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QUALITY OF HONEY FROM ARGENTINA: STUDY OF CHEMICAL COMPOSITION AND TRACE ELEMENTS.

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Abstract

Content of P, Fe, Al, Mn, Zn, Cu, Ca. Mg, Na and K trace elements, as well as moisture, pH, free acidity, lactone, reducing sugar, sucrose, diastase activity, ashes and hidroximethylfurfural, were analyzed in 38 samples of natural honey from different places of the centre of Argentina. The mean values for element content were (in μg g^{-1}): P: 28.80; Fe: 3.91; Al: 2.57; Mn: 0.33; Zn: 1.08; Cu: 0.19; Ca: 56.35; Mg: 23.38; Na: 25.56; K: 482.75. Pr was used as an internal standard in order to evaluate the recovery percentage of the trace elements.

Chemical content shows the following media results: moisture 16.24 %; pH 3.85; free acidity 30.15 mg g^{-1}; total acidity 31.65 mg g^{-1}; reducing sugar 68.08 %; sucrose 4.05 %; diastase number 19.73, ashes 0.11 % and hidroximethylfurfural: 8.98 mg kg^{-1}.
Keywords: Honey. Quality. Composition. Trace elements.

Resumen

El contenido de elementos vestigio tales como P, Fe, Al, Mn, Zn, Cu, Ca, Mg, Na y K, así como la humedad, el pH, acidez libre, lactona, azúcares reductores, sacarosa, actividad de diastasa, cenizas y hidroximetilfurfural, fueron analizados en 38 muestras de miel natural de diferentes lugares del centro de Argentina. Los valores medios para el contenido elemental fueron (en µg g^{-1}): P: 28.80; Fe: 3.91; Al: 2.57; Mn: 0.33; Zn: 1.08; Cu: 0.19; Ca: 56.35; Mg: 23.38; Na: 25.56; K: 482.75. Pr fue utilizado como estándar interno con el objetivo de evaluar el porcentaje de recuperación de los elementos trazas.

El contenido químico las muestras analizadas fueron los siguientes: humedad 16.24%; el pH 3.85; la acidez libre 30.15 mg g^{-1}; la acidez total 31.65 mg g^{-1}; azucares reductores 68.08 %; sacarosa 4.05 %; el número del diastasa 19.73, cenizas 0.11 % y hidroximetilfurfural 8.98 mg kg^{-1}.
Palabras claves: Miel. Calidad. Composición. Elementos vestigios.

Introduction

Honey is a natural food produced by bees from nectar or secretion of flowers. Honey has a content of 80-85 % carbohydrates, 15-17 % water, 0.3 % proteins, 0.2 % ashes, and minor quantities of amino-acids and vitamins as well as other components in low levels of concentration.

Argentina is an important honey-producer, which in 2007 exported about 80,341 ton [1]. Argentine honey has an international market due to its multifloral origin and it is recognized around the world because of its quality [2]. La Pampa province is located in the geographical centre of Argentina; it has an area of 143,440 km^2 which represents 5.1 % of the total area of Argentina. La Pampa presents three important regions based on rainfall: humid, semiarid and arid. The area of arable land involves 11,861 km^2 and corresponds mainly to the humid region. Apicultural practice is suitable in La Pampa due to the intensive agricultural activity and to the presence of an important number of blossoms from wheat, sunflower, oat, corn, sorghum, rye and alfalfa. Furthermore, 50 % of the total area of the province is covered by brush and underbrush vegetation, which produces a particular type of honey called brush honey. La Pampa produces about 8-9 ton/year of honey (doubling the value obtained ten years ago) which represents about 10 % of the total production of honey in Argentina [3]. For these reasons, to know characteristics and properties of honey, to maintain or improve its quality and to increase the share of honey from La Pampa for both, local and international markets constitutes a key fact.

There are an important number of works studying trace elements and chemical properties in honey around the world [4-9]. Microbiological and chemical properties of honey in Argentina have been studied previously [2], but only for honey produced in the south of the province of Cordoba, which represents only a little area of central Argentina. Furthermore, trace elements have not been studied until now in honey from central Argentina and for these reasons, it is important to determine these elements and their chemical composition as a preliminary study for quality control and to determine mineral contents.

This paper discusses the study of 10 trace elements (P, Al, Fe, Mn, Zn, Cu, Ca, Mg, Na and K), and 9 chemical parameters (moisture, pH, free acidity, lactone, inverse sugar, sucrose,

ashes, diastase activity and hidroximethylfurfural) in natural and fresh honey samples from beehives in the province of La Pampa. These chemical parameters are included in the Argentine Food Code [10] and are used as quality control standards to accept o reject honey for commercialization.

Experimental

Honey Samples

Thirty eight samples of natural honey were obtained from beehives of honey producers in the province of La Pampa. The botanical origin of these honey samples was unknown because honey produced in La Pampa is commercialized as multifloral honey. All samples were saved in glass flasks and cool places and analyzed within two months from sampling.

Trace elements analysis

Five grams of honey were accurately weighed in a 120 mL glass beaker. Then 25 mL of nitric, 10 mL of perchloric acid and adequate volume of Pr internal standard solution [11] were added and heated in a sand bath, to mineralize organic material. The solutions were then carried to almost dry. The acid clear solutions were transferred to 50 mL volumetric flasks and diluted with deionized water. Three replicates were analyzed per sample. Elements P, Al, Fe, Mn, Zn, Cu, Ca, Mg, Na and K were analyzed by emission measurements obtained by direct nebulization in an inductively coupled plasma optical emission spectrometer (ICP-OES), Baird model 2070 ICP (USA), with 100 cm optical length Czerny Turner monochromator. In the calibration step, standard solutions for all elements were prepared from a spectroscopic grade reagent (Merck).

Chemical analysis

All chemical analyses were carried out following official methods of analysis [12]. In all cases, three replicates were analyzed per sample.

Free acidity and lactone

Ten grams of honey were weighed in a glass beaker and then 75 mL of deionized water were added. This solution was titrated with NaOH 0.05 mol L^{-1} until reaching pH 8.5, and measured with a Horiba F42 (Japan) pH-meter. Then 10 mL of NaOH 0.05 mol L^{-1} were added and titrated again with 0.05 mol L^{-1} HCl until reaching pH 8.30. The results were expressed as meq kg^{-1} of acids.

Ash content

Ashes were obtained by ashing in a muffle furnace at 550 ºC for 5-6 hours to obtain constant weight.

Moisture content

Moisture was measured at 25 ºC using a Carl Zeiss (Germany) Abbe refractometer and using a Wedmore table [12].

Hidroximethylfurfural (HMF) content

HMF was analyzed following Winkler's method. Five grams of every sample were treated with a clarifying agent (Carrez solution), transferred to and completed with deionized water in 50 mL volumetric flasks. The absorbance of the filtered solution was measured at 284 and 336 nm using a blank produced with an aliquot of the solution treated with $NaHSO_3$ 0.1 %.

Reducing sugars and sucrose

Reduction of sugar was carried out using the Layne-Enyon method. 2.6 g of honey were weighed and then transferred to a 500 mL volumetric flask. Five milliliters of standardized Fehling A and B solutions were transferred to a 250 ml Erlenmeyer, with 7 mL of water and 15 mL of honey solution. The Erlenmeyer was heated and 1 mL methylene blue 0.2 % was added. Titration was carried out adding the diluted honey solution until the indicator was decolorized. Determining sucrose content was carried out by inversion, adding 10 mL of diluted HCl, 50 mL diluted honey solution and water to a 100 mL volumetric flask, heating in water bath, then cooling and diluting to mark. Finally the Lane-Enyon method was applied and sucrose content was obtained by difference.

Diastase activity

Five grams of honey were dissolved in 15 mL water; then 2.5 mL pH 5.3 acetate buffer solution were added and transferred to a 25 mL volumetric flask. Ten milliliters of this solution were mixed with 5 ml 2 % starch solution in a tube and incubated at 40 ºC during 15 minutes and then, it started a stopwatch. After 5 minutes, 1 mL of this solution was taken and an iodine solution 7. 10^{-4} meq L^{-1} was added. Absorbance was read in an Ocean Optic model Chemusb4-UVVis spectrophotometer (USA) at 660 nm until readings obtained were less than 0.235 absorbance units. Then diastase number (DN) was obtained by plotting absorbance against reaction time.

Results and discussion

Trace elements composition

Table 1 shows the mean, standard deviation and range of trace elements analyzed by ICP-OES. The r^2 regression coefficients obtained for all elements in the calibration step ranged from 0.989 to 0.996. A recovery degree of 99 % was obtained, using Pr as internal standard, similar to that reported in a previous work [11]. The most abundant elements were K, Na, P, Ca and Mg. P concentration has been only reported in Spanish thyme honey, with a higher mean value than the one found in this paper (51 µg g^{-1}) [13]. In comparison with Italy K, Na, Ca and Mg elements were 2 or three times lower to those found in Siena (K= 1195, Na= 96.6, Ca= 257 and Mg= 56.7 µg g^{-1}. [9], but similar to what was reported for the Latium region (K= 472, Na= 96, Ca= 47.7 and Mg= 37 µg g^{-1}) [14]. In comparison with honey from Anatolia (Turkey), La Pampa has honey with more K (K= 296 µg g^{-1}) and less content of Ca and Mg (Ca= 51 and Mg= 136 µg g^{-1}) but the reported concentration of Na was around 4 times higher (Na= 117 µg g^{-1}) than that in La Pampa [4,8]. In comparison with studies reported in Spain, La Pampa has lower concentration of K, Ca, Mg and Na (mean values of K= 1283.9, Na= 99.7, Ca= 92.2 and Mg= 55.3 µg g^{-1}) [7,13,15-17]. Fe, Mn, Zn and Cu elements for La Pampa honey, present values which are lower than those reported. Al has not been reported in regional honey analysis. In comparison with Italy, honey from Siena and Latium shows similar concentrations of Fe and Zn to those reported in this paper (3.8 and 2.5 µg g^{-1} for Siena; 4.5 and 3.1 for Latium respectively) but reported Mn and Cu are higher (1.54 and 0.91 µg g^{-1} for Siena and 3.0 and 0.31 µg g^{-1} for Latium respectively) [9,14]. Honey from La Pampa has about 2 times lower concentration of trace elements in comparison with Turkey (Fe= 6.6, Mn= 1.0, Zn= 2.7 µg g^{-1}) [4] and Egypt (Fe= 113.3, Mn= 1.7, Zn= 7.2 µg g^{-1}) [5]. However, there exists a more important difference with Cu= 1.9 µg g^{-1} [8]. With respect to Spanish honeys, there exist differences with Zn (mean 2.12 µg g^{-1}), Mn (mean 3.10 µg g^{-1}), Fe (mean 4.56 µg g^{-1}) [7,15-17], Cu (mean 0.60 µg g^{-1}) and Al (mean 21.48 µg g^{-1}) [16]. The Table 2 shows the composition of trace elements in honey from Argentina and other countries.

Table 1. Concentration of trace elements in fresh natural honey.

	Mean[a,b]	Standard Deviation[a,b]	Range[a]
P	28.80	20.64	1.17 – 100.66
Fe	3.91	2.52	1.13 – 10.32
Al	2.57	2.49	0.02 – 13.04
Mn	0.33	0.15	0.07 – 0.68
Zn	1.08	0.84	0.14 – 3.87
Cu	0.19	0.12	0.05 – 0.68
Ca	56.35	23.75	18.60 – 136.14
Mg	23.38	9.21	6.01 – 46.57
Na	25.56	15.29	6.10 – 89.98
K	482.75	466.67	90.92 – 1955.75

[a]Concentration expressed in μg g^{-1}.

[b] Mean and standard deviation of three determinations per sample.

Table 2. Comparison of the composition of trace elements between Argentina and countries producing of natural honey.

	Argentina[a]	Spanish[a]	Italy[a] (Siena)	Italy[a] (Latium)	Turkey[a]	Egypt[a]
P	28.80	51	---	---	---	---
Fe	3.91	4.56	3.8	4.5	6.6	113.2
Al	2.57	21.48	---	---	---	---
Mn	0.33	3.10	1.54	3.0	1.0	1.7
Zn	1.08	2.12	2.5	3.1	2.7	7.2
Cu	0.19	0.60	0.91	0.31	1.9	1.7
Ca	56.35	92.2	257	47.7	51	192
Mg	23.38	55.3	56.7	37	136	102
Na	25.56	99.7	96.6	96	117	378
K	482.75	1283.9	1195	472	296	1500

[a]Concentration expressed in μg g^{-1}.

Chemical composition

Table 3 shows results of the chemical analysis in natural honey samples, with mean, standard deviation and range. These results indicate that La Pampa honeys fall within the national regulations in most cases. Moisture shows a mean value of 16.24 %; this variable depends on climatic factors, season of production and maturity of honey. Only one sample has more than 18 % of moisture, maximum allowed by local regulations to avoid fermentation. pH shows a mean value of 3.85 with a range between 3.34 and 4.70, which is next to what is normally accepted [8]. Free acidity shows a mean value of 30.15 mg kg^{-1}; this value represents the organic acids content in honey. Two samples are over 40 mg kg^{-1}, maximum value allowed by national regulations, but mean value is similar to the one reported in Spain (27.2 meq kg^{-1}) [13]. However, lactone presents a very low mean value of 1.56 mg kg^{-1}, lower than in previous works, with 16.61 mg kg^{-1} [8] and 7.22 mg kg^{-1} [15]. Reducing sugar has a mean of 68.8 %, while sucrose has a value of 4.05 %; these results are slightly different to those obtained in Turkey (71.32 %) [8]. The obtained value for the diastase number was 19.73, showing a mean value higher than that reported in Turkey (16.27) [8] but accepted by national regulations. Moreover, an inverse relation between diastase number and P content (Figure 1) was found, which indicates that high P content produces a reduction of freshness in honey. Ashes mean value was 0.11 % and it represents the mineral content of honey. This value is higher in comparison with other works in the region [2], but lower in comparison with Turkey (0.25 %) [8] and Spain (0.32 %) [13]. HMF represents the freshness of honey and depends on adequate beehives and harvest practice. The mean value was 8.98 mg kg^{-1}, higher than the one obtained in Turkey (4.52 mg kg^{-1}) [8] but lower than that obtained in other places of Argentina (14.8 mg kg^{-1}) [2]. Table 4 summarizes the results of chemical composition in honey from Argentina and other countries. Figure 2 shows a direct relation found between free acidity and HMF, due to the formation of levulinic and metanoic acids from HMF. The maximum value obtained of HMF was 34.08 mg kg^{-1}, which agrees with national regulations (40 mg kg^{-1}).

Table 3. Chemical analysis in natural honey samples.

	Mean[a]	**Standard Deviation[a]**	**Range**
Moisture (%)	16.24	0.19	14.28 – 18.60
pH	3.85	0.34	3.34 – 4.70
Free acidity (meq kg^{-1})	30.15	5.86	21.23 – 43.20
Lactone (meq kg^{-1})	1.56	1.87	0.10 – 6.46
Reducing sugar (%)	68.08	2.27	63.20 – 73.24
Sucrose (%)	4.05	1.37	0.41 – 6.22
Diastase number	19.73	7.27	41.04 – 10.07
Ash (%)	0.11	0.04	0.06 – 0.21
HMF (meq kg^{-1})	8.98	9.20	1.48 – 34.08

[a]Mean and standard deviation of three determinations per sample.

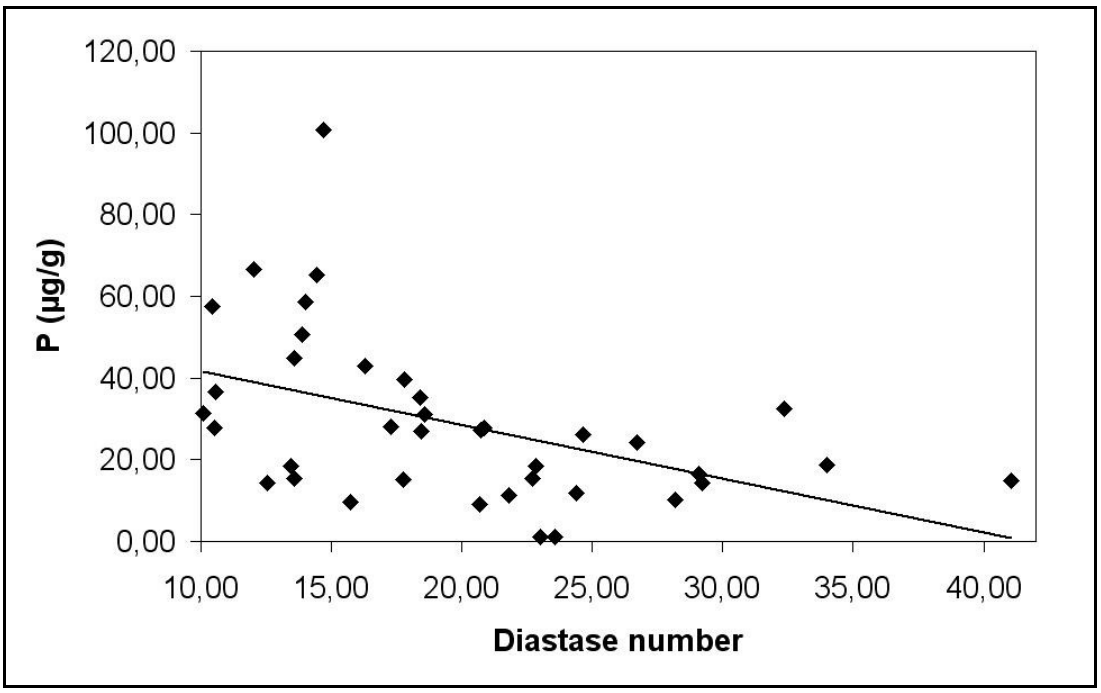

Figure 1. Plot of Diastase number as function of P content.

Table 4 . Comparison of Chemical analysis in natural honey between countries producing natural honey.

	Argentina	**Spanish**	**Italy**	**Turkey**
Moisture (%)	16.24	16.30	16.36	---
pH	3.85	4.2	4.32	---
Free acidity (meq kg^{-1})	30.15	27.2	---	---
Lactone (meq kg^{-1})	1.56	7.22	---	16.61
Reducing sugar (%)	68.8	---	---	71.32
Sucrose (%)	4.05	---	---	3.03
Diastase number	19.73	---	---	16.27
Ash (%)	0.11	0.32	---	0.25
HMF (meq kg^{-1})	8.98	---	---	4.52

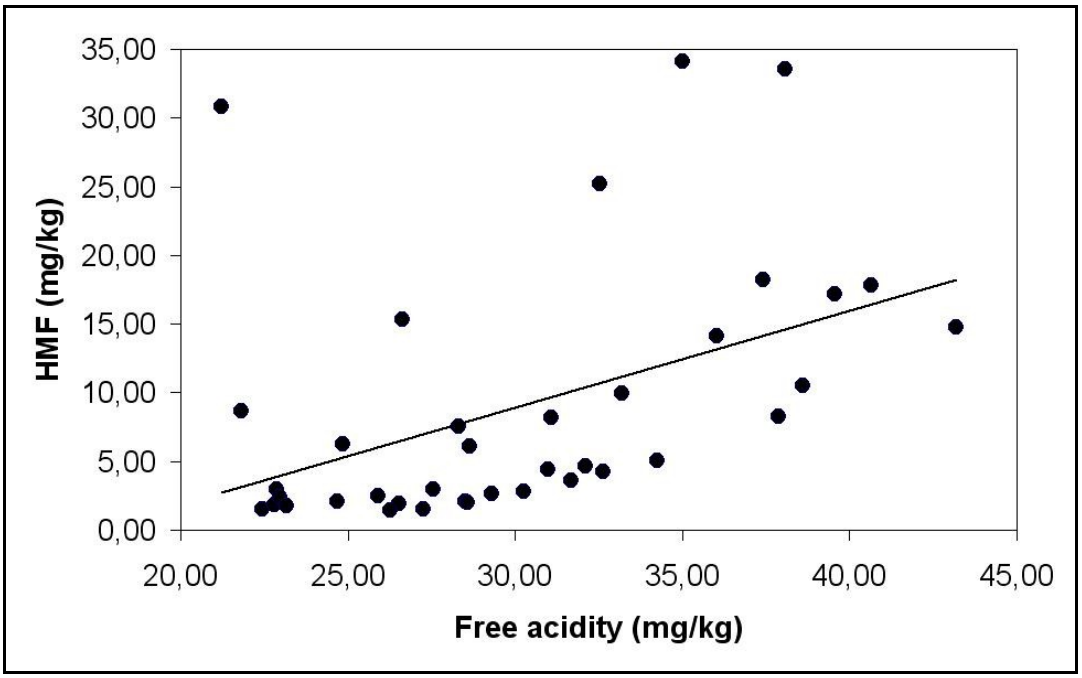

Figure 2. Plot of free acidity as function of HMF.

Conclusions

The results obtained indicate that trace elements in honey from central Argentina, have a low content of analytes in comparison with similar studies carried out in other regions. This work shows the following abundance order: K, Ca, P, Na, Mg, Fe, Al, Zn, Mn and Cu.

Chemical properties of honey from the center of Argentina indicate good quality. Low diastase activity, HMF and acidity values were found, which indicate honey freshness and good conservation. The low moisture content helps to protect honey from microbiological activity and thus it can be preserved for longer periods of time. Honey presents high reducing sugar content (68 %) and very low values of sucrose (4 %); the remaining quality parameters agree in general, with national regulations.

To summarize, this paper shows novel results of honey composition from central Argentina, (La Pampa province) since trace elements have not been studied until now in honey from this region. These results make the honey from central Argentina, a product that offers good quality, showing good beekeeping practices, which must be standardized to maintain or to improve quality in the future. These results are also very important for commercialization of Argentine honey both in the national and international markets.

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