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PALYNOLOGICAL, PHYSICOCHEMICAL, AND MICROBIOLOGICAL ATTRIBUTES OF ORGANIC LAVENDER *(LAVANDULA STOECHAS)* HONEY FROM PORTUGAL

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At the present time, the quality, integrity, sanitation, and nutritional value of honeys receive attention on an international level due to the increasing content of chemicals in the aforementioned matrix. The present study aims to characterize organic honey (n=73) from Northeast Portugal, with respect to floral nectar origin, physicochemical parameters, microbial safety, and commercial quality. All organic honey samples can be classified as monofloral lavender (*Lavandula stoechas* L.), exceed in quality the international physicochemical standards, and show low microbiological counts (yeast, moulds, and aerobic mesophiles), with negative results in respect to faecal coliforms, *Salmonella,* and sulphite-reducing clostridia. Correlating the palynological, physicochemical, and microbiological results is necessary in order to check the authenticity, quality, and sanitation of honey.

Keywords: honey, organic, physicochemical attributes, microbiological analysis

Honey is an amazing natural production, the first and most reliable sweetener and food used by human beings. The importance of honey in various areas of daily life has been appreciated for centuries and across civilizations. Moreover, when analysing and studying the therapeutic properties of honeys, modern science has made it possible to specify their medical significance for healing wounds and burns (Molan, 2001), oncology care (Bardy et al., 2008), as well as its antioxidant and antimicrobial factors (Gomes et al., 2010). It is clear that honeys to be used for therapeutic purposes should be harvested in areas with no contamination sources (Feás & Estevinho, 2011; Estevinho et al., 2011).

Today, having survived all kinds of climatic changes, bees are threatened again, and therefore global food security, if pollinators, mainly honeybee, decline or disappear (Cuthbertson & Brown, 2009). The cause of the problem is still unknown, which is why it is being described as Colony Collapse Disorder (CCD) and researchers suspect this may be due to a combination of various diseases, environmental pollution, and farming practices, mainly due to an abusive use of increasingly toxic phytosanitary products and large monoculture cropping (Oldroyd, 2007).

Organic honey production is an ecologically based system, which encourages the use of good agricultural practices to maintain the agricultural ecosystem balance and diversity, promoting the sustainable use of natural resources, environmental quality, animal welfare, and human health (EU, 2007). Research results indicate that the botanic origin of honey,

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different beehive types, and the material beehives are made of, have an influence on the development of bee diseases and the quality of honey (TUCAK et al., 2004).

These strict guidelines mean that it is almost impossible for any beekeeper to be certified as organic. Given the interest in organic apiculture, which has quite recently developed in Portugal, together with the diversity, distribution, basic characteristics, and the current status of the Portuguese apiculture sector, the transition towards the certification as organic may be an easy one, compared to other countries in Northern Europe. In fact, this is more easy due to (i) the so-called "backwardness" of Portuguese agriculture; (ii) the very diverse natural heritage thanks to its geographical location and geophysical conditions (ARAÚJO, 1999), the Portuguese continental territory has 29 Special Protected Areas and 60 Sites of Community Importance (EU, 1992), and finally (iii) Portugal is in position in the European honey market, with the highest number of honeys bearing the PDO (Protected Designation of Origin) logo, adding up to a total of nine (EU, 2006). The management of such areas must be ecological, economical, and socially sustainable; most of the land in the protected allocations can only be used for grazing, which makes the apiculture one of the most promising branch of both agriculture and land livestock production in this area.

The progressive increase in the market of imported honey, with lower prices and inferior quality, has recently led to a growing need to assess the authenticity of local, specially monofloral honeys (ANDRADE et al., 1999; AZEREDO et al., 2003; PIRES et al., 2009; SILVA et al., 2009; GOMES et al., 2010; FEÁS et al., 2010a). However, the full characterization of honey is not abundant and there is a lack of information about the characteristics of honey certified as organic (ESTEVINHO et al., 2012).

The main feature of organic honey for the consumer is its purity. The consumer hopes that organic honey does not contain pesticides, inorganic impurities, it is free of bee medicines, and derives from organic crops or uncultivated areas, far from the busy highways or smoky factories. The present study aims to characterize organic honeys harvested in Portugal with respect to: (i) floral nectar origin, (ii) physicochemical parameters, and (iii) microbial safety.

1. Materials and methods

1.1. Honey sampling

Seventy-three (n=73) organic honey samples from *Apis mellifera* were collected from July to August in 2009 by twenty-five beekeepers from separate apiaries. The samples were from 5 localities of North Portugal with the following distribution: Mogadouro (n=16); Milhão (n=15); Angueira (n=13); Bragança (n=15), and Vinhais (n=14). All honey samples from a single locality were from five different producers.

1.2. Sample floral-type identification

Even though the beekeepers themselves declared honey as monofloral lavender, all the samples were subjected to pollen analysis as per the acetolysis method of Erdtman and reported previously in detail (FEÁS et al., 2010a). The following terms were used for pollen frequency classes: predominant pollen (P, more than 45% of pollen grains counted), secondary pollen (S, 16–45%), important minor pollen (IM, 3–15%), and minor pollen (M, 1–3%).

1.3. Physicochemical analysis

Analyses of the physicochemical properties of organic honey samples were performed in accordance with The *Official Methods of Analysis* of the Association of Official Analytical Chemists and The Harmonised Methods of the European Honey Commission, reported previously in detail (Methods: 969.38, 980.23, 962.19 and 958.09) (Pires et al., 2009; Feás et al., 2010b). Samples were analysed during the same time period by three different analysts to ensure uniform conditions and comparability. Three replicate analyses were made from each sample to obtain the reported data. The evaluated parameters were: water content (M, g per 100 g), ash (g per 100 g), electrical conductivity (EC, mS cm^{-1}), hydroxymethylfurfural content (HMF, mg kg^{-1}), free acidity (FA, meq kg^{-1}), diastase activity (DA, Gothe degrees), reducing sugars (RS, g per 100 g), apparent sucrose (AS, g per 100 g), and pH.

1.4. Microbiological determinations

Ten grams of each honey sample were homogenized with 90 ml of peptone water solvent. Decimal dilutions were made with the same solvent. Aerobic mesophilic bacteria were counted on standard plate count agar (PCA) and incubated at 30 ºC for 48 h (NP-3788, 2002). Mould and yeast counts were determined following the protocol of ISO (2008). Microbial counts were expressed as colony-forming units per gram of honey (CFU g^{-1}). For the counting of sulphite-reducing clostridia, aliquots of 10, 5, 1, and 0.1 ml of the initial suspension were added to empty tubes, thermally treated at 80 ºC for 5 min, and covered with SPS (sulphite–polymixin–sulphadiazine) agar media. The tubes were incubated at 37 ºC for 5 days. Faecal coliforms and Salmonella were also detected. Faecal coliforms were enumerated by the Most Probable Number technique defined in the protocol ISO (2006). Salmonella detection followed the protocol ISO (2002). All microbial tests were performed in triplicate.

2. Results and discussion

2.1. Pollen analysis

Consumers prefer monofloral honeys, which means that they also have a higher commercial value for the producers. The identified pollens and their frequency in the analysed organic honeys are presented in Table 1. Monofloral status generally refers to the presence of a single pollen type in quantities greater than 45% of the total pollen content in the spectrum. For honey samples having under-represented pollen grains, botanical classification may be achieved with a minor pollen frequency percentage, as for example, lavender honey needs 15% of *Lavandula* sp. pollen to be monofloral. The analysed samples had always *Lavandula* sp. as S (16–45%) and can be classified as monofloral lavender. Other pollens found in the total samples were: *Echium* sp. and *Rubus* sp. (both in 100% of the total honeys as S), and *Cistus* sp. (present in 92% and 8% of the total honeys as S and IM pollen, respectively). Next *Prunus* sp. was found as IM (9.3%) and M (60%); and *Erica* sp. pollen was found as M pollen in 37.3% of the total honeys. Variations in nectar content, together with other factors, such as climatic conditions, soil type, and beekeeper activities, contribute to the existence of different types of honeys (Anklam, 1998). Bees forage different plants; thus, honey is always a mixture of several sources. Differences in their composition also mean differences in the organoleptic and nutritional properties of these honeys. Portuguese lavender honeys are generated from the nectar of *Lavandula stoechas*, whereas French lavender honeys are exclusively derived from *Lavandula angustifolia*, *Lavandula latifolia*, or hybrids of these two species (Guyot-Declerck et al., 2002).

The contribution of the scientific-technical melissopalynology may in the future be a partner in commercial transactions, certifying the differentiation of honey for its floral origin as an added value that certainly should not be wasted. Moreover, melissopalynology also allows scientists to infer the vegetation present in an area, and to date and ascertain any biodiversity changes, as for example the presence and distribution of invasive and/or exotic plants (Feás et al., 2010a).

Table 1. Frequency classes, presence, range, and media of the pollen types in the honeys

Geographic area		Plant species					
		Erica	*Lavandula*	*Cistus*	*Echium*	*Rubus*	*Prunus*
Mogadouro (n=16)	presence[a]	7 M	15 S	13 S, 2 IM	15 S	15 S	3 IM, 7 M
	%[b]	46.7	100	86.7, 13.3	100	100	20.0, 46.7
	mean± SD	0.5±0.7	29.6±1.9	17.4±1.4	25.6±2.8	25.3±1,2	1.7±1.1
	range	0.0–2.0	25.6–33.0	15.0–20.0	20.0–28.5	22.8–27.0	0.2–3.3
Milhão (n=15)	presence	2 M	15 S	13S, 2 IM	15 S	15 S	9 M
	%	13.3	100	86.7, 13.3	100	100	60.0
	mean± SD	0.1±0.3	30.1±1.7	18.2±1.6	26.7±1.1	23.6±1.5	1.3±0.6
	range	0.0–1.4	27.0–32.6	15.2–20.2	25.0–28.5	20.8–27.0	0.3–3.3
Angueira (n=13)	presence	3 M	15 S	15 S	15 S	15 S	1 IM, 12 M
	%	20.0	100	100	100	100	6.7, 80.0
	mean± SD	1.2±0.7	26.9±2.0	19.5±1.5	24.7±1.9	25.6±1.8	2.1±0.7
	range	0.0–2.0	23.9–30.0	17.2–22.3	23.0–28.2	23.3–28.5	0.7–3.0
Bragança (n=15)	presence	9 M	15 S	13 S, 2 IM	15 S	15 S	12 M
	%	60.0	100	86.7, 13.3	100	100	80.0
	mean± SD	1.3±1.2	28.3±2.9	17.8±1.4	24.9±2.1	25.8±1.7	1.9±0.9
	range	0.0–3.0	23.9–32.0	15.6–19.5	22.7–28.6	23.8–28.5	0.4–2.8
Vinhais (n=14)	presence	7 M	15 S	15 S	15 S	15 S	3 IM, 5 M
	%	46.7	100	100	100	100	20.0, 33.3
	mean± SD	0.6±0.7	26.9±2.8	18.8±0.8	25.7±1.8	26.4±0.7	1.6±1.2
	range	0-2	23.0-30.9	17.2-19.6	22.8-28.2	25.0-27.3	0.2-3.1
Mean (n=73)	presence	28 M	75 S	69 S, 6 IM	75 S	75 S	7 IM, 45 M
	%	37.3	100	92.0, 8.0	100	100	9.3, 60.0
	mean±SD	0.8±0.9	28.3±2.6	18.3±1.5	25.5±2.1	25.3±1.7	1.7±0.9
	range	0.0–3.0	23.0–33.0	15.0–22.3	20.0–28.6	20.8–28.5	0.2–3.3

The following terms were used for pollen frequency classes: P: predominant pollen (>45%); S: secondary pollen (16%–45%); IM: important minor pollen (3%–15%); M: minor pollen (1%–3%).
[a]n=Sample size; [b]percentage of representation in the studied samples

2.2. Physicochemical parameters

Table 2 summarises the results obtained (mean, range, and standard deviation, SD) from the physicochemical analysis of the organic honey samples. The M (%) varied from 16.0 to 18.3 (mean value±SD=17.2±0.6%). The small variation observed in the water contents of these samples may be due to the similar bee-hive handling practices applied by the Portuguese beekeepers. The M of honey depends on various factors, for example: the harvesting season, the degree of maturity reached in the hive, and environmental factors. The maximum amount of M present in honey is the only composition criterion that, as part of the Honey Standard, has to be met for all world trade honeys. In Codex Alimentarius (2001) and EU Council directives (2002) the maximum M content value of pure floral honey is given as 23% for heather honeys and not more than 20% in general.

HMF and DA are widely recognized parameters for the evaluation of honey freshness and/or overheating. International regulations set a minimum value of 8 on Gothe's scale for DA, and a maximum HMF content of 40 mg kg^{-1}. (Codex Alimentarius, 2001; EU, 2002). The HMF content of the honeys analysed ranged from 0.8 to 1.5 mg kg^{-1} (mean value±SD=1.1±0.2 mg kg^{-1}). The HMF content is indicative of honey freshness and, from this point of view, most of the analysed samples are fresh, coinciding with the information provided by the beekeepers. The DA of honey samples is 15.4 (Gothe degrees) (average) with a range of 13.9 to 16.4 and a SD of 0.6. Values obtained for HMF and DA are typical of unprocessed honey. In honey, these parameters are related to its quality and heat processing but have not been related to the origin of the samples (Anklam, 1998). No sample exceeded the limits established for these variables (Codex Alimentarius, 2001; EU, 2002).

Ash and EC values depend on the mineral content of the honey: ash gives a direct measure of inorganic residue after carbonisation, while electric conductivity measures all ionisable organic and inorganic substances. The honeys considered in this study had ash contents ranging from 0.1 to 0.5%. Ash values were below 0.6%, as expected for nectar honeys (Codex Alimentarius, 2001; EU, 2002). The EC values of the honeys analysed ranged from 0.1 to 0.4 mS cm^{-1} (mean value±SD=0.3±0.1 mS cm^{-1}). The electrical conductivity of honey may be explained by taking into account the ash and acid content of honey, which reflects the presence of ions and organic acids; the higher their content, the higher the resulting conductivity is.

The honey samples presented a pH from 3.4 to 4.0, with an average of 3.7. The low pH of honey inhibits the presence and growth of microorganisms and makes honey compatible with many food products in terms of pH and acidity. This parameter is of great importance during the extraction and storage of honey as it influences its texture, stability, and shelf-life. Published reports indicate that pH should be between 3.2 and 4.5 (Bogdanov et al., 1999). The values of pH in honey help to determine its origin: flower or forest; the latter showing higher values.

The FA of honey samples is 29.8 meq kg^{-1} (mean) with a range of 38.9 to 41.3 and a SD of 0.7 meq kg^{-1}. Variation in FA among different honeys can be attributed to floral origin or to variation because of the harvest season (Pérez-Arquillué et al., 1995). The FA of honey may be explained by taking into account the presence of organic acids in equilibrium with their corresponding lactones, or internal esters, and some inorganic ions. All of the samples investigated met the demands set out in the regulations that require, in general, not more than 50 meq kg^{-1} and not more than 80 meq kg^{-1} (baker's honey); this indicates the absence of unwanted fermentations (Codex Alimentarius, 2001; EU, 2002).

Table 2. Physicochemical parameters of analysed organic honey samples

	Moisture (% w/w)	Electrical Conductivity (mS cm^{-1})	Ash (% w/w)	HMF (mg kg^{-1})	Diastase activity (Gothe scale)	pH	Free acidity (meq kg^{-1})	Reducing Sugars (%)	Apparent sucrose (%)
Mogadouro (n=16)	17.3±0.6 (16.5–18.2)	0.2±0.1 (0.2–0.3)	0.2±0.1 (0.2–0.4)	1.1±0.2 (0.9–1.5)	15.3±0.7 (14.0–16.2)	3.8±0.1 (3.6–4.0)	40.5±0.6 (39.7–41.2)	67.8±0.4 (66.9–68.3)	7.9±0.2 (7.6–8.3)
Milhão (n=15)	17.8±0.6 (16.6–18.2)	0.3±0.1 (0.2–0.4)	0.3±0.1 (0.2–0.4)	1.2±0.2 (1.0–1.5)	15.5±0.4 (14.9–16.0)	3.7±0.1 (3.5–4.0)	40.2±0.7 (39.4–41.3)	67.9±0.2 (67.4–68.3)	8.1±0.2 (7.7–8.2)
Angueira (n=13)	17.2±0.7 (16.4–18.3)	0.3±0.1 (0.3–0.4)	0.3±0.1 (0.2–0.4)	1.1±0.1 (0.9–1.2)	15.6±0.4 (15.0–16.2)	3.8±0.1 (3.6–4.0)	40.3±0.8 (38.9–41.2)	67.9±0.7 (65.9–68.9)	8.2±0.1 (7.9–8.3)
Bragança (n=15)	17.2±0.6 (16.5–18.1)	0.3±0.1 (0.2–0.4)	0.3±0.1 (0.2–0.5)	1.2±0.2 (0.8–1.5)	15.2±0.6 (14.0–16.2)	3.8±0.1 (3.6–4.0)	40.5±0.6 (39.5–41.3)	67.9±0.4 (67.1–68.3)	8.0±0.2 (7.6–8.3)
Vinhais (n=14)	17.0±0.7 (16.0–18.0)	0.2±0.1 (0.1–0.3)	0.1±0.1 (0.1–0.2)	1.1±0.2 (0.8–1.4)	15.2±0.8 (13.9–16.4)	3.7±0.2 (3.5–3.9)	40.0±0.6 (38.9–41.2)	67.5±0.8 (65.6–68.5)	7.9±0.2 (7.5–8.2)
Mean (n=73)	17.2±0.6 (16.0–18.3)	0.3±0.1 (0.1–0.4)	0.3±0.1 (0.1–0.5)	1.1±0.2 (0.8–1.5)	15.4±0.6 (13.9–16.4)	3.7±0.1 (3.5–4.0)	40.3±0.7 (38.9–41.3)	67.8±0.5 (65.6–68.9)	8.0±0.2 (7.5–8.3)

n=Sample size

Table 3. Microbial analyses of organic honey samples.

	Mogadouro (n=16)	Milhão (n=15)	Angueira (n=13)	Bragança (n=15)	Vinhais (n=14)	Mean (n=73)
Moulds and Yeasts[a]	15±5.0	16.0±5.0	12.0±3.0	15.0±4.0	15.0±4.0	14.1±4.5
Aerobic mesophiles[a]	$1.9\times10^2\pm4.3\times10^1$	$2.1\times10^2\pm3.9\times10^1$	$1.9\times10^2\pm2.4\times10^1$	$1.9\times10^2\pm2.5\times10^1$	$1.9\times10^2\pm2.5\times10^1$	$2.0\times10^2\pm3.2\times10^1$
Faecal coliforms[b]	<1	<1	<1	<1	<1	<1
Sulphite-reducing clostridia[c]	nd	nd	nd	nd	nd	nd
Salmonella[d]	nd	nd	nd	nd	nd	nd

[a]Colony-forming units per gram of honey (CFU g^{-1}); [b]faecal coliforms were enumerated by the Most Probable Number (MPN); [c](in 0.01 g); [d](in 25 g); n=sample size; nd: not detected

Honey is mainly composed of the monosaccharides glucose and fructose. The RS (%) content of the honeys analysed ranged from 65.6 to 68.9% (mean value±SD=67.8±0.5) and the mean percentages of AS is 8.0% with a range of 7.5 to 8.3 and a SD of 0.2 (sucrose content by European Directives must be under 5%). These two parameters confirm that the honey samples studied were floral honeys. In respect to reducing sugars (fructose and glucose), EU Directive (2002) imposes values ≥60 g/100 g per except for honeydew honey, which is ≥45 g per 100 g. These samples do not only meet the standards but also correspond to the levels observed in other studies (Terrab et al., 2002; Küçük et al., 2007). Non-reducing sugars (apparent sucrose) are set to be ≤5 g per 100 g for the majority of honeys, except for citrus and eucalyptus honeys, which have higher limits (≥10 g/100 g), as well as lavender honeys (≥15 g per 100 g). Higher sucrose contents could be the result of an early harvest of honeys, i.e. the sucrose has not been converted to fructose and glucose. The sucrose determined for the organic lavender honey (8.0%) can be justified by its floral origin. Modern techniques, based on chromatography, are desirable for the determination of honey sugar composition. The reducing sugar and apparent sucrose determination may be acceptable, especially because floral honeys usually do not contain high amounts of trisaccharides and starch, which appear as apparent sucrose.

2.3. Microbiological analyses

Microbial counts in organic honey samples are presented in Table 3. Honey, in spite of its usefulness, is known to contain certain microbes. The microorganisms that survive in honey are those that withstand the concentrated sugar, acidity, and other antimicrobial characters of the honey. In any case, this natural reservoir for microbes′ status does not diminish the many important uses that honey is known for.

Levels of quantification for the commercial quality parameters (aerobic mesophiles and moulds and yeasts) in the analysed honey samples were generally lower than those reported by other authors. Yeast and moulds were detected in low counts, with a mean value of 14.1±4.5 CFU g^{-1}. The total aerobic mesophilic counts in the samples ranged from 1.9×10^2 to 2.1×10^2 CFU g^{-1}, with a mean value of $2.0\times10^2\pm3.2\times10^1$ CFU g^{-1}. The presence of fungi in honey is linked to contact with the intestinal contents of bees, bee hive and grass (Gillian & Prest, 1987). The primary sources of sugar tolerant yeast are flowers and soil. From the microbiological point of view, these low values of moulds and yeasts would be most probably related to environmental conditions, and are indicative of an appropriate management of organic apiaries. Iurlina and Fritz (2005) found higher levels of contamination for both aerobic mesophiles (average 244 CFU g^{-1}) and moulds and yeasts (average 34 CFU g^{-1}) counts.

In respect to sanitary quality (faecal coliforms) and safety (sulphite-reducing clostridia and *Salmonella*), all our samples were negative. In contrast, other authors detected coliform contamination in one tested sample (Iurlina & Fritz, 2005), and reported that 70% of 23 honey samples were contaminated with sulphite-reducing clostridia (Finola et al., 2007). Having reviewed the scientific literature, *Clostridia* appear to be the main microorganisms in honey concerning human health. Its presence in honey is especially dangerous for babies under one year old, since they do not have a fully developed immune system, and honey is the only dietary vehicle so far definitively linked to infant botulism. Honey samples collected at retail level were found to be contaminated with *Clostridium botulinum* spores in the USA (10% of the analysed samples), Japan (8.5%), Brazil (7.5%) and Italy (6.5%); with contamination levels between 5 and 80 spores/g of product (Nakano & Sakaguchi, 1991).

EU legislation lacks specifications concerning microbiological contamination and hygiene of the product. In fact, numerous studies have been reported on the physicochemical parameters of honeys from all over the world, but microbiological contamination studies are few and are essentially devoted to *C. botulinum*.

3. Conclusion

Concerns about traces of numerous toxic substances have prompted some demand for honey that is certified as organic. The present study has characterized organic honey harvested in Portugal, in respect to floral origin, physicochemical parameters, and microbial safety. All organic honey samples can be classified as monofloral lavender (*L. stoechas*) and all of the values obtained for the physicochemical and microbiological parameters exceed in quality the limits imposed by the present legislation. Biodiversity is vital for the well-being of humans today as well as in the future, and bees must be seen as the guardians of the environment, thus a clean and healthy environment should be provided for our apiculture to be viable. One of the fundamental aspects of quality that affects its commercial value is its botanical and geographical declaration of origin; as in its food chain: from harvesting to packaging, the product should not be submitted to any treatment that modified its composition. Using suggestive names, labels, and descriptions in the marketing of honey products sometimes reaches almost fraudulent dimensions, creating false hopes and expectations in consumers. Such practices are untruthful, unethical and should be avoided. Results reported in this study should be introduced in the organic honey label, and may help beekeepers, industry, researchers, and consumers to better understand organic honey properties.

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