Adulteration study in Brazilian honey by SNIF and ^{1}H NMR

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Introduction

The apiculture in Brazil are increasing in the last years and will be possible to ingress in the set of the honey exportation country selling to Europe, United States and Japan[1]. Therefore, it is very important to have a good quality control to attend the exigency of the consuming market.

Honey adulteration is easily carried out by several ways like a simple sucrose addition (of a C_4 plant like sugar cane), a mixture of honeys from different source and more recent, to feeding the bees with a sucrose solution or sucrose syrup with vegetal extract when they are harvesting the nectar. The deuterium/hydrogen ratios measured at the methyl site of ethanol $(^2H/^1H)_I$, obtained from honey fermentation, differ significantly according to the nectar origin. By feeding the bees with sucrose solutions from sugar-cane will increase the $(^2H/^1H)_I$ value if compared with that produced from C_3 plant, like nectar from eucalyptus or citrus[2].

This work describe the use of SNIF-NMR techniques to confirm the honey precedence and investigate the product adulteration. ^{1}H NMR was also used together with chemometric methods, Hierarchical Cluster Analysis (HCA), to complement this study. The HCA method examines the distances between all of the samples and represents the information in the form of a dendrogram[3].

Materials and Methods

The ^{1}H and ^{2}H NMR spectra were recorded in a DRX 400 BRUKER spectrometer operating at 9.4 Tesla in triplicate and in a 5 mm probe at 298K. The internal reference was TMS (tetramethylsilane) for ^{1}H NMR spectra and TMU (tetramethylurea) 99% to establish the isotopic relationship on the methyl and methylene groups of ethanol.

The isotopic ratios at the methyl $(^2H/^1H)_I$ and methylene $(^2H/^1H)_{II}$ sites were determined according to the equation[4]

$$\left(\frac{^2H}{^1H}\right)_i^A = \frac{I_i^A}{I^P} * \frac{P^P}{P_i^A} * \frac{m^P}{m^A} * \frac{M^A}{M^P}\left(\frac{^2H}{^1H}\right)^P$$

where I_i^A and I^P are the areas of signal i of A and of the methyl signal of TMU in the ^{2}H NMR spectrum. P_i^A and P^P are the stoichiometric numbers of hydrogens at site i and in the TMU. M^A, m^A and M^P, m^P are the molecular weight and mass of the investigated compound, A, and the reference, respectively.

Results and Discussion

In figure 1, we can observe the ^{1}H and ^{2}H NMR spectra of ethanol obtained from fermentation of a wildflower honey, where can be visualised the signals of ethanol, TMU and water. The isotopic ratios $(^2H/^1H)_I$ and $(^2H/^1H)_{II}$ of ethanol samples obtained from honey fermentation are shown in table 1.

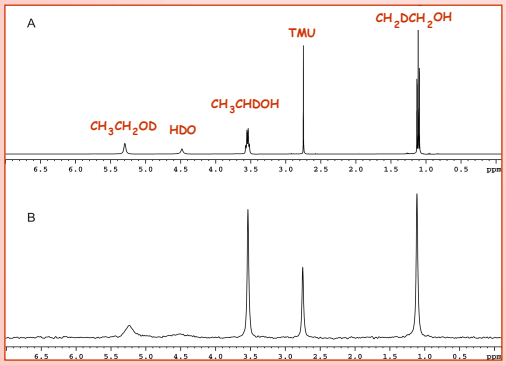

Figure 1. Natural abundance NMR spectra of ethanol from honey sample A) ^{1}H and B) ^{2}H

Table 1. $^2H/^1H$ relations value for methyl(I) and methylene(II) sites of ethanol

Honey	Isotopic Relation (ppm)** $(^2H/^1H)_I$	$(^2H/^1H)_{II}$
Eucalyptus 1*	96.9 (± 0.1)	128.3 (± 1.1)
Eucalyptus 2	96.9 (± 1.0)	128.4 (± 0.2)
Eucalyptus 3*	97.3 (± 0.8)	129.6 (± 0.5)
Eucalyptus 4	97.9 (± 0.2)	128.2 (± 0.1)
Eucalyptus 5	99.2 (± 0.7)	127.5 (± 0.6)
Eucalyptus 6	99.4 (± 0.2)	128.8 (± 0.2)
Eucalyptus 7*	99.7 (± 0.8)	128.8 (± 0.7)
Eucalyptus 8	99.7 (± 1.0)	129.1 (± 0.5)
Eucalyptus 9	98.7 (± 0.5)	128.1 (± 0.5)
Eucalyptus 10	**112.2 (± 0.5)**	**128.0 (± 0.3)**
Citrus 1	100.3 (± 0.1)	127.6 (± 0.3)
Citrus 2	100.5 (± 0.3)	129.1 (± 0.5)
Citrus 3	100.7 (± 0.4)	128.0 (± 0.4)
Citrus 4*	100.7 (± 0.6)	128.2 (± 0.2)
Citrus 5*	100.9 (± 0.6)	127.3 (± 0.4)
Citrus 6	101.3 (± 0.9)	128.0 (± 0.5)
Citrus 7*	101.8 (± 0.3)	127.9 (± 1.1)
Citrus 8	101.8 (± 0.4)	128.1 (± 0.6)
Citrus 9	102.1 (± 0.2)	127.2 (± 0.2)
Citrus 10*	103.4 (± 0.2)	128.1 (± 0.9)
Citrus 11	**112.0 (± 0.3)**	**129.2 (± 0.5)**
Citrus 12*	102.4 (± 0.1)	127.6 (± 0.5)
Wildflower 1*	97.1 (± 0.4)	128.9 (± 1.0)
Wildflower 2	97.4 (± 0.4)	127.5 (± 1.0)
Wildflower 3*	98.0 (± 0.4)	128.0 (± 2.0)
Wildflower 4	98.5 (± 1.4)	127.4 (± 2.0)
Wildflower 5	99.4 (± 0.4)	127.7 (± 0.4)
Wildflower 6	99.4 (± 0.7)	128.5 (± 0.3)
Wildflower 7	99.8 (± 0.5)	128.2 (± 0.6)
Wildflower 8	100.1 (± 0.1)	127.5 (± 0.6)
Wildflower 9	100.4 (± 0.6)	127.6 (± 0.6)
Wildflower 10*	100.4 (± 0.7)	129.1 (± 0.7)
Wildflower 11	100.5 (± 0.4)	128.4 (± 0.4)
Wildflower 12	101.1 (± 0.6)	127.7 (± 0.1)
Wildflower 13	102.0 (± 0.1)	128.4 (± 0.6)
Wildflower 14	102.5 (± 0.4)	127.7 (± 0.4)
Wildflower 15*	103.5 (± 0.2)	129.3 (± 0.2)
Assa-peixe 1*	101.2 (± 0.1)	127.8 (± 0.3)
Assa-peixe 2*	101.7 (± 0.1)	127.9 (± 0.4)
Sugar-cane*	**111.8 (± 0.2)**	**128.0 (± 0.5)**

* standard samples. ** triplicate medium values

The application of HCA in the ^{1}H NMR spectra (figure 2) became possible the discrimination of citrus and eucalyptus honeys, and moreover separated other honey kinds. The dendrogram obtained is shown in figure 3.

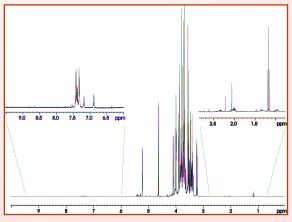

Figure 2. ^{1}H NMR spectrum of a wildflower honey

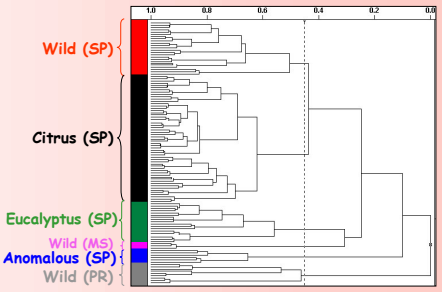

Figure 3. HCA dendrogram obtained from ^{1}H NMR spectra from different kinds of honeys

Conclusions

The SNIF-NMR method discriminated honeys from different origin as C_3 (eucalyptus and citrus) and C_4 (sugar-cane) plants, and chemometric analysis applied to ^{1}H NMR spectra separated eucalyptus and citrus honeys.

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