Abstract

Honey, consumed by people for thousands of years due to its organoleptic and therapeutic properties, is the product of the union of two worlds, the animal (the bee *Apis mellifera*), and the plant (nectar from flowers and/or sweet secretions from plants and insects). Today, the honey packaging and retail business must classify honey before the manufacturing process, taking into account quality and safety criteria in order to meet legal and commercial requirements. In addition to the requirements relating to compliance with the levels of physico-chemical parameters obligatory by national and international law; the quality criteria require classification according to botanical origin, which results in added value for the companies. In this regard, looking for new analytical tools that facilitate the botanical differentiation of honey would be useful for the beekeeping sector. This is because the traditional method based on the quantification of the pollen content not only has the disadvantage of requiring skilled technicians, but is also sometimes subject to interference, especially when the pollen content is underrepresented, as occurs in some types of honey.

Another essential aspect for the sector, is that related to the possible presence of chemical residues (antibiotics or pesticides) in honey, as a direct consequence of veterinary treatments or indirectly due to agricultural treatments. In this regard, guaranteeing compliance with the legislation and reducing risks to consumers is an essential requirement in the field of food safety. For this reason, on receiving batches of raw honey, the packaging industry must conduct proper analysis using proven and validated methods.

Taking this into account, the present PhD thesis has two distinct objectives: 1. To evaluate the techniques that have been used routinely in the quality control of honey, at both an industrial and commercial level, and to compare them with other unconventional alternatives, and 2. To evaluate the effectiveness of monitoring the raw material in the packaging industry (carried out on receiving batches of raw honey,) to meet the legal limits regarding the presence of chemical residues. Also, to assess the risk to the consumer as a result of exposure to such residues when there is a legally established maximum residue limit (MRLs).

Based on the results obtained it is concluded that, in general, the physicochemical parameters that have been used conventionally in the classification of honey do not permit good differentiation in terms of monoflorality. While the botanical origin of honey has a clear impact on some of them, such as the color and electrical conductivity, levels of certain physicochemical parameters may vary depending on the year of harvest (especially color) and beekeeping practices. In this line, the beekeeper has an important role in the variability of some of these parameters, especially in regard to HMF and moisture, and even in the characteristic varietal color that the market requires.

Therefore, good beekeeping practices are essential to obtain the product that the consumer expects and legislation requires.

The alternative techniques tested in this study, such as identifying characteristic volatile compounds in the volatile fraction of honey, and the application of an electronic tongue made with metals, have provided useful and promising results in the classification of honey to complement the information obtained by pollen analysis.

The use of chemical fingerprinting, such as for methyl anthranilate in citrus blossom honey, is particularly useful when the percentage of pollen is particularly low, as in the case of sterile hybrids or when pollen and nectar production is not simultaneous. However, inexplicably commercial transactions are sometimes more demanding with this type of honey than with others, as they not only require the presence of a minimum percentage of *Citrus* spp. pollen (at least 10-20%), but also require a minimum presence of methyl anthranilate (2 mg/kg). This PhD thesis suggests reconsidering the level of this compound required in Spanish citrus honey; proposing a minimum value of 1.2 mg/kg (greater than that recommended in other studies for Italian citrus honey). However, only taking this parameter into consideration in the case of honey with a surprising low percentage of citrus pollen, and after evaluating its organoleptic and physicochemical properties.

The presence of certain compounds, in the volatile fraction of the honey, is decisive in its differentiation; botanical origin having the greatest influence on discrimination and to a lesser extent the geographical origin. For example, carvacrol and α-terpinene are characteristic of tilia honey; α-pinene and 3-methyl-2-butanol of sunflower honey; and cis-linalool oxide of acacia honey.

The information obtained with an electronic tongue (made with metal sensors) in combination with appropriate multivariate statistical techniques (Principal Component Analysis and Neural Networks) has demonstrated that this system allows the differentiation of honey by botanical origin with a success rate of 100%. A good correlation between the electronic tongue and the antioxidant capacity of honey has also been confirmed (0.9666).

With regard to the control of chemical residues, the results confirm that proper quality control on receiving batches of raw honey, applying appropriate validated analytical methods, is effective in reducing the risk of exposure to sulfonamides in commercialized honey. In this respect, it can be considered that honey consumer safety, with regard not only to the presence of sulfonamides, but also to other chemical residues such as antibiotics and pesticides is guaranteed, as the control that companies carry out routinely covers all this aspects.

Regarding the risk of exposure to pesticides through consumption of honey, it is concluded that, although in this study, carried out with commercial samples, the

Maximum Residue Level (MRL) was not exceeded for any of the pesticides analyzed; the consumer is exposed to many of them at concentrations below these limits (especially acaricides used against varroa). However, the "hazard index" (HI) for the presence of pesticides in honey obtained as the addition of the individual risk of each pesticide (Hazard Quotient: HQ) present in them, was in the worst case 500 times lower than the value of 1, considered as the limit of acceptability. Although consumers are not exposed to toxic levels of pesticides through consumption of honey; the principle that exposure to residues has to be "as low as reasonably possible", means that the primary sector, beekeepers and farmers, has to strive to improve their practices as these directly influence the problem of the presence of residues in honey