



Trace elements content and antioxidant capacity in honey from protected phytogeographical regions in Uruguay

Contenido de oligoelementos y capacidad antioxidante en mieles de regiones fitogeográficas protegidas de Uruguay

Teor de oligoelementos e capacidade antioxidante em méis de regiões fitogeográficas protegidas do Uruguai

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Abstract

Honey is a natural sweet substance produced by honey bees, from the nectars of plant flowers and honey dew. The components of honey vary depending on the geographical site and the existing flora. The objective of this work was to characterize Uruguayan honeys produced in different regions by composition of trace elements (Cu, Fe, Mn, Mo, Zn, I, Se), the antioxidant capacity, the total polyphenols, and the palynological profile. Sixty-six honey samples were obtained in two seasons, spring-summer and summer-autumn, from three regions of protected areas, and a fourth region associated with human activities. The results obtained show that the contents of Cu and Mn were significantly higher ($P < 0.05$) in region C (1.785 $\mu\text{g/g}$ and 4.668 $\mu\text{g/g}$). For Mo, the Q region presented 1.060 $\mu\text{g/g}$, being significantly different ($P < 0.05$) from the other regions. The total polyphenol content from region C was significantly higher ($P < 0.05$) (66.442 mg GAE/100g). Likewise, the antioxidant capacity presented significant differences ($P < 0.05$) for region C in the spring-summer season. Some species, genus or botanical families were only found in a particular region. This work allows us to affirm that honey can be differentiated by its micronutrient content associated with the total polyphenol content, and a higher antioxidant activity for certain regions, given by the floral species present in the region and the type of soil.

Keywords: honey, micro-minerals, antioxidant capacity, palynology

Resumen

La miel es una sustancia dulce natural producida por las abejas melíferas a partir del néctar de las flores de las plantas y de secreciones de partes vivas de estas. Los componentes de la miel varían según el sitio geográfico y la flora existente. El objetivo de este trabajo fue caracterizar las mieles uruguayas producidas en diferentes regiones por composición de oligoelementos (Cu, Fe, Mn, Mo, Zn, I, Se), capacidad antioxidante, polifenoles totales y perfil palinológico. Se obtuvieron 66 muestras de miel en dos temporadas, primavera-verano y verano-otoño, de tres regiones de áreas protegidas y una cuarta región asociada a actividades humanas. Los resultados obtenidos muestran que los contenidos de Cu y Mn fueron significativamente mayores ($P < 0,05$) en la región C (1,785 $\mu\text{g/g}$ y 4,668 $\mu\text{g/g}$). Para Mo, la región Q presentó 1,060 $\mu\text{g/g}$, siendo significativamente diferente ($P < 0,05$) de las demás regiones. El contenido de polifenoles totales de la región C fue significativamente mayor ($P < 0,05$) (66,442 mgGAE/100g). Asimismo, la capacidad antioxidan-





te presentó diferencia significativa ($P < 0,05$) para la región C en la época primavera-verano. Algunas especies, géneros o familias botánicas solo se encontraron en una región en particular. Este trabajo permite afirmar que la miel se puede diferenciar por su contenido de micronutrientes asociados al contenido de polifenoles totales y una mayor actividad antioxidante para ciertas regiones, dada por las especies florales presentes en la región y el tipo de suelo.

Palabras clave: miel, microminerales, capacidad antioxidante, palinología

Resumo

O mel é uma substância doce natural produzida pelas abelhas a partir do néctar das flores das plantas e das secreções das partes vivas das plantas. Os componentes do mel variam de acordo com o local geográfico e a flora existente. O objetivo deste trabalho foi caracterizar méis uruguaios produzidos em diferentes regiões por composição de oligoelementos (Cu, Fe, Mn, Mo, Zn, I, Se), capacidade antioxidante, polifenóis totais e perfil palinológico. Sessenta e seis amostras de mel foram obtidas em duas estações, primavera-verão e verão-outono, de três regiões de áreas protegidas e uma quarta região associada a atividades humanas. Os resultados obtidos mostram que os teores de Cu e Mn foram significativamente maiores ($P < 0,05$) na região C (1,785 ug/ge 4,668 ug/g). Para Mo, a região Q apresentou 1.060 ug/g, sendo significativamente diferente ($P < 0,05$) das demais regiões. O teor total de polifenóis da região C foi significativamente maior ($P < 0,05$) (66,442 mgGAE/100g). Da mesma forma, a capacidade antioxidante apresentou diferença significativa ($P < 0,05$) para a região C na estação primavera-verão. Algumas espécies, gêneros ou famílias botánicas foram encontrados apenas em uma determinada região. Este trabalho permite-nos afirmar que o mel pode ser diferenciado pelo seu teor de micronutrientes associado ao teor de polifenóis totais e uma maior atividade antioxidante para determinadas regiões, dada pelas espécies floral presentes na região e pelo tipo de solo.

Palavras-chave: mel, microminerais, capacidade antioxidante, palinologia

1. Introduction

Honey is understood as the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants, from secretions of living parts of these or from excretions of plant sucking insects that remain on living parts of them and that bees collect, transform and combine with specific substances of their own, and deposit, dehydrate, store and leave in the honeycomb to mature and age⁽¹⁾. The main compounds of honey are carbohydrates, proteins, aminoacids, macro-minerals and trace elements⁽²⁾, and others particular compounds with antioxidant properties⁽³⁾. The components responsible for the redox properties of honey are probably phenolic acids, flavonoids, vitamins and enzymes, as well as a small amount of trace elements content, particularly copper and iron⁽⁴⁾. Previous works have shown that honey can improve the defense system against oxidative stress⁽⁵⁾. Phenolic compounds, such as aromatic acids and flavonoids present in plants, are considered the cause of the antioxidant effect and the anti-radical capacity of honey⁽⁶⁾. A straight relation between the botanical origin and the antioxidant properties of honey is demonstrated, and this may differentiate honeys from different regions in the world⁽⁷⁾. Honey production in Uruguay has had a sustained growth since last years, with 3,000 beekeepers who own 500,000 colonies. In spite of this little volume, 90% of this production is exported⁽⁸⁻⁹⁾. This activity has a social and economic role besides the essential

role of bees in biodiversity and agricultural production. The quality of Uruguayan honey is in accordance with the requirements of an increasingly demanding national and international market⁽¹⁰⁾. Although local honey comfortably meets, in the vast majority of cases, with international quality requirements, unfortunately, all of the production is exported in bulk, being enough that it complies with certain physicochemical conditions and that it does not exceed established minimum levels of contamination⁽¹¹⁾. With the increase in the intensification of land use, especially in the area dedicated to monocultures and industrialization, a latent risk for the safety of honey appears⁽⁸⁾. Furthermore, the country has developed a National System of Protected Areas (SNAP in Spanish) with important richness in biodiversity where there is honey production in natural conditions. For this reason, it is an interesting opportunity as well as extremely important to characterize honey from this protected regions, since the country could be located as an alternative source of organic honey and generate new strategies for the valorization of Uruguayan honey. The limited volume to be exported could be counter with a high quality associated to a natural product, and this would be the future opportunity to market honey with its own biological and quality traits well identified⁽¹²⁾. Based on this background and the importance of determining parameters that allow qualifying and differentiating some honey produced in Uruguay, this paper aimed to charac-



terize the Uruguayan honey produced in different phytogeographical regions by the content in trace elements (Cu, Fe, Mn, Mo, Zn, I, Se), the total polyphenols and the antioxidant capacity related to the palynological profile.

2. Materials and methods

2.1 Sample honey

Honey samples were collected from four different regions of Uruguay in two seasons, spring-summer (S-S) and summer-autumn (S-A), with a difference of three months between samples. Three of them correspond to protected areas of the National System of Protected Areas of Uruguay. The regions were Quebrada de los Cuervos (Q; located in the east), Valle del Lunarejo (VL, located in the north), and Esteros de Farrapos (EF, located in the west). A fourth region selected was Los Cerrillos (C, located in the south), associated with human activities such as horticulture and agriculture, and closer to populated centers. Two months before sampling beeswax foundations were placed in each hive. This ensures that the honey samples correspond to the study season. The samples were obtained by cutting two pieces of honeycomb of approximately 3 cm wide by 5 cm long, with 100% operculated on both sides for each hive. Ten samples were collected from each region (Q, VL, EF, C) and in each season (S-S and S-A). The samples were placed in sterile polypropylene bottles and subsequently transported to the laboratory with refrigerated conditions, then stocked at -20 °C until analysis. For region Q, due to low production in the beekeeper, 7 and 9 samples per season were obtained. Also, in EF region only a season (Spring-Summer) was sampled. The total number of samples obtained was 66.

2.2 Palynological analysis

To determine the botanical origin of honey, 20 g of each sample were diluted with distilled water⁽¹³⁾. After centrifugation the pellet was quantitatively analyzed by the pollen grains in each sample of honey, using a 400x magnification microscope. Six-hundred pollen grains were counted to determine the abundance of each family, genus or species depending on the capacity of identification⁽¹⁴⁾. Also, the presence of honey dew elements (HDE) was determined, indicative of an extra-floral origin of honey. The melissopalynology process without acetolysis was performed, based in the reference collection⁽¹³⁾. Analysis was performed in the Palynological

Laboratory of the Faculty of Sciences, University of the Republic, Montevideo, Uruguay.

2.3 Determination of trace elements

The honey samples were prepared as follows. Briefly, 2 g of honey were solubilized in acid solution, with ultrapure HNO₃ (1M, 65% Merck, ppa, distilled by sub boiling) and HCl (6M, Merck, ACS ppa) in Erlen with steam trap device, in hot plate, for 1 hour, and then taken to volume with distilled H₂O, less than 18 MΩ-cm of resistivity, in volumetric flask. The content of Iron (Fe), Zinc (Zn), Copper (Cu), Manganese (Mn), Molybdenum (Mo) and Selenium (Se) in honey was quantified by Atomic Absorption Spectrometer, AAS (Perkin Elmer, A Analyst 300, USA), equipped with Deuterium lamp as background corrector, with flame (air-acetylene; 8.0 l/min and 1.4 l/min) for Fe, Zn, Cu, Mn. For Mo and Se, it was quantified by AAS with graphite furnace (HG-800, Perkin Elmer, USA) and auto-sampler (AS 800, Perkin Elmer, USA). Suitable standards were used for each analyte. Standard single element solutions of Fe, Zn, Cu, Mn, Mo and Se containing 1000 mg/l (Perkin Elmer or Fluka) and diluted with deionized H₂O containing 2.5% ultrapure HNO₃, according to the expected values of each analyte in the sample of honey, were used. For Fe, Cl₂Ca, PPA to eliminate interference, and for Se, a chemical matrix modifier based on Palladium (500 µg/ml) and NO₃Mg were used to avoid interference with the matrix. The content of iodine (I) was determined as iodide with appropriate ionic chromatography and standards of iodine. Briefly, I was measured in the blank, honey samples, and calibration curve solutions using ion exchange chromatography (Dionex Integron HPLC system, Thermo Fisher Scientific, USA)⁽¹⁵⁾. The identification of I was based on the retention time, and quantification was performed by integrating the peak area. The sample peaks of each sample were compared to those produced by the calibration solution. Detection limit was 20 µg/l (EPA, 2016; calculated as: 2.8 × standard deviations of blanks + average of 10 blanks).

2.4 Determination of total polyphenols and antioxidant capacity

The total phenolic content (TPC) of honey was determined by using Folin-Ciocalteu reagent, based on electron transfer and providing a reduction capacity, which is normally expressed as phenolic content⁽¹⁶⁾. The method consists in a reduction/oxidation reaction (redox) between polyphenols present in samples of honey and Folin-Ciocalteu reagent. A quantity of 0.1 ml of aqueous



extract of honey was added with 0.6 ml of sodium carbonate 20%. After 2 minutes, 0.15 ml of Folin-Ciocalteu diluted 1/6 with distilled water (2N, Folin & Ciocalteu's phenol reagent working solution, Sigma Diagnostics) was added. A standard curve was also prepared with gallic acid (Gallic acid monohydrate 98+%, reagent grade, Sigma-Aldrich), with concentrations of 0 to 0.30 mg/l, and were treated as the samples. All solutions were incubated at room temperature, in darkness for 30 minutes. After that, the absorbance at 750 nm was measured in a spectrophotometer (Spectronic Genesys 6, Thermo, USA). The TPC was expressed as mg of gallic acid equivalent by 100 g of raw honey (mg GAEq/100g honey). The antioxidant activity was determined by free radical elimination activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH). A honey solution was prepared containing 0.04 g of honey in 1 ml of methanol and homogenizing the sample completely and carefully, and making the dilutions with methanol until the desired concentration was obtained, between 20-40 mg/ml, in a final volume of 1 ml. Initial absorbance of DPPH solution (60 μ M in methanol) and absorbance of samples with honey and DPPH were measured at 520 nm (Spectronic Genesys 6, Thermo, USA), and inhibition % was calculated. The capacity of honey to reduce the radical DPPH can be expressed as Trolox equivalent (TE). For that, a calibration curve (0-50 μ M) was prepared using the Trolox reagent (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), absorbance was measured, and inhibition % was calculated. A graph was made with the % inhibition versus the TE concentration and the equation was calculated. Calculation was performed for each sample of honey and the results of antioxidant capacity were expressed in μ moles TE/100 g of honey.

2.5 Statistical and chemometric analysis

From each region (C; EF; Q; VL) a number of 7 to 10 hives (repetition) were used. In each one of the seasons (Spring-Summer or Summer-Autumn) the same hives were sampled. For each variable measured, trace elements, TPC and TEAC, the results were expressed as mean \pm standard error of the mean (SEM). Data were analyzed by ANOVA, with the GLM procedure, with the region and season as main effects. When differences were statistically significant, means were compared using the Tukey-Kramer Test with a p value <0.05. Also, an ANOVA one way and a post hoc a Tukey-Kramer Test (p<0.05) were performed for all regions and seasons, because in the EF region only one season was sampled. The statistical analysis

of the data was carried out through the NCSS software⁽¹⁷⁾. For the trace elements results a chemometric method was applied, such as exploratory data analysis, to grouping minerals in the different honeys, by HPA and PCA. These two types of chemometric techniques, hierarchical cluster analysis (HCA) and principal component analysis (PCA), were performed to explore the relationships that may exist between trace elements and samples⁽¹⁸⁾.

3. Results

3.1 Palynological analysis

Through palynological analysis it was possible to determine different families, genus or species of pollen types. The results are presented in Table 1 expressed as a percentage and frequency of classes. The region C in season S-A was characterized by monofloral honeys of *Lotus sp.* (72%), observing a total of 14 pollen types. For the same region, in the season S-S, the honeys were multifloral, presenting 24 types of pollens. The pollens detected were from *Xylosma tweedianum* (14.1%), *Salix sp.* (14%), *Lotus sp.* (11.5%) and *Eucalyptus sp.* (10.3%), as the most important ones. In honeys of the Q region in the S-A season 14 types of pollen were found, and in the S-S season there were 13 types of pollen.

In the S-A season 18 types of pollen were detected, with the highest percentage being from *Schinus longifolius* (31.8%), followed by *Baccharis articulata* (24%), *Baccharis spp.* (24%), and *Tripodanthus acutifolius* (22.4%). For the S-S season 9 types of pollen were detected, principally pollens of *Myrcianthes cisplatensis* (38.6%), *Lithraea brasiliensis* (32.4%), and *Gleditsia triacanthos* (12.5%). The honeys of the EF region were also characterized by being multifloral honeys, but as in the Q and VL region, they could be considered monofloral honeys because there are no reports for this classification for this type of species, being pollens of *Myrcianthes cisplatensis* (34.8%), *Salix sp.* (34.8%), *Eucalyptus sp.* (30.7%), *Schinus longifolius* (26.2%), *Parkinsonia aculeata* (19.5%), *Trifolium pratense* (16.9%). A total of 14 types of pollen were observed in this region.

3.2 Content of trace elements in honey

Contents of Cu, I, Mo, Mn, Zn and Se were significantly different between regions (p<0.05). However, no difference was found for Fe between regions (Table 2), although significant differences between seasons were observed (Table 2).



The average honeys' Cu content from the four regions was between 1.45 and 0.19 mg/kg of honey. The highest values were found in the honeys of region C in the S-S season. Like Cu, Mn contents were higher in honeys from region C in the S-S season (5.71 mg/kg). The honeys from the Q region in the S-S season had higher Mo content (1.17 mg/kg). For I, significant differences were found between honey in the EF region with respect to VL ($p < 0.05$) (Table 2). The Se content was higher in the honeys of the Q region in the S-A season. Such amount present in the honey samples from the four regions is between 0.12–0.52 mg/kg of honey.

The result of the cluster analysis (dendrogram) is reported in Figure 1. The y axis depicts the production site discriminated by season, while the x axis indicates the calculated distances among sampling sites.

A principal component analysis (PCA) was also carried out to study the influence of trace elements, the total polyphenols and the antioxidant capacity with respect to the regions and seasons studied (Figure 2). The main components 1 and 2 (CP1 and CP2) explained 42.9% and 23.1% of the variability between samples, respectively.

Table 1. Pollen content expressed in percentage and frequency of classes: predominant (D), secondary (S), important minor (I) and minor pollen (M) in honeys' protected region (Q, EF, VL) and not protected region (C) in two harvest seasons (S-S or S-A) in Uruguay

Family	Scientific name	Region and Season							
		C S-S	C S-A	Q S-S	Q S-A	VL S-S	VL S-A	EF S-S	
Myrtaceae	<i>Eucalyptus sp.</i>	10.3 (I)	4.3 (I)	1.7 (M)	1.7 (M)	4.5 (I)	0.5 (M)	27.6 (S)	
Myrtaceae	<i>Myrcianthes cisplatensis</i>	8.7 (I)	6.9 (I)	6.5 (I)	3.1 (I)	38.6 (S)	1.6 (M)	34.8 (S)	
Fabaceae	<i>Parkinsonia aculeata</i>							17.6 (S)	
Rosaceae	-							0.8 (M)	
Fabaceae	<i>Lotus sp.</i>	11.5 (I)	72.2 (D)					0.1 (M)	
Fabaceae	<i>Acacia sp.</i>	1.6 (M)						1.0 (M)	
Scrophulariaceae	-	0.6 (M)						0.2 (M)	
Fabaceae	<i>Trifolium pratense</i>	0.4 (M)	2.0 (M)				0.3 (M)	10.1 (I)	
Asteraceae	<i>Senecio sp.</i>		0.3 (M)	4.4 (I)	2.9 (I)	0.2 (M)	2.4 (M)	0.6 (M)	
Asteraceae	<i>Cirsium vulgare</i>		0.1 (M)				0.1 (M)	0.4 (M)	
Asteraceae	<i>Senecio sp.</i>		0.6 (M)		1.9 (M)		0.8 (M)	0.5 (M)	
Fabaceae/Papilionoidea	<i>Fabaceae</i>		4.8 (I)				0.2 (M)	0.4 (M)	
Salicaceae	<i>Salix sp.</i>	14.0 (I)						3.5 (I)	
Anacardiaceae	<i>Schinus longifolius</i>	6.9 (I)		11.0 (I)	0.6 (M)	7.3 (I)	15.9 (S)	2.4 (M)	
Fabaceae	<i>Glycine max</i>		1.6 (M)						
Fabaceae	<i>Trifolium repens</i>	3.4 (I)	3.6 (I)						
Apiaceae	<i>Eryngium sp.</i>		0.2 (M)			0.3 (M)	0.2 (M)		
Apiaceae	<i>Ammi viznaga</i>	0.2 (M)	0.2 (M)						
Boraginaceae	<i>Echium plantagineum</i>	7.4 (I)	2.4 (M)			1.5 (M)			
Lamiaceae	<i>Menta piperita</i>	0.7 (M)	0.8 (M)						
Loranthaceae	<i>Tripodanthus acutifolius</i>			25.8 (S)	27.4 (S)			22.4 (S)	
Asteraceae	<i>Baccharis articulata</i>	5.9 (I)		27.4 (S)	31.6 (S)	2.7 (M)		24.0 (S)	
Asteraceae	<i>Baccharis spp.</i>	3.6 (I)		9.0 (I)	22.2 (S)			24.0 (S)	
Asteraceae	<i>Baccharis spp.</i>	2.1 (M)		7.6 (I)	5.8 (I)			4.5 (I)	
Poaceae	-			1.3 (M)	0.5 (M)			1.5 (M)	
Anacardiaceae	<i>Lithraea brasiliensis</i>			1.2 (M)	0.4 (M)	32.4 (S)		0.5 (M)	
Fabaceae	<i>Gleditsia triacanthos</i>	2.6 (M)		1.6 (M)	1.1 (M)	12.5 (I)		0.5 (M)	
Caprifoliaceae	<i>Lonicera japonica</i>			0.4 (M)	0.1 (M)				
Myrtaceae	<i>Eugenia uniflora</i>			2.1 (M)	0.7 (M)			0.4 (M)	
Iridaceae	<i>Iridaceae</i>							0.2 (M)	
Euphorbiaceae	<i>Manihot grahamii</i>								
Oxalidaceae	<i>Oxalis sp.</i>	5.0 (I)							
Rhamnaceae	<i>Discaria americana</i>	0.5 (M)							
Rhamnaceae	<i>Scutia buxifolia</i>	0.3 (M)							
Brassicaceae	-	0.2 (M)							
Salicaceae	<i>Xylosma tweedianum</i>	14.1 (I)							

Data represents percentage and frequency of pollen classes pollen in honey ($n = 7-10$) for each region (C, Q, VL, EF) by season (S-S, S-A). Honey in the EF region was collected only in one season. D = dominant pollen (>45%), S = secondary pollen (16-45%), I = important minor pollen (3-15%), M = minor pollen (<3%).



Table 2. Content of trace elements (mg/kg raw honey) in honeys' protected region (Quebrada de los Cuervos, Q; Estero de Farrapos, EF; Valle del Lunarejo, VL) and not protected region (Los Cerrillos, C) in two harvest seasons (S-S or S-A) in Uruguay

Region	Season	Trace Elements (mg/kg raw honey)						
		Cu	Fe	I	Mo	Mn	Zn	Se
C	S-S	1.45 ± 0.35 a	2.27 ± 0.92 a	1.11 ± 0.80 ab	0.09 ± 0.01 b	5.71 ± 0.30 a	0.42 ± 0.17 c	0.29 ± 0.04 bcd
	S-A	0.71 ± 0.29 bc	3.56 ± 1.64 a	0.06 ± 0.09 b	0.08 ± 0.01 b	3.24 ± 0.30 b	1.47 ± 0.31 ab	0.34 ± 0.04 abc
Q	S-S	0.25 ± 0.19 d	2.63 ± 0.61 a	0.08 ± 0.11 b	1.17 ± 1.11 a	1.87 ± 0.32 c	0.94 ± 0.51 bc	0.12 ± 0.04 d
	S-A	0.27 ± 0.20 cd	3.29 ± 1.56 a	1.81 ± 3.10 a	0.09 ± 0.01 b	3.46 ± 0.36 b	1.19 ± 0.71 b	0.52 ± 0.05 a
VL	S-S	0.20 ± 0.11 d	2.24 ± 1.04 a	0.09 ± 0.09 b	0.12 ± 0.01 b	2.38 ± 0.30 bc	2.12 ± 0.31 a	0.31 ± 0.05 bc
	S-A	0.19 ± 0.08 d	3.50 ± 1.39 a	0.11 ± 0.13 b	0.05 ± 0.02 b	1.38 ± 0.32 c	1.43 ± 0.25 ab	0.26 ± 0.04 cd
EF	S-S	0.81 ± 0.56 b	2.61 ± 0.75 a	1.59 ± 0.95 ab	0.06 ± 0.01 b	1.57 ± 0.30 c	1.02 ± 0.85 bc	0.45 ± 0.04 ab
Main effects		Cu	Fe	I	Mo	Mn	Zn	Se
Region		p < 0.0001	ns	p = 0.0086	p < 0.0001	p < 0.0001	p < 0.0001	p = 0.0099
		C,EF > Q,VL		EF > VL	Q > C,VL,EF	C > Q,VL > EF	VL > Q,EF,C	EF > C,Q,VL
Season		p = 0.0016	p = 0.0009	ns	p = 0.0033	p = 0.0031	Ns	p = 0.0011
		S-S > S-A	S-A > S-S		S-S > S-A	S-S > S-A		S-A > S-S

Data represents mean ± SEM (n = 7-10) for each region (C, Q, VL, EF) by season (S-S, S-A). Data were analyzed by ANOVA GLM, considering as main effects the region and the season and post hoc Tukey-Kramer's Test (p < 0.05). As in the EF region honey was collected only in one season, all honeys were also analyzed with a one-way ANOVA and post hoc Tukey-Kramer's Test (p < 0.05).

Different letters indicate significant differences (p < 0.05) between region and seasons. ns: not significant.

Figure 1. Dendrogram of classification of the types of honey studied according to the content of trace elements by region and season. Euclidean distance and ward aggregation method were used. C: Los Cerrillos; Q: Quebrada de los Cuervos; EF: Esteros de Farrapos; VL: Valle del Lunarejo; S-S: spring-summer; S-A: summer-autumn

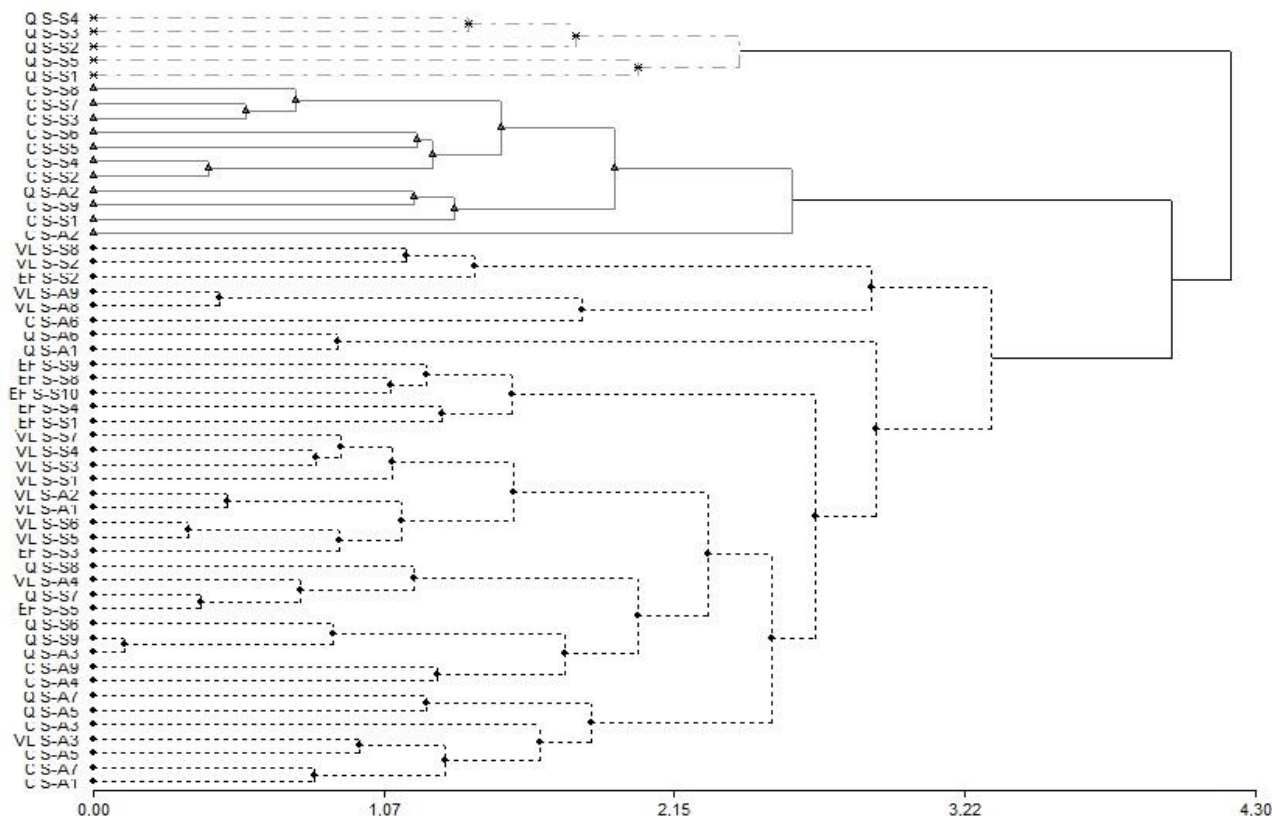
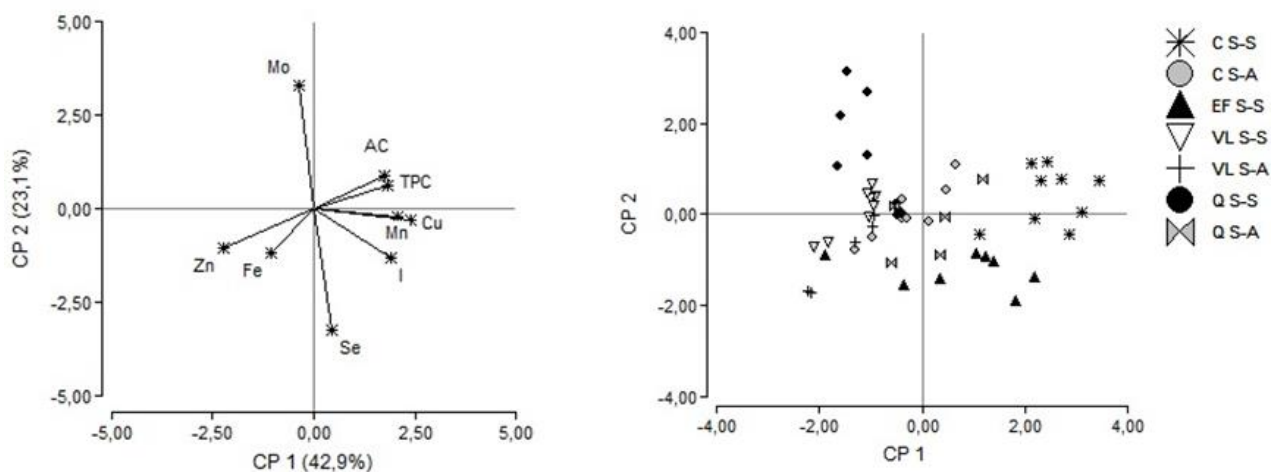




Figure 2. Analysis of main components (PCA) of the types of honey studied according to the content of trace elements, total polyphenols and antioxidant capacity by region and season. (Left: PCA distribution of variables; right: PCA distribution of honey samples). C: Los Cerrillos; Q: Quebrada de los Cuervos; EF: Esteros de Farrapos; VL: Valle del Lunarejo; S-S: spring-summer; S-A: summer-autumn



Main components 1 and 2 (CP1 and CP2) explain 66% of the variation found.

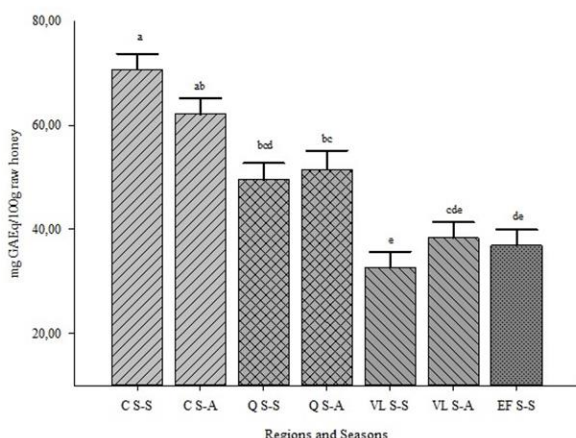
3.3 Total polyphenols

The results for total polyphenols show a variation between 35 to 66 mg GAEq/100g (Figure 3).

The region with the highest value was C (66.44 mg GAEq/100g), being significantly different from the values obtained for regions Q (50.417 mg GAEq/100g), EF (36.876 mg GAEq/100g), and VL (35.526 mg GAEq/100g).

When comparing honeys from the S-A season with respect to S-S season for the same region, no significant differences were found (Figure 3).

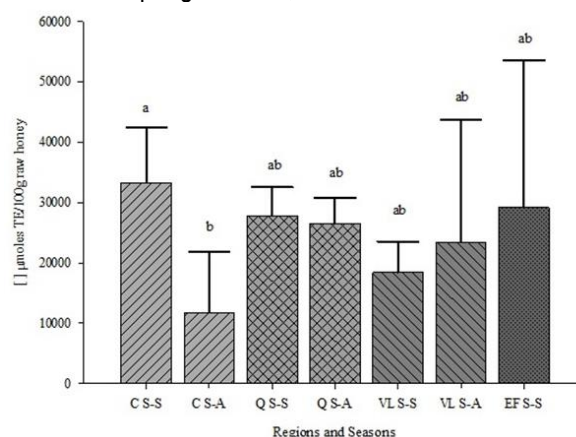
Figure 3. Total polyphenol content (TPC; mg GAEq/100g) by region and season. The values represent the mean \pm SEM (n=7-10). Different letters indicate significant differences between regions and seasons ($p < 0.05$). C: Los Cerrillos; Q: Quebrada de los Cuervos; EF: Esteros de Farrapos; VL: Valle del Lunarejo; S-S: spring-summer; S-A: summer-autumn



3.4 Antioxidant capacity

The results can be seen in Figure 4. The honeys from the C region in season S-S registered greater antioxidant capacity (33274 μ moles TE/100g) compared to the same region in season S-A (11802 μ moles TE/100g). No significant differences were observed for the other regions and between seasons (Figure 4).

Figure 4. Antioxidant capacity (μ moles TE/100g honey) by region and season. The values represent the mean \pm SEM (n=7-10). Different letters indicate significant differences between regions and seasons ($p < 0.05$). C: Los Cerrillos; Q: Quebrada de los Cuervos; EF: Esteros de Farrapos; VL: Valle del Lunarejo; S-S: spring-summer; S-A: summer-autumn



4. Discussion

4.1 Palynological analysis

Both seasons were characterized by multifloral honeys considering the classification criteria described above. Honeys with pollen contents of native species, such as *Baccharis articulata* (27.4%-31.6%), *Baccharis spp.* (25.9%), *Tripodanthus acutifolius* (25.8%-27.4%), were found.

In both seasons, the botanical origin was similar, except for the presence of *Senecio* sp. in season S-A. Likewise, in the S-S season, a higher percentage of *Schinus longifolius* (12.4%) was observed compared to the S-A season (1.1%). Honeys from the VL region were characterized as multifloral honeys in both seasons.

Palynological analysis of honey shows that there are regions with similarities in species plants in both seasons (Q region), while for the southeast region (C), there is a difference in honey botanical origin mainly due to the presence of improved crops (legumes particularly) and meadows with the presence of introduced species where the flowering period is shorter (Fabaceae family particularly).

The botanical origin of the honeys turned out to be different according to the geographical site and the season. Some species, genres or botanical families were only found in a particular region. Indeed, *Glycine max*, *Scutia buxifolia*, *Ammi viznaga*, *Trifolium repens*, *Xylosoma twedianum*, *Oxalis* sp., *Discaria americana* and *Raphanus sativus* were found in honeys from C region. In honeys of the Q region, the presence of *Lonicera Japónica* stands out. Honeys from the VL region presented *Manihot grahammi* and *Iridaceae* species in their pollen content. The presence of *Parkinsonia aculeata* and *Rosaceae* stands out in honeys from the EF region. Regions Q and VL shared pollen species such as *Tripodanthus acutifolius*, *Lithraea brasiliensis* and *Eugenia uniflora*. Finally, through the palynological analysis, differences in the pollen content were observed in the four regions studied as well as for each season.

4.2 Content of trace elements in honey

Honeys from region C had higher contents of Cu and Mn. This could be explained because the C region is close to areas associated with horticultural and agricultural activity, with applications of cupric inorganic products for the control of plant diseases and fertilizer applications with Mn. However, the values found per season had no significant differences.

Cu values in honeys from the four regions are within those reported by other authors, with ranges between 0.228 and 3.317 mg/kg⁽¹⁹⁻²⁰⁾. For multifloral honeys in Argentina, average values of 0.29 mg/kg with a range of 0.09 to 1.19 mg/kg have been found⁽²¹⁾.

The Mn content is within the ranges cited in the literature (0.14 and 3.13 mg/kg)⁽²¹⁾. The Zn content in the VL region honeys was higher with respect to Q, EF and C regions, coinciding with the lowest Cu values found in this region. High concentrations of Zn in the soil solution depress the absorption of Cu by the roots and may exacerbate Cu deficiency⁽²²⁾.

The Mo content was higher in the honeys of the Q region, being significantly different from the honeys of the other regions (C, VL and EF). This could be explained by the types of soil associated with the geological formation of the region, where calcareous soils predominate. The availability of Mo for the plant, unlike other micronutrients, increases with the decrease in soil acidity⁽²²⁾. The differences found between the honeys of the EF and VL regions for iodine could be due to different factors and several approaches can be used for the analysis of trace element data⁽²³⁻²⁴⁾.

The variation in the content of Se may be due to the flower source (botanical origin) and the region (geographical origin) where they are obtained, especially due to the type of soil.

Hierarchical cluster analysis was applied to the data set (66 samples and 7 trace elements measured) to classify the distribution of honey samples according to their production areas⁽²¹⁾. Based on the different trace elements measured, associated with each region and season, regions with similar characteristics were grouping. There are three very different large groups or cluster. One cluster is formed by the C region for the S-S season (cluster A), and another cluster is formed by the Q region for the S-S (cluster B). A third group is a cluster integrated by the regions VL, in both seasons, the region EF for the season S-S, and the regions Q and C for the season S-A. The explanation for this grouping based in the trace elements was given by geology, and by the biodiversity and the natural ecosystem⁽²⁵⁾.

According to PCA (Figure 2), it is possible to associate a particular region according to a trace element. The EF region is associated with the I content, while Zn is associated with the VL region. The Q region is characterized by higher Mo values and despite there being no significant differences between regions for Fe, it can be seen that in this



region the values obtained from Fe are associated with this same region.

Through this study we can emphasize that the content of trace elements of the honeys of Uruguay vary according to each geographical site and each season of the year, allowing the classification of the honeys by their geographical origin. From an applied point of view, the southern region C would produce honey with a higher content of Cu, Mn, and I in the season S-S. For protected areas, the VL region in the S-S season would produce honey with a higher Zn content, and the Q region in the S-S season would produce honey with a higher Mo content.

4.3 Total polyphenols

The presence of legumes from the Fabaceas family, such as Lotus in C region, may be the reason for the high level of total polyphenols found in honey.

In other studies compounds with phenolic bioactivity were found in *Lotus corniculatus*, of the quercetin and kaempferol type with antioxidant activity in humans, which indicates that more studies should be directed to quantify and identify these compounds in honey because of their importance in health⁽²⁶⁾.

They are probably not the same compounds with phenolic activity in all the regions in which the PTCs were measured, since other phenolic compounds could be present in the flora that predominates in those regions, and that still being of the flavonoid family have another chemical structure⁽²⁷⁻²⁸⁾.

4.4 Antioxidant capacity

The Antioxidant Capacity could be positively associated with the greater presence of trace elements such as Cu, Mn, I, and a higher Total Polyphenol content.

Taken together, Total Polyphenols Content and Antioxidant Capacity for honey from the southern region (C) in the S-S season were higher related to the VL and EF honeys, which could be positively associated with the greater presence of trace elements such as Cu, Mn, I and also with a higher content of legumes from the Fabaceae family⁽²⁹⁾.

5. Conclusions

This work allows us to affirm that honey can be differentiated by its micronutrient content associated with the total polyphenol content and the antioxidant activity for different regions, given by the

floral species present in the region and the type of soil. Through palynological analysis, differences in pollen content could be observed in the four regions studied, as well as for each season. The botanical origin of the honeys turned out to be different according to the geographical location and the season of the year. Some species, genus or botanical families were only found in a particular region. Region C would produce honeys with a higher content of Cu, Mn, I in the S-S season. The VL region in the S-S season would produce honeys with a higher Zn content, and the Q region in the S-S season would produce honeys with a higher Mo content. The honeys from the C region in the S-S season presented higher values of Total Polyphenols and Antioxidant Capacity, compared to the honeys from VL and EF, probably due to a higher content of legumes and/or trace elements such as Cu, Mn, and I.

Transparency of data

Available data: The entire data set that supports the results of this study was published in the article itself.

Author contribution statement

Moreni, A. conceived the collected samples, contributed to data or analysis tools, performed the analysis, and wrote the article.

Cabrera, MC. conceived and designed the analysis, performed the mineral analysis and polyphenols analysis.

Cracco, P. conceived and designed the collected samples, performed the mineral analysis.

Cadenazzi, M. contributed to analysis tools.

Pirotti, F. performed the capacity antioxidant analysis.

Santos, E. performed the palynological analysis.

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