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Color and electrical conductivity of honeys produced by *Apis mellifera* in Uruguay

Color y conductividad eléctrica de las mieles producidas por *Apis mellifera* en Uruguay

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Resumen
La apicultura está en desarrollo en Uruguay, donde más del 90% de la producción es vendida para exportación. El objetivo de este trabajo fue investigar la relación entre el origen botánico de la miel y la conductividad. La conductividad es una medida indirecta del contenido de cenizas y desde un punto de vista nutricional es un indicador de la composición mineral de la miel, importante suplemento de la dieta del hombre. Se estudiaron cuadros de colmenas de diferentes áreas geográficas de Uruguay. La miel se extrajo mediante prensa en laboratorio y filtrado con tejido sintético. Se analizaron el color, la humedad, la conductividad eléctrica y la melisopalinología. Las mieles más oscuras presentaron mayores valores de conductividad. La mayor conductividad fue de bosque nativo, bosque de *Eucalyptus* spp. y de mielada. La miel de monte nativo uruguayo es única en el mundo y tiene un sabor típico y altos niveles de conductividad, lo que la hace una buena fuente de minerales.
Palabras clave: miel, conductividad eléctrica, color.

Abstract
Beekeeping is in development in Uruguay, where more than 90% of the production is sold for export. The aim of this work was to investigate the relation between the botanical origin and honey conductivity. Conductivity is an indirect measurement of the ash, and from the nutritional point of view, it is an indicator of the mineral composition of the honey, which is an important supplement of human diet. Honeycombs frames were studied from different geographic areas from Uruguay. The honey was extracted by a press at laboratory and filtered by a synthetic cloth. Colour, moisture, electrical conductivity, and pollen content were analysed. The darker honeys presented higher conductivity values. The higher electrical conductivity observed was from native bush, *Eucalyptus* spp. forest and Honeydew. Uruguayan native bush honey is unique in the world and has typical flavour. Moreover, this type of honey has higher levels of conductivity that makes it a good source of minerals.
Keywords: honey, electric conductivity, color.

Introduction

In Uruguay honey is produced since 1834 (Cordara, 2005) and at the moment it is the second product from farms, after citrus, that sells with exportation aims more than 90% of its production (Ministerio de Ganadería, Agricultura y Pesca, 2016). International markets are becoming more demanding regarding food quality; hence, it is more frequently required that physical and chemical parameters are determined so the honeys can be selected. There are a few studies in the country that determined some botanical, physical or chemical properties of the honey (Bazurro, et al., 1996; Daners and Telleria, 1998; Cozzolino and Corbella, 2003; Corbella, et al., 2005; Corbella and Cozzolino, 2006; Gámbaro, et al., 2007; Tejera, et al., 2013).

The color is an appreciated characteristic of the honey when stablishing commercial contracts, and it is variable depending on factors such as botanical origin, the management of the beekepers of the wax frames in the apiary, temperature and time of honey storage (Terrab, et al., 2004; Turkmen, et al., 2006; Bogdanov, 2007). On the other hand, the electric conductivity is a parameter related to the mineral content in the honey, therefore a measure that gives knowledge of the nutritional value of the honey, regarding trace elements (Acquarone, et al., 2007; Bogdanov, et al., 2004, 2007), and it has been used in several studies to corroborate the botanical origin of the honeys (Mateo and Bosch-Reig, 1998; Nanda, et al., 2003; Bogdanov, 2007). The aim of this work was to obtain information for determining the botanical origin, the color and electric conductivity of honeys produced in Uruguay, looking for a predictive relationship between these parameters. The hypothesis of this work was that at higher values of honey color, higher values of electric conductivity of the honey would be observed.

Material and Methods

Sampling

With the objective of knowing the kind of honey produced in Uruguay, periodical meetings with beekeeping groups from different areas of the country were performed. Sixty samples of honey were obtained from apiaries located in states of higher production: Artigas, Rivera, Salto, Paysandú, Río Negro, Soriano, Colonia, San José, Canelones and Florida (Figure 1).

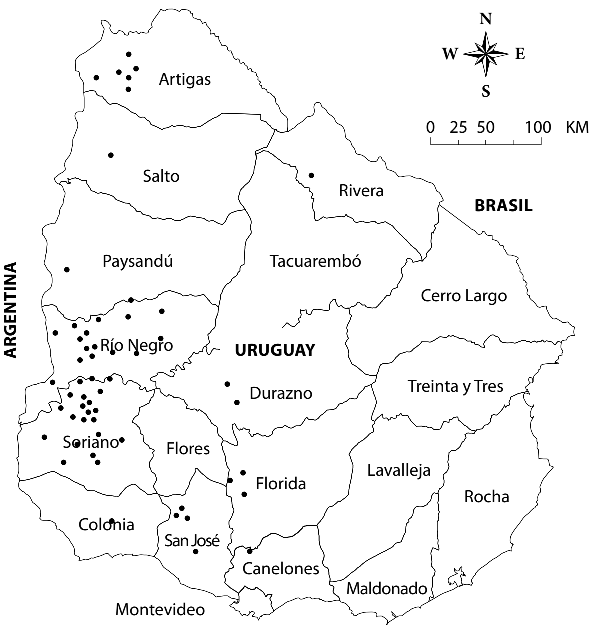

Figure 1. Study area. Sampling sites are represented by dots.

The vegetation of interest for beekeeping in these zones are: Eucalyptus forestation, prairies cultivated for cattle industry (*Lotus* spp., *Medicago sativa*, *Trifolium pratense y Trifolium repens*), natural forest, fruit trees (*Citrus* spp., *Malus domestica)* and natural prairies (*Baccharis* spp, *Senecio* spp., *Eryngium* spp.).

For obtaining mono-floral honey the honeycombs were collected directly from apiary and were extracted at the laboratory with plastic press. The honeycombs arrived at the laboratory in a box specially designed for this purpose, with the aim of avoiding contamination with metals in the transport or extraction. Once the honey samples arrived at the laboratory, the extraction of the honey was done through pressing from small pieces of honeycomb discarding zones with pollen reserves. After this, the honey was filtered to separate the wax pieces (mesh of 1 mm size) and was stored in glass jars until analysis. Honey samples from private honey extraction factories, authorized by the MGAP (Livestock, Agriculture and Fisheries Ministry from Uruguay) were also received. The samples were identified by the name of the beekeeper, locality, date of the harvest, partner vegetation in the apiary and botanical origin of honey proposed by beekeepers.

Reference collection

A pollen collection was produced for reference in the botanical characterization of honeys, with botanical material collected in several sampling fields around apiaries that provided honey for this study. For pollen processing, the technique of pollen mounting from the microscopical analysis of sediment, from the Spanish Food Legislation, was used with some modifications. The flower antenna were extracted and washed with ethylic ether for degreasing the pollen removed. The ether was evaporated in the gas extraction chimney and once dried 10 mL of distillated water was added, removing the surplus parts of the flowers. It was centrifuged for 10 m at 2500 rpm and the surplus was moved away for making the mounting of the pollen residue in the hot glycerin over a microscope slide. It was covered with a slide cover and sealed with paraffin over a hot iron for conservation.

Botanical origin

For the botanical determination, 20 g of honey were processed with distilled water according to Louveaux et al. (1978). After the final centrifugation, the residual was mounted over a microscope slide to be analyzed under a microscope (400X augment) and the counting of 600 pollen grains was done according to Von der Ohe et al. (2004) for determining the relative abundances of each pollen type.

Honeys that presented dominance of a particular kind of pollen with a percentage of pollen higher or equal than 45% were considered mono-floral. When there was no dominance of a floral type, the honey was considered to be poly-floral (Louveaux, et al., 1978; Bogdanov, et al., 1997). Exceptions were considered for the mono-floral honey of citrus and Eucalyptus, being of 18% and 75% respectively.

Electric conductivity and color

The electric conductivity (µsm/cm) was determined in a solution of honey, the equivalent of 20 g of dry matte, in 100 mL of deionized water and using a conductimeter. (Orion Model 105) (Bogdanov, 1999). For determining the honey color, the sample was placed in a manual colorimeter cell, in its liquid state at environmental temperature. The sample color was visually compared with the color scale installed at the colorimeter (Pfund scale, Fell, 1978).

Results and Discussion

Reference collection

It was possible to collect 198 botanical samples that were used for the pollen extraction. Samples of repeated species, feminine specimens, flower buttons immature or too mature (without pollen) were discarded. A collection of pollen of 99 botanical species was conformed. This collection may be used in future studies and is stored at the Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

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35% of the species present in the collection corresponded to the category natural prairies, 31% to natural forest, 19% to species of agriculture interest (harvested), 12% corresponded to the ornamental category and 2% corresponded to forestry species.

In the palynological analysis, 26 botanical species were registered in the total of the samples, and 6 pollen types were not identified. In 41,7% of the cases, the pollen result determined at the laboratory did not coincide with the botanical origin proposed by the beekeeper that sent the samples.

The majority of the honeys were mono-floral (78,3%). The kind of mono-floral honey most common was from *Lotus* spp. (Lotus), from the 47 mono-floral samples 34 corresponded to this kind (72%) and from all the honey samples, Lotus occurred in 49 (81,7%). The second most represented mono-floral honey (13,3%) corresponded to *Eucalyptus* spp. The rest of the mono-floral honeys corresponded to *Brassica napus* (Colza), *Baccharis* spp. ("Chirca") and *Citrus* sp. (Citrus). These results highlight the importance of the floral fodder species to produce honey in Uruguay. Results coincide with Díaz and Raudovinche (2010), who signal that the extensive cattering progress that is occurring at present (plantations of *Glycine max,* soybean) has decreased the area destined to fodder grass affecting the apiary performance (Table 1).

	Extracted at the laboratory	Extracted at the Beekeper Factory	Total
Monofloral	23	24	47
Multi-floral	3	9	12
Honeydew	1	0	1
Total	28	33	60

Table 1. Samples analyzed in this study.

From the natural forest honeys (multi-floral) the botanic species more represented were *Blepharocalyx salicifolius* (Arrayán) and *Myrcianthes cisplatensis* (red Guava tree), grouped in the table as native Mirtaceas.

It was also verified that the botanical origin of one of the honeys was not floral. In this sample that was sent by a beekeeper there were no pollen grains that allowed to identify it, but there were abundant elements of honeydew that would indicate an extrafloral origin.

Conductivity and color

Conductivity values ranged between 184 and 1306 µsm/cm. Darker honeys came from Eucaliptus forests and natural forest, and these also present high values of electric conductivity, higher than 800 µS/cm (Table 2).

The majority of the honeys that were analyzed came from the west of the country (Figure 1), area characterized by prairies, hence the honeys were not dark in this study.

Taking into consideration only honeys extracted directly from honeycombs at laboratory, under proper conditions that did not affect the composition of the honey (n=28), the conductivity values ranged between 184 and 889 µS/cm. Honeys with values over 800µ S/cm came from *Eucalyptus* spp., honeydew, multifloral (with high content of *Eucalyptus* spp.) and *Lotus* spp. (with high content of *Eryngium* spp.) (Table 2).

Considering only honeys extracted at the laboratory, it was observed that the color values oscillated between 12,8 and 88,9 mm Pfund (Extra White and Ambar respectively), with a mean value of 54 mm Pfund, that corresponds to Light Amber. This color category was the most represented between the honeys (Figure 2).

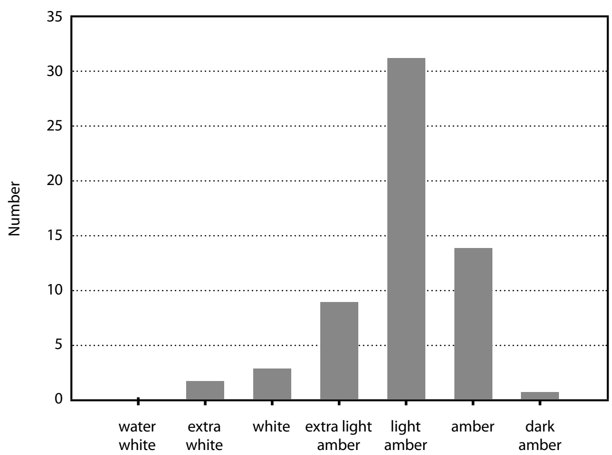

Figure 2. Quantity of honeys for color category.

Pollen (%)																			
Origin	K µS/cm	Colour mm Pfund	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Prairy	805	88,9	46,2	0,4	0,7	1,4	-	-	5,1	31,1	10	-	-		2		3,1	-	-
Eucaliptus forest	889	78,5	-	-	-	-	96	-	-	-	-	-	4	-	-	-	-	-	-
Honeydew	818	79,1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Multifloral	867	76,4	-	-	-	4,7	54,5	1,1	8,3	-	1,3	2,3	1,2	0,7	1,3	2,3	-	11	10,9
Natural forest	814	80,3	14	-	-	-	78	-	4,8	-	-	-	-	-	-	-	-	-	-

1-*Lotus* spp., 2-*Medicago sativa*, 3-*Trifolium pratense*, 4-*Trifolium repens*, 5-*Eucaliptus* spp., 6-*Senecio* spp., 7-*Echium plantagineum*, 8-*Eryngium* spp., 9-*Blepharocalyx salicifolius*, 10-*Schinus longifolius*, 11-*Bacharis* sp., 12-*Ammi biznaga*, 13-*Acacia* sp., 14-*Salix* sp., 15-*Prosopis alba*, 16-*Brassicaceae*, 17-*Malus domestica.*

Table 2. Pollen content from honeys extracted at the laboratory, with electric conductivity values higher than 800 µS/cm.

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Dominant pollen	Secondary pollen	Minor pollen	Color (mm Pfund)	Conductivity (µsm/m)
93% *Brassica napus*	6% *Eucalyptus* spp.	1% *Lotus* spp.	35,4	257
51% *Brassica napus*	39,7% *Eucalyptus* spp.	3,6% *Echium plantagineum*	61,7	314

Table 3. *Brassica napus* honey.

Regarding the botanical origin, the results showed that honey of *Lotus* sp. present some color variation, but always in the lower colours in the Pfund scale. Only one sample was from the Amber category (89 mm Pfund) and its darker color (more than the other *Lotus* sp.) was explained because of the partner flora registered in the pollinic analysis: *Eryngium* spand *Blepharocalyx salicifolius)* with important proportions (Table 2). It was verified that at higher purity in the percentage of pollen, the lighter the honey classified in the categories Extra White and White.

The same occurred with two samples obtained of colza (*Brassica napus)*. Among them, there was high variability of the color, instead of coming from the same region and dates, approximately. However, it was observed that the companion flora in the pollinic analysis presented a high proportion of *Eucalyptus* spp. that makes natural color and the conductivity of the Colza honey variated (Table 3).

From the ordination analysis performed with samples of *Lotus* sp. it was possible to observe that the samples from Soriano presented higher dispersion than samples from Río Negro. The variables that contributed to the Principal Component Analysis (PCA) were color and conductivity. The cumulated variance in the first axis was 47,1% and in the second axis 67,4%. Samples with higher content of *Echium plantagineum*, *Medicago sativa* and *Trifolium pratense*, as companion flora, presented minor color and conductivity. Samples with higher values of color presented higher content of *Eucalyptus* sp., sunflower and soybean (Figure 3).

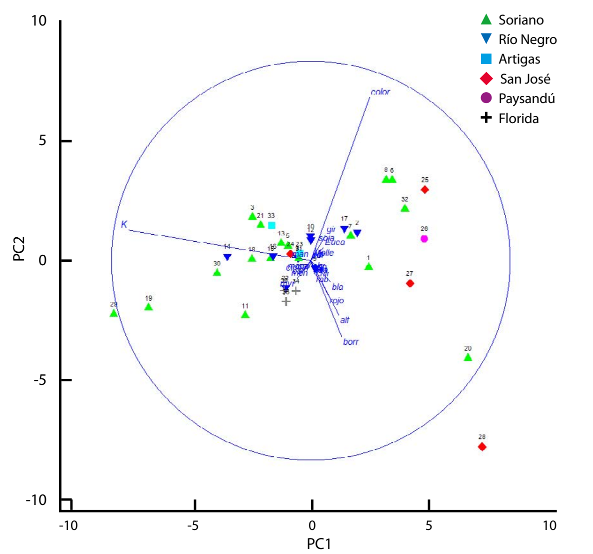

Figure 3. Principal component analysis of the honey samples from Uruguay (k: conductivity, Euca: *Eucalyptus* spp., borr: Echium plantagineum, gir: sunflower, alf: Medicago sativa, rojo: *Trifolium pratense*).

On the other hand, *Eucalyptus* sp. honeys presented light amber color, similar to a sample of natural forest and *Baccharis trimera* sample. Despite the low number of these floral type samples, we might propose that the color do not vary from what we obtained, since samples were monofloral.

Honey color and electric conductivity presented a positive relationship (Figure 4).

The darker the color of the honey, the higher the electric conductivity it presented, independently of the locality or botanical origin.

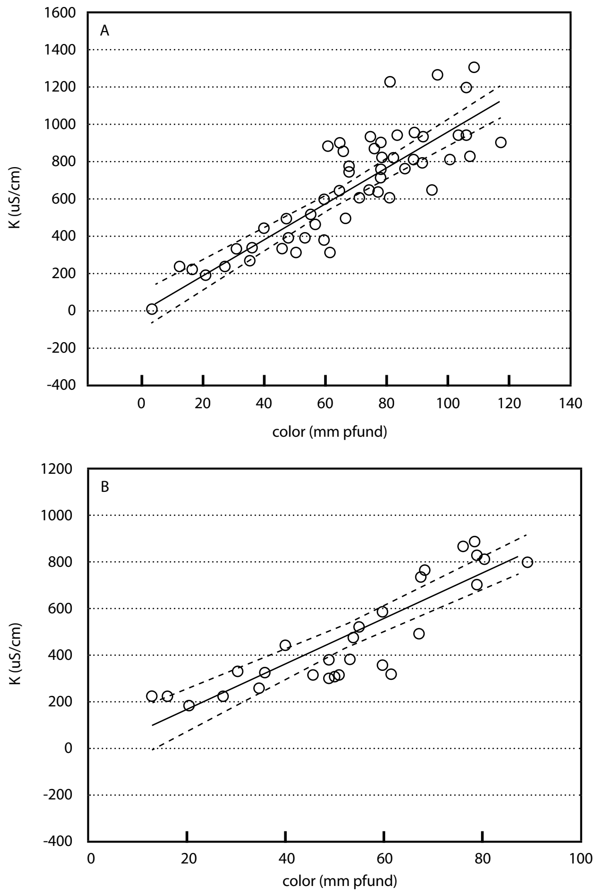

Figure 4. Linear regression and confidence interval between electric conductivity (K) and color value of all the samples (A) and from samples from honeycombs (B).

Conclusion

Uruguay is a honey exporter since last century, and honey represents one of the most exported farm products. In this work, we obtained an important number of monofloral honey. Monofloral honey was obtained without implementing special management strategies, which indicates the possibility of producing this kind of honey for differential sale, such as prairie honey (*Lotus* spp.) and *Eucalyptus* spp.

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The conductivity and color values that characterize each type of honey were determined. Each kind of honey was different in a sensorial way (color, smell) (Gámbaro, et al., 2007), but also from a physico-chemical point of view.

The botanical analysis allowed us to understand which is the flora that conforms different colors of the honey. In almost half of the cases, the pollen result determined at the laboratory did not coincide with the botanical origin proposed by the beekeeper that sent the samples. This shows that in many cases the Uruguayan beekeepers do not know the flowering origin of their honey. Thanks to studies like this, the beekeepers can decide where to place the apiaries. The fact that beekeepers do not know the botanical origin of the honey limits its production potential, because they do not move the apiaries to blooms with higher honey productivity.

Honeys with high conductivity indicate that they present high mineral content and, from a nutritional point of view, more trace elements. This is an important aspect to take into consideration for characterizing dark honeys produced in Uruguay (*Eucalyptus* spp., natural forest and honeydew). The color is indicative of the conductivity of honey, hence it is possible to use this parameter as predictive of honey conductivity.

Eucalyptus spp. honey and honeydew are considered by international regulations as honey with higher values than the maximum standard of 800 µS/cm, because of their high mineral content.

In Uruguay it is common that natural forest and Eucalyptus forest honey surpass 800 µS/cm (Corbellaand Cozzolino, 2006), but the natural forest honey produced in Uruguay is not considered at this moment. It would be interesting to conduct more research with this kind of honey to characterize and enter them to international regulations as typical honey from Uruguay.

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