meq/kg in honey from Morocco and Nair & Maghraoui
254 (2017) from 10.0 to 25.0 meq/kg in honey from Algeria.

255 The lactonic acidity, considered as the acidity reserve when the honey becomes alkaline, (Baroni et
256 al., 2009), ranged from a minimum of 0.1 meq/kg in one Nampula sample to 17.6 meq/kg in one
257 Zambezia sample. These values are similar to those found in honey samples from Central and South
258 of Mozambique (1.2 to 15.3 meq/kg) (Zandamela, 2008) as well as in honey from other African
259 countries such as Algeria (2.0 to 5.1 meq/kg) (Nair & Maghraoui, 2017); Morocco (0.5 to 18.5 meq/kg)
260 (Terrab et al., 2002). Ethiopia (Belay, Solomon, Bultossa, Adgaba, & Melaku, 2013), Tanzania
261 (Gidamis, Chove, Shayo, Nnko, & Bangu, 2004); and from Burkina Faso (Paul, et al., 2013).

262 In the present study, in general, pH values ranged from 3.5 to around 4.5. Other authors reported
263 similar pH values in African honeys: 3.50–4.43 from Algeria; 3.80–4.50 Morocco; 3.80–4.50 Tunisia;
264 3.58-4.84 Burkina Faso and 3.87 to 5.12 from South Africa. However, several samples in Sofala
265 province showed values of around 6.3, which could be considered as characteristic of certain types of
266 honey (Zandamela, 2008).

267 In summary, and despite the different outliers specific values shown in some cases, the variability of
268 the quality parameters evaluated in Mozambiquean honey was within the range reported in other types
269 of African honey.

3.2. Pollen analysis

There is very little data available about the types of pollen that should be present in honey from Mozambique. For this reason, classifying these honeys according to its botanical origin was a difficult challenge. With this in mind, everything possible was done to reach the maximum classification detail: the taxonomic level (taxa) of identification achieved in this type of honey was always to family, and to genus, species or pollen type whenever possible. Pollen type includes species and/or genus present in the area, which have the same floral spectra and the same, or similar, pollen morphology (Acebes Ginove's et al., 2001).

In the present study the simplest qualitative analysis was carried out by identifying the most numerous pollen grains and those grains with specific morphologic characteristics (Moar, 1985). The abundance of each taxa (frequency of pollen appearance) was categorized as follows: dominant or predominant pollen (D:> 45%); secondary pollen (S: 16-45%); important minor pollen (I: 3-15%); minor pollen (m: 1-3%) and pollen present (p: <1%) (Louveaux, Maurizio, & Vorwohl, 1978).

A total of 25 taxa were recorded belonging to 16 families (Acanthaceae, Anacardiaceae, Asteraceae, Brassicaceae, Campanulaceae, Caryophyllaceae, Celastraceae, Combretaceae, Convolvulaceae, Cyperaceae, Euphorbiaceae, Fabaceae, Myrtaceae, Nymphaeaceae, Pedaliaceae and Poaceae) as well as one taxa unidentified. The most common family that occurred in the samples was Fabaceae (80%), followed by Asteraceae (65%) and Poaceae (60%). The family with the most recorded taxas was Fabaceae with 5 representatives (*Acacia* sp., *Brachystegia* sp., Astragalus type, Vicia type and *Mimosa* sp.) followed by Asteraceae with 4 (*Vernonia* sp., *Sigesbeckia* sp. and two unidentified). Despite the high conductivities found in these types of honey (which leads to think that those belong to a honeydew honey type), however, honeydew elements were not observed in a significant amount in the microscope. This fact has been observed in Mozambiquean honey as well as in other countries with low levels of humidity (Zandamela, 2008).

294 The melissopalynologycal analysis permitted classifying the honey samples in terms of their
295 geographical and botanical origin. Taking into account the similarity of the pollen spectrum of the
296 honeys analysed in each region, it was possible to cluster them. In this sense, within the Nampula
297 region (N), the 20 samples analysed were grouped into four types corresponding to their similarity in
298 floral origin: group I. Predominant pollen, Astragalus type (samples N-11 to N-20); group II.
299 Predominant pollen, *Acanthus* sp. (samples N-3 to N-6); group III. Predominant pollen, Celastraceae
300 family with a presence greater than 85% (samples N-7 to N-10) and group IV. Predominant pollen,
301 Brassicaceae family with a presence greater than 90% (samples N-1 to N-2*).* In the Sofala region (S),
302 the 20 samples were clustered into 3 different groups: group V. Predominant pollen, Anacardiaceae
303 family and Astragalus type (samples S-1 to S-14); group VI. Predominant pollen, Astragalus type and
304 Myrtaceae family (samples S-15 and S-16) and group VII. Predominant pollen, Asteraceae family
305 (samples S-17 to S-20). In the Manica region (M), the 15 samples were placed into three different
306 groups: group VIII. Predominant pollen, *unknown 1* (samples M-7 and M-8). The rest of the Manica
307 samples (M-1 to M-4 and M-11, M-14 and M-15) and (M-5, M-6, M-9, M-10, M-12 and M-13) share
308 their pollen spectrum with the previously mentioned groups V and VII respectively. Samples from the
309 Zambezia region (Z) (Z-1 to Z15) also showed similar pollen spectrum of group V.
310 It is important to mention that the sediment of the sample belonging to Group IV showed an anomaly
311 of high pollen abundance and should be interpreted with caution. This is because the presence of a
312 given pollen type in a honey could not only come to the nectar but also to other sources: inclusion of
313 pollens inside the hive; inclusion of pollens during the extraction process of the honey or even aerial
314 contamination. For this reason, in the case of Group IV pollen analysis does not provide any reliable
315 information about botanical source.

316 It was observed in the present study that the honey from Nampula could be classified into four
317 exclusive types (group I to IV) not found in the other regions. This is logical since the area of Nampula
318 presents specific tropical vegetation due to the high level of humidity in this geographical area. The

319 rest of the provinces, located towards the south-central part of the country, share, in a certain way, a
320 characteristic and similar vegetation which is very different from the northern zone (Nampula). This
321 fact implies that the pollen spectrum of honeys from these zones sometimes share similarities.

322 Table 1 shows the summarized result of the 8 types of honey according to the similar pollen spectrum
323 (group I to group VIII). Within each group the taxa found have been categorized by their abundance.
324 In short, 4 different types of honey were found in the Nampula region, 3 in Sofala and Manica, and 1
325 in Zambezia. Figure 2 shows, as an example, the light microscope photomicrographs (20x
326 magnification) of the eight pollen spectrums found in the Mozambiquean provinces studied.

327 *3.3. Volatile compounds*

328 Table 2 shows the maximum and minimum values of the volatile compounds found in the
329 Mozambiquean honeys regarding the province and the pollen spectrum (group I to group VIII). In
330 addition, this table indicates the ANOVA results (F-ratio and significant differences) obtained for these
331 two factors. Of the 48 identified compounds, 28 showed statistical significant differences referring to
332 the factor "province" and 29 to "the pollen spectrum". Volatile fraction, as was the case for the
333 physicochemical quality parameters, colour and sugar content, contains potentially usable information
334 for the differentiation of the studied honeys. Group I (from Nampula) presents outstanding abundance
335 of certain compounds: Alcohols [ethanol (36.85-133.10); propan-2-ol (11.53-41.17); 2-methylpropan-
336 1-ol (9.52/38.16); pentanol-1 (78.03/235.40)]; Aldehydes [acetaldehyde (n.d.-3.30)]; Esters [2-
337 methylpropanoic acid, ethyl ester (0.32/1.70); ethyl acetate (7.67/34.56); 3-methylbutanoic acid, ethyl
338 ester (1.05/4.86); acetic acid, 2-phenyl ethyl ester (0.34/3.23); Acids[ethanoic acid (0.84/7.36); 2-
339 methyl propanoic acid (0.34/3.23)]; Terpenes [β-linalool (0.27/1.68). The other Nampula groups
340 (group II, III and IV), in general, also showed significant amounts of most of these compounds in
341 relation to the other provinces, but in smaller quantities than group I. The special abundance in groups
342 III and IV of 5 carbon methyl alcohols, such as 3-Methylbutan-1-ol, could contribute to the freshness
343 of the aroma of these honeys (Castro-Vázquez, L., Díaz-Maroto, M. C., & Pérez-Coello, M. S. (2007).

With the only exception of some samples from Group I, in general honey from Nampula are poor in aldehydes, whereas Sofala and Manica are mainly abundant in butanal, decanal and 2-methyl-butanal. It is important to mention the presence of furan derived compounds largely identified, especially in Sofala and Manica honey (7 and 6 compounds out of the 7 identified, respectively). These compounds could be present as a consequence of inadequate thermal procedures and storage conditions. Also, these furan-derived compounds could be associated with the smoke that beekeepers use to hone the bees and extract the honeycombs from the hive and to minimise their aggressiveness. Therefore, if these practices are not implemented well, they could be a source of contamination to the honey and cause unpleasant flavours. Tananaki, Gounari, & Thrasyvoulou (2009), reported the presence of certain compounds in honeys that were also present in the smoke used by beekeepers in the combs. They also observed a direct relationship between the type of fuel used (pine needles, cypress leaves, fungus, sawdust, etc.) and the amount and type of compounds generated, five of which were identified by these authors that coincide with those found in the honeys of the present study: 1-(2-furanyl)-ethanone; 2-methyl dihydro 3(H) furanone, 5-methyl furacarboxaldehyde and 5-ethenyl tetrahydro-5-trimethyl 2-furanmethanol.

3.4. Effect of province and pollen spectrum on the parameters analysed

With the aim of evaluating the general effect that the province of harvest and the pollen spectrum has on all the parameters studied, the principal component analyses (PCA) statistical method was chosen to synthesize the great quantity of information obtained from all these parameters. In this way, an easy and understandable form using an exploratory graphical description permitted to know the relationships between variables. Three separate PCA were performed considering in each case different groups of the variables studied: PCA-1. the physicochemical quality parameters, colour and sugar content exclusively; PCA-2. the volatile compounds exclusively and PCA-3. both groups of variables jointly. In these analyses the average values from the three repetitions for each sample of honey were used. The HMF values were not considered in these analyses because, as mentioned before,

this parameter is mainly related to the freshness of honey, and therefore is not useful for the differentiation among types of honey.

The PCA-3 obtained the best separation between groups, which corresponds to all the variables together (physicochemical quality parameters, colour, sugar content and volatile compounds). PCA-1 and PCA-2 information is not shown. Figure 3 shows PCA-3 in which the code for each point refers to: province-batch number (e.g: N-5 code refers to the batch number 5 from Nampula province). In the score plot, samples proximity means a certain similarity among them. It was found that two principal components explained 67% of the variations in the data set: PC1 47% of the variability, PC2 20%-The groupings observed by the pollen spectrum are overlapped (from group I to VIII). The group I from Nampula (located on the right side of the plane), is clearly differentiated from the rest of the groups by PC1. The rest of the groups are mainly in the left quadrant. This fact reveals, as previously mentioned, the vast difference between the honey of Nampula from group I and the rest. The general behaviour is that a better grouping is obtained considering the pollen spectrum over the provinces.

The information provided by ANOVA and PCA analyses of the physicochemical parameters and volatile compounds, shows that certain variables contribute more in the differentiation honeys according to the pollen spectrum. A discriminant analyses was applied to identify the variables with the highest discriminant power, considering grouping by pollen spectrum. Table 3 shows the standardized canonical discriminant function coefficients obtained for the model. In the construction of the two first discriminant functions, 14 variables were used. Considering that the higher the absolute value of a standardized canonical coefficient, the more significant a variable is, that most contributed to the discrimination of honeys according to the groups were: electrical conductivity and moisture followed by the volatile compound 3-Methylbutan-1-ol and the free acidity.

The classification results (expressed as percentages) of the discriminant analysis carried out by cross validated procedure, permitted the correct classification of 96.7% of honeys (supplementary Table 1). Within group VII (from Sofala and Manica), 14.3% of samples were incorrectly classified placing

394 them in group VIII (only from Manica). It could be considered that the proximity between both
395 provinces would imply a certain similarity in the flora and therefore very little difference in the
396 characteristics of the harvested honeys. However, as seen in the pollen analysis, these groups (VII and
397 VIII) do not share the pollen spectrum, at all. Therefore, this confusion could be caused by exogenous
398 components of honey, such as specific volatile compounds (from the smoking practices) or some
399 physicochemical parameters (moisture, colour, etc.).

400 **4. Conclusion**

401 Pollen spectrum more than geographical origin permits distinguishing different types of honey from
402 Mozambique. Not only vegetation but also the apicultural practices contribute to the variability of the
403 physicochemical quality parameters, compositional and aromatic characteristics. All this, confers a
404 certain singularity to honeys that belong to the same group. High levels of certain quality parameters
405 such as HMF and moisture, as well as the presence of certain characteristic smoke compounds,
406 highlights the importance of beekeeping practices in improving the quality of Mozambiquean honey.
407 Therefore, having a good knowledge of the environmental and human implications belonging to this
408 country, the quality characteristics of honeys is of great help to propose a program of future actions
409 when the apiculture wants to be exploited more thoroughly. These considerations are of great
410 importance when developing beekeeping activities.

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415 **Figure captions**

Figure 1. Box and whisker plots for all the physicochemical parameters considered in this study: [moisture, a_w, hydroximethylfurfural (HMF), electrical conductivity, colour Pfund, glucose, fructose, F/G ratio, pH and acidity] for honey from Nampula (N), Zambezia (Z), Manica (M) and Sofala (S).

Figure 2. An example of light microscope photomicrographs (x 20) of the eight-pollen spectrum (groups I to VIII) found in the Mozambiquean regions (Nampula, Zambezia, Manica and Sofala). Scale bars = 20 μm.

Figure 3. PCA scores (province-batch number) and loadings (physicochemical quality parameters, colour, sugar content, and volatile compounds) plots of the first two components. Nampula (N), Zambezia (Z), Manica (M) and Sofala (S)

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534

535 **Table 1**. Types of honey according to the similar pollen spectrum (group I to group VIII). Taxas were classified by abundance (category).

	POLLEN SPECTRUM CATEGORIZATION							
CATEGORY	**Group I**	**Group II** Nampula	**Group III**	**Group IV**	**Group V** Sofala, Manica, Zambezia	**Group VI** Sofala	**Group VII** Sofala, Manica	**Group VIII** Manica
Dominant pollen (>45%)		*Acanthus* sp. (acantaceae)	Celastraceae	Brassicaceae				Unknown
Secondary pollen (16–45%)	Astragalus type Vicia type				Astragalus type Anacardiaceae	Myrtaceae Astragalus type	Asteraceae (*Vernonia* sp., *Sigesbeckia* sp., 2 unidentified)	
Important minor pollen (3–15%)	Poaceae	*Justicia* sp. (acantaceae) Asteraceae	Vicia type		Myrtaceae Poaceae	Asteraceae	Myrtaceae Astragalus type	Combretaceae
Minor pollen (<3%)	*Acacia* sp. Asteraceae Carex type *Brachystegia* sp. Combretaceae Caryophyllaceae	Poaceae			Combretaceae Asteraceae Euphorbiaceae	Poaceae	*Brachystegia* sp. Unknown Anacardiaceae	Anacardiaceae Poaceae
Present pollen (<1%)					Carex type *Acacia* sp. *Mimosa* sp. *Brachystegia* sp. *Sesamum* sp. Nymphaeaceae	Convolvulaceae	Caryophyllaceae Campanulaceae *Acacia* sp. Poaceae	Cyperaceae

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Table 2. Volatile compounds (maximum and minimum expressed as μg/100g of honey) in honey from Mozambique regarding the province and the pollen spectrum (group I to group VIII). ANOVA results (F-ratio and significant differences) obtained for the factors: "province" and "pollen spectrum group".

		PROVINCES					POLLEN SPECTRUM GROUPS								
Volatile compounds μg/100g (min/max)	KI	Nampula	Sofala	Manica	Zambezia	F-ratio	I	II	III	IV	V	VI	VII	VIII	F-ratio
Acids															
Ethanoic acid	1471	0.23/7.30	0.28/4.39	0.19/2.41	0.79/3.00	3*	0.84/7.36	0.25/0.39	0.62/1.14	0.23/0.27	0.28/3.00	0.40/0.41	0.20/4.39	0.46/0.67	8**
2-Methyl propanoic acid	1602	n.d./3.20	n.d./0.37	n.d./0.67	0.07/0.38	10***	0.34/3.23	n.d./0.04	0.49/1.43	0.09/0.22	0.03/0.67	0.09/0.13	n.d./0.18	0.06/0.07	14***
3-Methyl butanoic acid	1698	n.d./2.38	n.d./0.66	n.d./2.96	n.d./0.38	ns	n.d.	0.05/0.16	0.81/2.39	0.10/0.34	0.04/2.97	0.04/0.05	n.d./0.33	0.31/0.37	7***
Alcohols															
Ethanol	951	6.44/133.1	0.05/31.1	0.89/7.00	11.67/34.6	14**	36.85/133.10	6.45/21.80	16.23/22.23	18.77/23.60	0.89/34.62	0.05/0.07	1.69/31.11	1.44/1.62	26***
Propan-2-ol	1050	n.d./41.16	n.d./3.64	n.d.	n.d./6.92	7***	11.53/41.17	0.11/0.25	n.d.	n.d.	n.d./6.92	n.d.	n.d./3.64	n.d.	41***
Butan-2-ol	1063	n.d./1.29	n.d./0.08	n.d.	n.d./0.44	5**	n.d./1.29	n.d.	n.d.	n.d.	n.d./0.57	n.d.	n.d.	n.d.	8***
2-Methyl 3-buten-2-ol	1069	n.d.	n.d.	n.d./0.07	n.d.	ns	n.d.	n.d.	n.d.	n.d	n.d./0.08	n.d.	n.d.	n.d.	ns
2-Methylpropan-1-ol	1125	1.11/41.16	0.03/3.60	0.02/0.85	1.27/6.92	10***	9.52/38.16	1.11/2.73	2.40/3.49	2.65/3.99	n.d./6.92	0.08/0.21	0.28/3.64	0.69/0.82	38***
Butan-1-ol	1186	n.d./0.64	n.d./1.8	n.d./0.77	0.15/0.44	ns	0.18/0.64	n.d./0.08	n.d.	n.d.	n.d./0.77	0.05/0.05	0.24/1.87	0.36/0.38	8***
3-Methylbutan-1-ol	1218	n.d./32.12	n.d./2.92	0.42/2.06	n.d./6.37	5*	n.d./0.44	1.60/2.42	24.05/32.13	9.62/17.59	0.53/6.38	0.16/0.18	n.d./0.78	1.55/1.69	141***
Pentanol-1	1226	n.d./235.10	n.d./5.4	n.d.	n.d./160.10	8***	78.03/235.4	n.d.	n.d.	n.d.	n.d./7.47	n.d.	n.d./5.54	n.d.	43***
4-Methyl-3-penten-1-ol	1421	n.d./0.43	n.d.	n.d.	n.d./0.15	8***	n.d./0.43	n.d.	0.03/0.24	n.d.	n.d.	n.d.	n.d.	n.d.	7***
Aldehydes															
Acetaldehyde	756	n.d./3.28	n.d./0.18	n.d./0.11	0.08/0.29	4**	n.d./3.28	n.d.	n.d./n.d.	n.d.	n.d./0.30	n.d.	n.d.	n.d.	7***

Butanal	881	n.d.	n.d./1.01	0.27/0.17	n.d.	ns	n.d.	n.d.	n.d.	n.d.	n.d./0.18	n.d.	0.07/1.01	0.11/0.13	3*
2-Methyl butanal	931	n.d.	n.d./0.64	n.d./0.25	n.d.	3*	n.d.	n.d.	n.d.	n.d.	n.d./0.19	0.62/0.64	n.d./0.50	n.d./n.d.	14***
3-Methyl butanal	938	n.d./4.46	n.d./3.42	0.20/1.47	n.d./0.27	ns	0.16/4.47	n.d.	n.d./0.17	n.d.	n.d./1.48	0.36/0.42	0.20/3.42	1.24/1.30	3*
Decanal	1535	n.d.	n.d.	n.d./0.06	n.d.	ns	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d./0.06	n.d.	ns
Benzaldehyde	1581	n.d./0.84	n.d./0.07	0.02/0.49	n.d./0.16	3*	n.d./0.84	n.d.	n.d.	n.d.	n.d./0.47	0.01/0.02	n.d./0.49	0.06/0.09	ns
Esters															
Acetic acid methyl ester	830	n.d./0.10	n.d./0.42	n.d./0.04	n.d.	ns	n.d./0.11	n.d.	0.0/n.d.	n.d.	n.d./0.04	n.d.	n.d./0.42	n.d.	ns
Ethyl acetate	898	1.3/34.50	n.d./1.34	0.07/0.47	1.42/9.34	11***	7.67/34.56	1.37/2.94	1.44/2.77	2.73/5.25	0.08/9.35	n.d.	0.07/1.90	0.14/0.17	23***
2-Methyl-1,3-pentanedioic	1054	n.d.	n.d.	n.d.	n.d./0.09	64***	n.d.	n.d.	n.d.	n.d.	n.d./0.09	n.d.	n.d.	n.d.	ns
2-Methylbutanoic acid, ethyl ester	1086	n.d./0.72	n.d.	n.d.	n.d./0.13	10***	0.16/0.72	n.d.	n.d.	n.d.	n.d./0.13	n.d.	n.d.	n.d.	26***
3-Methylbutanoic acid, ethyl ester	1090	n.d./4.86	n.d.	n.d./0.07	0.11/0.47	11***	1.05/4.86	n.d.	0.39/0.68	0.15/0.34	n.d./0.48	n.d.	n.d.	n.d.	27***
2-Hydroxy propanoic acid ethyl ester	1282	n.d./1.23	n.d./0.32	n.d./0.30	0.24/1.12	31***	n.d./1.24	0.28/0.48	0.11/0.79	n.d.	n.d./1.12	n.d.	n.d./0.10	n.d.	3*
Benzeneacetic acid, ethyl ester	1750	n.d.	n.d.	n.d./1.10	n.d./0.13	4*	n.d.	n.d.	n.d.	n.d.	n.d./0.13	n.d.	n.d./1.11	n.d.	3*
Acetic acid, 2-phenyl ethyl ester	1847	n.d./3.22	n.d.	n.d.	n.d./0.38	8**	0.34/3.23	n.d./0.18	0.08/0.18	n.d.	n.d./0.38	n.d.	n.d.	n.d.	17***
Furanes															
2,5-dimethylfurane	990	n.d.	n.d./0.27	n.d.	n.d.	ns	n.d.	n.d.	n.d.	n.d.	n.d./0.27	n.d.	n.d.	n.d.	ns
2-Methyl-dihydro-3(H)-Furanone	1252	n.d.	n.d./0.47	n.d./0.19	n.d.	ns	n.d.	n.d.	n.d.	n.d.	n.d./0.52	0.32/0.47	n.d./0.11	n.d.	8***

Linalool oxide	1464	n.d./10.0	n.d./0.83	0.17/3.6	0.97/3.43	7***	n.d./10.06	0.14/0.17	0.70/1.58	0.13/0.20	0.08/3.63	0.05/0.06	n.d./0.66	0.28/0.36	5**
2-Furaldehyde	1502	n.d./0.17	n.d./1.28	0.05/13.43	n.d./3.85	13***	n.d.	n.d.	n.d./0.16	0.04/0.18	0.02/13.43	0.51/0.71	n.d./5.00	1.71/2.22	ns
1-(2-Furanyl)-ethanone	1547	n.d./0.11	n.d./0.05	n.d./0.68	n.d./0.13	4*	n.d.	n.d.	n.d./0.11	n.d.	n.d./0.69	0.03/0.05	n.d./0.15	n.d.	ns
2-Furaldehyde, 5-methyl-	1627	n.d./0.12	n.d./0.35	0.02/1.13	n.d./0.20	4*	n.d.	n.d.	n.d.	0.07/0.13	n.d./1.14	0.19/0.35	n.d./0.32	0.06/0.09	ns
2-Furanmethanol	1646	n.d.	n.d./0.18	n.d./1.53	n.d./0.39	ns	n.d.	n.d.	n.d.	n.d.	n.d./1.54	0.12/0.18	n.d./0.27	n.d.	ns
Hydrocarbons															
Octane	796	n.d./0.22	n.d./0.15	n.d./0.09	n.d.	ns	n.d.	n.d.	n.d./0.22	n.d.	n.d./0.09	0.10/0.16	n.d./0.05	0.04/0.05	5**
Toluene	1060	n.d./1.0	n.d./0.39	n.d./0.08	n.d.	8**	n.d./0.71	0.78/1.03	n.d.	n.d.	n.d.	0.04/0.05	n.d./0.39	n.d.	34***
Ethylbenzene	1142	n.d./0.26	n.d.	n.d.	n.d.	ns	n.d.	n.d./0.27	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4*
Ketones															
Acetone	850	n.d./0.71	n.d./0.78	n.d./0.29	0.02/0.60	ns	n.d./0.72	n.d./0.37	n.d.	n.d.	n.d./0.62	n.d.	n.d./0.78	n.d.	ns
2-Butanone	929	n.d./0.73	n.d./0.05	n.d.	n.d.	5*	n.d./0.74	n.d.	n.d.	n.d.	n.d.	0.02/0.05	n.d.	n.d.	6**
2-Pentanone	1010	n.d.	n.d./0.08	n.d./0.12	n.d.	ns	n.d./0.40	n.d.	n.d.	n.d.	n.d./0.11	n.d.	n.d./0.08	n.d.	ns
Limonene	1214	n.d.	n.d.	n.d./0.60	n.d.	ns	n.d.	n.d.	n.d.	n.d.	n.d./0.26	n.d.	n.d.	n.d.	ns
3-Hydroxy 2-Butanone	1261	n.d./1.39	0.02/1.21	0.04/0.88	0.10/0.20	ns	0.30/1.40	0.22/0.43	0.19/0.46	n.d.	0.02/0.88	0.30/0.39	0.21/1.21	0.12/0.14	4***
1-Hydroxy 2-Propanone	1270	n.d./0.38	n.d/0.65	0.07/2.11	n.d./0.16	4*	n.d.	n.d.	0.18/0.38	n.d.	n.d/2.11	0.62/0.66	n.d./0.29	0.38/0.44	ns
Nitrogen compounds															
2-Methylpropanenitrile	1040	n.d.	n.d.	n.d./0.10	n.d.	5*	n.d.	n.d.	n.d.	n.d.	n.d./0.11	n.d.	n.d./0.07	n.d.	ns
2-Methylbutanenitrile	1153	n.d./0.57	n.d./0.94	n.d./0.26	n.d.	ns	n.d./0.57	n.d.	n.d.	n.d.	n.d./0.27	n.d.	n.d./0.95	0.23/0.25	2***
Sulfur compounds															
Dimethyl sulfide	775	n.d./1.11	n.d./0.4	n.d./0.60	0.02/1.93	ns	n.d./1.12	n.d.	n.d.	n.d.	n.d./1.94	0.01/0.03	n.d./0.56	0.20/0.26	ns
Terpenes					n.d.				n.d.	n.d.		n.d.			

Limonene	1214	n.d.	n.d.	n.d./0.60	n.d.	ns	n.d.	n.d.	n.d.	n.d.	n.d./0.26	n.d.	n.d.	n.d.	ns
β-linalool	1695	n.d./1.68	n.d./2.86	n.d./0.7	n.d./0.94	ns	0.27/1.68	n.d.	0.20/0.45	n.d.	n.d./0.95	n.d.	n.d./2.86	n.d.	4*

538 (ns: non significant; * p<0.05; ** p<0.01; ***p<0.001)

539

Table 3. Standardized canonical discriminant function coefficients

Variables	**Function 1**	**Function 2**
	68%	**18%**
Butan-2-ol	-0.593	0.286
2-Methyl-1,3-pentanedioic	-0.789	0.862
Toluene	-0.587	-0.071
2-Methybutanenitrile	0.082	0.867
Pentan-1-ol	0.269	-0.266
3-Methylbutan-1-ol	2.877	-0.977
Decanal	0.070	0.294
Moisture	-3.226	-0.076
Electrical conductivity	5.197	-2.186
Fructose	0.358	1.146
Glucose	-1.289	-1.867
pH	0.527	0.770
Free acidity	-1.319	1.334
Lactonic acidity	-0.680	0.599

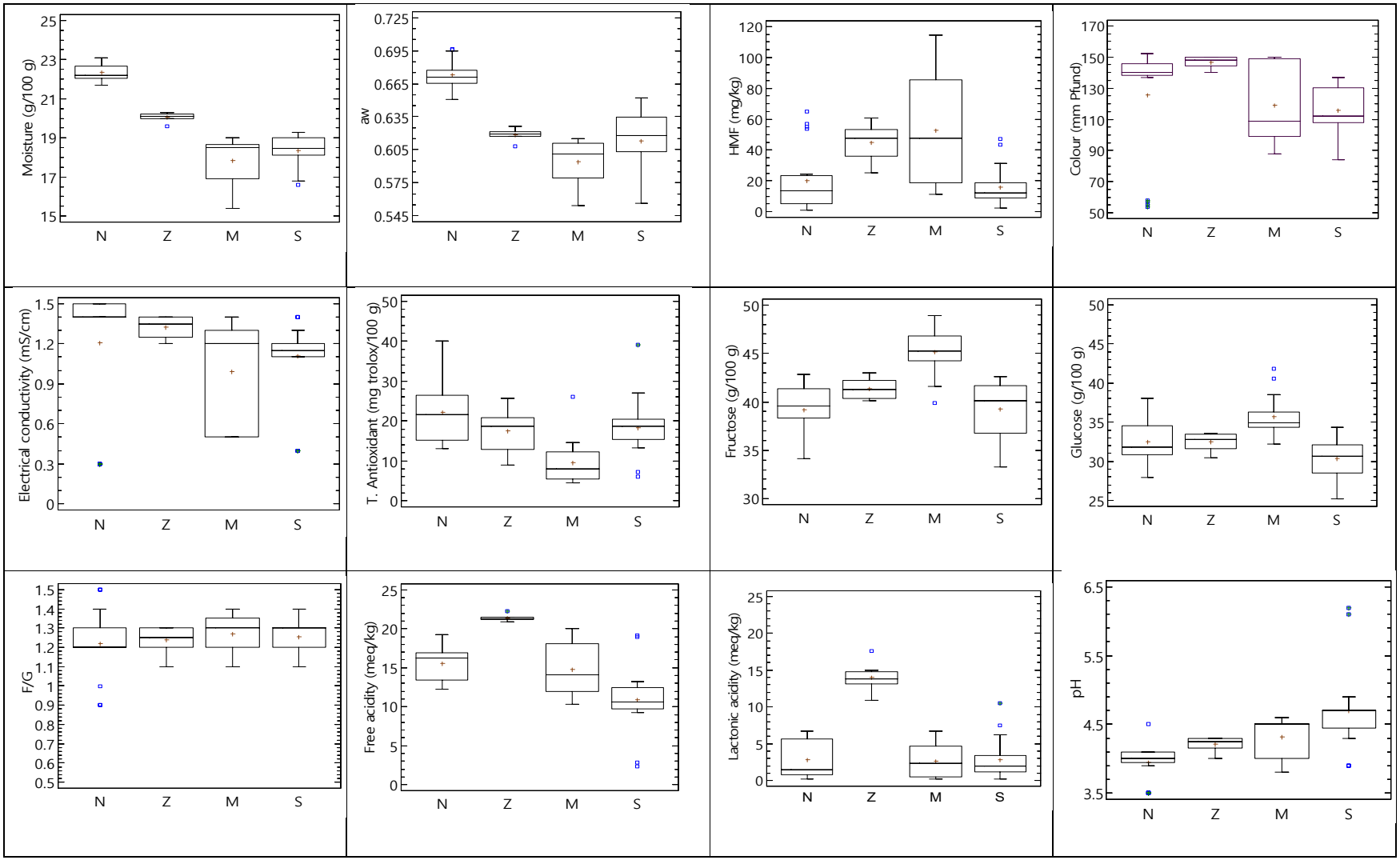

Moisture (g/100 g)
aw
HMF (mg/kg)
Colour (mm Pfund)
Electrical conductivity (mS/cm)
T. Antioxidant (mg trolox/100 g)
Fructose (g/100 g)
Glucose (g/100 g)
F/G
Free acidity (meq/kg)
Lactonic acidity (meq/kg)
pH
N
Z
M
S

Figure 1.

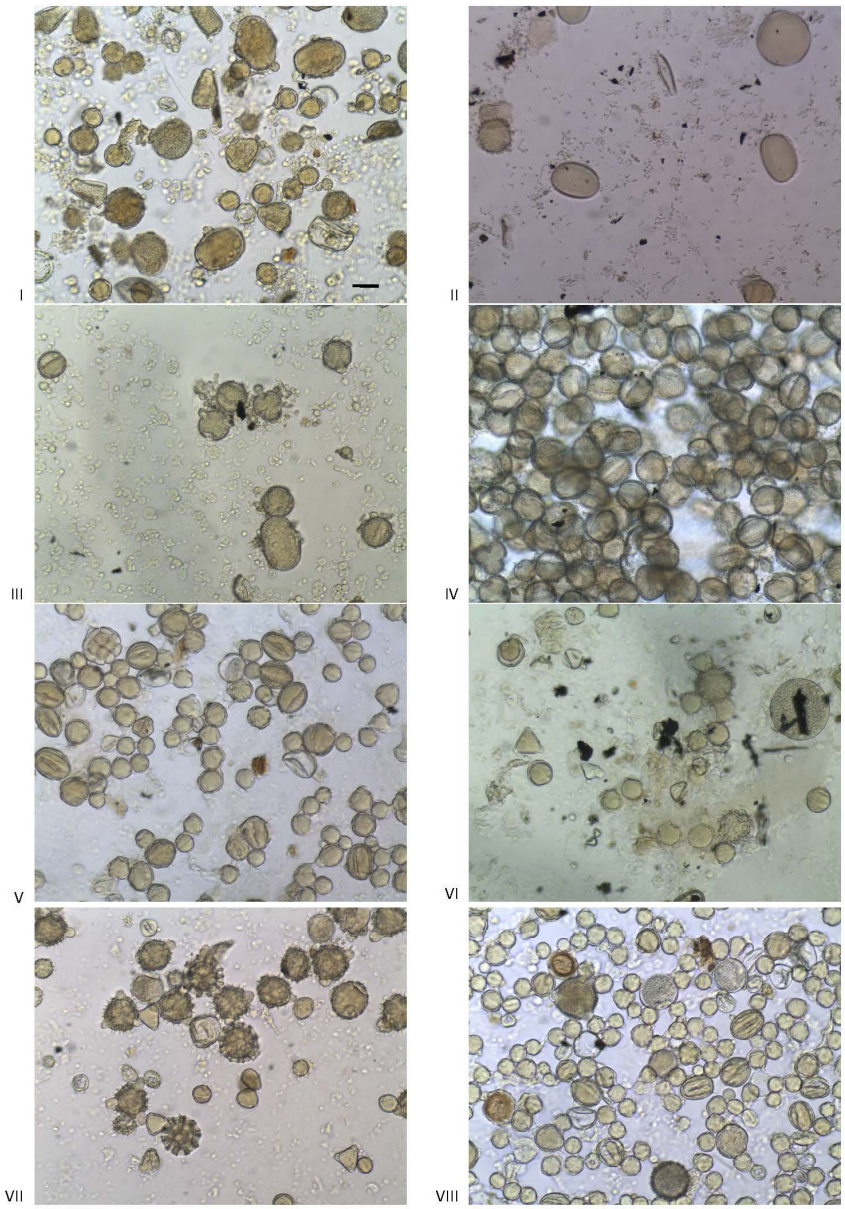
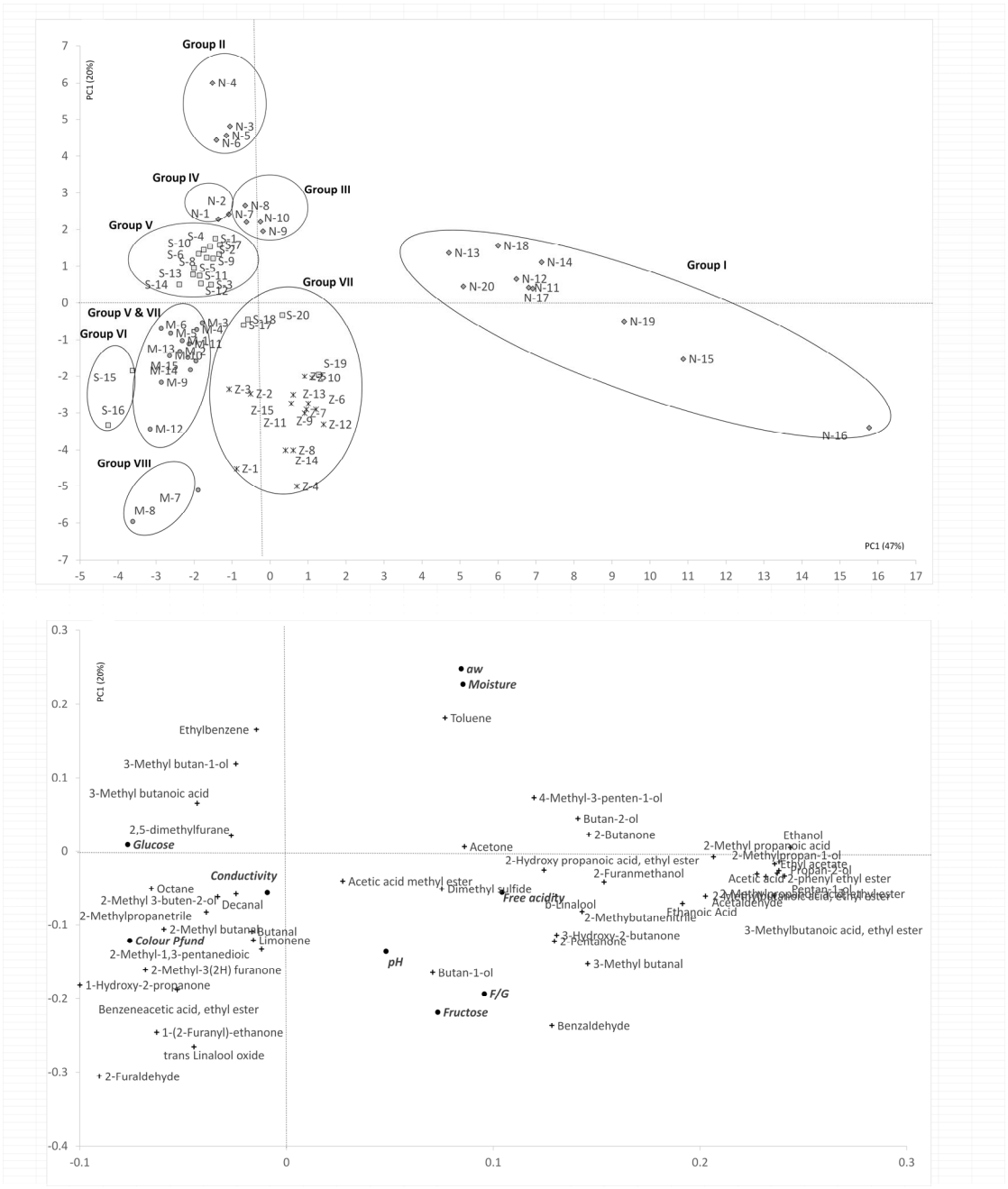


Figure 2.



546

547 **Figure 3.**