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THE ANTIOXIDANT ACTIVITY OF CHILEAN HONEY AND BEE POLLEN PRODUCED IN THE LLAIMA VOLCANO'S ZONES

ENRIQUE MEJÍAS[1] and GLORIA MONTENEGRO

Department of Vegetable Sciences, Faculty of Agricultural and Forestry Sciences, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Macul, Santiago 6904411, Chile

[1]Corresponding author. TEL: 56-2-3547216; FAX: 56-2-5520780; EMAIL: egmejias@uc.cl

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ABSTRACT

Chile produces a variety of bee products with different botanical origins and several biological characteristics inherited from specific floral sources. Plants and beehives in areas polluted with metals may increase the levels of these elements in beehive products. Thus, it is expected that products that are produced in such places have higher metal content. Furthermore, all of these honey and bee pollen samples have modified biological properties, but their antioxidant activities are determined by their botanical origin and metal content. The honey and bee pollen samples were produced near the Llaima Volcano (South of Chile) and studied to determine metal contents as well as their antioxidant activities. In honey, the highest values for Cu, Mn and Fe were 0.851, 1.043 and 1.82 μg/g, respectively. In bee pollen samples, the higher metal content was for Fe, with a 194.0 μg/g maximum value. Furthermore, in those samples, Pb (0.17–0.28 μg/g), Cd (0.03–0.05 μg/g) and Cr (0.37–0.47 μg/g) were detected. The total phenolic content ranged from 1,000.0 to 1,255.4 mg/kg in honey and from 347.7 to 1,286.5 mg/kg in bee pollen. The selected beehive products herein had higher metal content as well as lower phenolic content and antioxidant activity.

PRACTICAL APPLICATIONS

By determining the metal contents in bee products produced in a potentially polluted zone, it is possible to have a good monitoring of the real impact of natural pollution such as volcano's eruptions. In the same way, these preliminary results allow one to open an alternative option to estimate the impact on the environment of the contaminant activities of humans by using those natural bioindicators, which production occurs in zone exposed to metals. Also, the modification of biological properties of these products can be useful for determining alterations in food compositions of natural products with undesirable presence of toxic elements.

INTRODUCTION

Honey is a natural product made by bees (*Apis mellifera*) from the nectar of melliferous plants, which is processed and modified. Afterward, it is stored in beehives for further use, such as for food (Codex Stan 1981). Honey inherits all of its properties from plants; therefore, its biological properties are related to the plant species that produced the nectar (Montenegro *et al.* 2003, 2004).

Similarly, bee pollen is a conglomerate of pollen grains that bees bring to the beehive from flowers in a cavity in their third pair of legs. Bee pollen has been described as a product with high levels of nutritional compounds, several molecules of which have interesting biological properties, such as antioxidant activity. The botanical origin is responsible for the nature and properties of the bioactive compounds found in bee pollen (Dreller and Tarpy 2000; Thorp 2000; Montenegro *et al.* 2008). Several bee pollen samples

have been analyzed to determine the physicochemical characteristics, such as pH, ash and moisture content. pH ranges from 3.4 to 5.1 have been reported for honey and other substances made by bees. Such samples had a similar level of inorganic content, regardless of their botanical origin. This level was approximately 3% of the net weight for the bee pollen studied. It is important to note that the primary minerals detected in bee pollen samples were iron, magnesium, copper, zinc, calcium, sodium and potassium (Roulston and Cane 2000; Pernal and Currie 2002; Gergen *et al.* 2006; Vit and Santiago 2008). Likewise, the phenolic compound content, antioxidant activity and antibacterial properties have been described for bee pollen to establish differences among the analyzed samples. From these results, the bee pollen from natural parks in Portugal was characterized and the analyzed samples were shown to be a suitable source of healthy compounds (Morais *et al.* 2011).

On the other hand, if either the melliferous species are exposed to chemical pollutants or beehives are near the areas with metal pollution, then the final composition of the honey and bee pollen produced in such areas could contain these elements. Several studies have attempted to establish a correlation between the geographic origin of honey and its inorganic content. The primary aim of these studies was to assess the use of honey as an environmental marker (Jones 1987; Przybylowski and Wilczynska 2001).

An analysis of the mineral content in 81 Spanish honey samples showed concentrations of alkali and alkaline earth metals at specific levels. Based on these results, certification of the geographic origin of honey by determining the inorganic pattern in honey samples was recommended (Hernández *et al.* 2005). Avocado honey samples have been characterized using either a similar procedure (Terrab *et al.* 2005) or parameters such as the total mineral composition and physicochemical properties (Gonzalez Paramas *et al.* 2000).

Chile produces a variety of bee products with different botanical origins and several biological characteristics that are inherited from specific floral sources (Montenegro *et al.* 2008). All of these properties are related to phenolic content, which is obtained from the metabolic pathways of melliferous plants (Gheldof *et al.* 2002). Phenolic compound profiles for bee products change based on the presence of metals, and therefore, the metal-containing product's properties could differ compared with lower metal content products (Küçük *et al.* 2007). Nevertheless, a relationship between the botanical origin and metal content has been difficult to establish in other honey studies (Fredes and Montenegro 2006; Silici *et al.* 2008). As honey inherits the properties of the plants where it originates, it is possible that honey produced in metal-polluted areas has such elements and yet has different antioxidant properties than honey with the same botanical origin but no metal content.

In this study, unifloral honey and bee pollen samples produced in beehives near the Llaima Volcano Zone in south Chile, Cherquenco, IX Region of Chile, were analyzed for lead, cadmium, chrome, copper, iron, manganese and zinc content. Furthermore, the antioxidant and antiradical activities were determined for each honey and bee pollen sample. This exploratory work is aimed to detect the effect of the Llaima Volcano's activity on the metal content in bee products that are produced in the surrounding area. In addition, the honey and bee pollen antioxidant activity was measured to elucidate any relationship between bee product's metal content and antioxidant properties.

MATERIALS AND METHODS

Honey and Bee Pollen Samples

Five unifloral honey and five total bee pollen samples were collected during the spring of 2010 (September–December) from five different beehives in Cherquenco (38°40′48″S–71°58′46″W) near the Llaima Volcano (38°41′30″S–71°44′00″W). A honey sample with a similar botanical origin was used as a control in the honey analyses. A bee pollen sample with similar botanical species content was used as a control in the bee pollen analyses. Both controls were produced in the same region of Chile, but outside the Llaima Volcano zone, where the selected samples for this work were obtained (Vilcún 38°40′23″S–72°13′74″W).

The Botanical Origin of Honey Using Melissopalynological Analysis

For the quantitative analyses, the method described by Loveaux *et al.* (1978) was followed, and all of the botanical elements were counted. For the qualitative analyses, acetolyzed slides were prepared with either 20 g of honey.

For each honey, an aliquot was diluted with 20 mL of warm distilled water (40C). The solution was placed in an appropriate tube and centrifuged at 3,500 rpm for 10 min. The supernatant was discarded and the pollen residue was deposited at the bottom of the tube and was resuspended in 100 μL of distilled water. An aliquot of this suspension (20 μL) was added to a slide, 10 μL of Calberla solution was added (a solution of either basic fuchsine or diamond) and the slide was gently dried. Finally, 15 μL of melted glycerinated gelatin was added to the mixture.

The isolation of bee pollen grains was made by color. Each bee pollen grain was separated from the total bee pollen sample according to its natural color and kept in a tube. After that, a similar procedure was followed for pollen samples. In this case, 5 g of bee pollen samples was used for the analysis. Bee pollen grains were directly placed onto the slides. The following steps were the same until the microscopic identification.

For each sample (honey and bee pollen), the pollen grains were identified using an optical microscope with total magnifications of 400× and 1,000×. The control samples were analyzed using the same criteria. For the reference pollen, the pollen collection at Laboratory of Botany of Pontificia Universidad Católica de Chile was used.

Determination of the Metal Content in Honey and Pollen: Sample Digestion

The samples were digested using a validated honey-specific method, as described previously (INN 2008). Honey and bee pollen samples (10 g) were dehydrated at 120C for 5 h. After this step, the bee products were placed on a hot plate (45C) for wet digestion using a mixture of inorganic acids (HNO_3/HCl 1:1). This procedure ended when the organic matrix of the bee products was destroyed. The excess acid was removed by evaporation until the sample was dry. Finally, 10 mL of 2% HNO_3 was added to each sample to generate aqueous solutions of the inorganic elements (Pb, Cd, Cr, Cu, Fe, Mn and Zn). All of the solutions that were obtained were analyzed using the inductively coupled plasma–optical emission spectrometer (Mejías *et al.* 2008).

Preparation of Honey Solutions for Colorimetric Assays

Fifty grams of honey was diluted with 150 mL of distilled water to obtain a homogeneous solution. All of the samples were stored at room temperature until the colorimetric analyses were performed. The pH range for all of the solutions was 5.3–5.5.

Preparation of Bee Pollen Solutions for Colorimetric Assays

One gram of bee pollen was suspended in 2.0 mL of distilled water at pH 2 and then vortexed. The pH range for all of the suspensions was 4.8–5.5.

Colorimetric Assays

A Shimadzu (Sao Paulo, Brazil) UV-1700 UV-visible spectrophotometer was used for absorbance measurements.

Total Phenolic Compound Content. The procedure described by Singleton and Rossi (1965) and Buratti *et al.* (2007) was used with minor modifications. Two-hundred microliters of either a honey solution or bee pollen suspension was mixed with 50 μL of Folin–Ciocalteu reagent (Merck, Darmstadt, Germany) and then 150 μL of 20% Na_2CO_3 (Merck) was added. Finally, distilled water was added to 1.00 mL. The absorbance at 765 nm was determined after 30 min. Gallic acid (Sigma-Aldrich, St. Louis, MO) was used as a standard to derive the calibration curve (0–150 μg/mL). The results define the phenolic content expressed as the mg equivalent of gallic acid/kg of sample.

Determination of Antiradical Activity. The procedure described by Meda *et al.* (2005) was followed to determine the antiradical activity.

The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay allowed us to establish the antiradical properties of the chemical compounds in honey and bee pollen by inhibiting or decreasing the oxidant activity of the DPPH. Seven hundred fifty microliters of either honey solution or bee pollen suspension was mixed with 1.5 mL of the DPPH (Merck) radical in methanol (0.02 mg DPPH/mL MeOH). The absorbance was determined after 15 min at 517 nm. A blank sample was prepared with methanol. Ascorbic acid (Calbio-Chem, Darmstadt, Germany) was used as a standard to derive the calibration curve (1–10 μg/mL). The values for antiradical activity were expressed as μg of ascorbic acid equivalents/g of sample.

Determination of Antioxidant Activity in Honey. The ferric reducing/antioxidant power (FRAP) assay was performed according to Bertoncelj *et al.* (2007). FRAP reagent was prepared by mixing 2.5 mL of 2,4,6-tripyridyl-s-triazine (TPTZ) (Sigma-Aldrich) (10 mM TPTZ/40 mM of HCl) with 2.5 mL of 20 mM $FeCl_3$ (Merck). Finally, 25.0 mL of 0.3 M acetate buffer at pH 3.6 was added to the mixture.

The FRAP reagent was made each time the assay was performed. To measure the antioxidant capacity of the bee products, 0.2 mL of each sample (either honey solution or bee pollen suspension) was mixed with 1.8 mL of the FRAP reagent. The absorbance was determined after 10 min at 593 nm. $FeSO_4{\cdot}7H_2O$ (Riedel de Haën, Seelze, Germany) was used as a standard to derive the calibration curve (50–1,000 μM). The values were expressed as mM equivalents of Fe^{+2}/g of sample.

Statistical Analysis

For each beehive, the analyses were performed in triplicate. An exploratory data analysis based on statistical graphics was carried out for choosing appropriate analysis and checking assumptions. Statistical analysis was carried out by using software R version 2.13 (R Development Core Team 2011).

TABLE 1. NUMBER OF UNIFLORAL HONEY SAMPLES FROM EACH SELECTED BEEHIVE PLACED IN THE POLLUTED AREA AROUND THE LLAIMA VOLCANO WITH THE PERCENTAGE OF THE PRIMARY AND SECONDARY BOTANICAL SPECIES

	Llaima honey 1	Llaima honey 2	Llaima honey 3	Llaima honey 4	Llaima honey 5	Honey control
% *Lotus uliginosus*	65.7 ± 0.01a	68.9 ± 0.03a	64.1 ± 0.02a	66.6 ± 0.01a	66.4 ± 0.02a	69.1 ± 0.04a
% *Escallonia rubra*	27.0 ± 0.01a	26.6 ± 0.01a	28.1 ± 0.04a	29.2 ± 0.02a	27.5 ± 0.04a	26.7 ± 0.01a

The means of triplicates reported in the same row are significantly different according to Tukey's test ($P \leq 0.05$) if denoted by different letters.

RESULTS AND DISCUSSION

The Botanical Origin of the Honey and Bee Pollen Samples

In this study, unifloral honey samples from *Lotus pedunculatus* (formerly *Lotus uliginosus*) were identified after melissopalynological analysis. This species is an introduced and naturalized melliferous plant. Nevertheless, a native species *Escallonia rubra* was identified with a secondary percentage in honey samples (Table 1).

Thus, bee pollen from those species were identified and isolated from total pollen samples collected for this study and used for chemical determinations. Before this study, there were no previous analyses for bee products obtained from those plants. Nevertheless, the primary aim of this work was to characterize the metal content and antioxidant activity in honey and bee pollen from *L. pedunculatus* and *E. rubra*, which were produced near the Llaima Volcano. The purpose was to assess the effect of this volcano, with reported signs of activity in the last 20 years, on these bee products. Control samples for honey and bee pollen allowed us to establish the metal content and antioxidant profiles for these bee products, and therefore, we could compare these with the samples selected for investigation.

Metal Content

All of the honey samples analyzed showed high levels of Mn (0.705–1.043 μg/g) and Fe (1.14–1.82 μg/g) compared with the control sample (Mn = 0.263 μg/g; Fe = 0.52 μg/g). Honey samples had a copper content higher than the control, with a 0.851 μg/g maximum value. Even when Zn was detected, the levels between samples and control did not differ (Table 2).

For the bee pollen samples, the ranges of values for copper content were 11.8–13.4 and 11.8–12.9 μg/g for *L. pedunculatus* and *E. rubra*, respectively. For iron content, the ranges of values for those samples were 174.5–176.5 and 175.0–194.0 μg/g. Both Cu and Fe levels were higher than in the control samples. In contrast, the Mn (47.1–58.1 and 51.4–58.7 μg/g) levels were lower than in the control bee pollen used for each species. Zinc content in bee pollen was similar to the control samples, showing the same trend observed for the selected honey in this work.

The Pb, Cd and Cr levels were also assessed (Table 2) and these elements were not detected in the honey. This result suggests that for bee products produced from the same beehive, the presence of metals in honey and in bee pollen may be different. In this case, the instrumental detection limit for Pb was ≤0.09 μg/g, Cd ≤ 0.01 μg/g and Cr ≤ 0.19 μg/g. These results allowed us to characterize bee products according to their geographic origin in regions of apicultural activity. However, it is necessary to study many honey and bee pollen samples to provide a good estimate of the mineral elements present (Silva *et al.* 2009). In most cases, where the mineral content of honey has been used as a parameter to identify the geographic origin, there are no data available for other bee products from either the same areas or beehives (Hernández *et al.* 2005; Baroni *et al.* 2009; Pohl 2009). Without this information, the confirmation of the geographic origin obtained after analytical determination of metal content could be complicated and confusing. In this study, the differences in metal content for honey and

TABLE 2. CU, MN, FE, ZN, PB, CD AND CR CONTENT IN THE HONEY AND BEE POLLEN FROM THE LLAIMA VOLCANO ZONE

Matrix	Zone	Cu (μg/g) SD	Mn (μg/g) SD	Fe (μg/g) SD	Zn (μg/g) SD	Pb (μg/g) SD	Cd (μg/g) SD	Cr (μg/g) SD
Honey	Llaima	0.602 (0.083)	0.838 (0.06)	1.32 (0.13)	0.586 (0.04)	ND	ND	ND
	Control	0.332 (0.002)[a]	0.263 (0.004)[b]	0.52 (0.06)[c]	0.623 (0.04)	ND	ND	ND
Lotus pollen	Llaima	12.52 (0.33)	52.1 (2.10)	175.4 (0.35)	63.5 (0.29)	0.227 (0.007)	0.040 (0.002)	0.427 (0.008)
	Control	9.83 (0.32)[a]	61.0 (0.45)[b]	159.4 (0.30)[c]	63.2 (0.30)	0.180 (0.01)[d]	0.015 (0.001)[e]	0.513 (0.006)[f]
Escallonia pollen	Llaima	12.21 (0.33)	55.4 (1.49)	181.8 (3.8)	63.9 (1.0)	0.233 (0.023)	0.417 (0.016)	0.043 (0.002)
	Control	8.82 (0.19)[a]	62.9 (0.32)[b]	146.7 (1.5)[c]	62.7 (0.8)	0.180 (0.012)[d]	0.413 (0.015)	0.013 (0.006)[f]

[a,b,c,d,e,f] The means reported in the same column are significantly different according to Tukey's test ($P < 0.05$) if denoted by these letters.
ND, not detected; SD, standard deviation.

TABLE 3. TOTAL PHENOLIC CONTENT AS WELL AS THE ANTIRADICAL AND ANTIOXIDANT ACTIVITIES OF UNIFLORAL HONEY AND BEE POLLEN FROM THE LLAIMA VOLCANO ZONE

Matrix	Zone	Phenolic (eq/kg) SD	FRAP* (eq/g) SD	DPPH** (eq/g) SD
Honey	Llaima	1,093 (47)	0.260 (0.015)	1.97 (0.57)
	Control	1,703 (30)[a]	0.424 (0.001)[b]	4.55 (0.06)[c]
Lotus Pollen	Llaima	504 (41)	67.8 (1.3)	112.1 (4.7)
	Control	760 (10)[a]	82.9 (0.6)[b]	139.3 (2.1)[c]
Escallonia Pollen	Llaima	1,249 (15)	69.5 (0.9)	119.9 (1.9)
	Control	1,679 (7.4)[a]	90.3 (0.9)[b]	140.3 (2.2)[c]

[a,b,c] The means reported in the same column are significantly different according to Tukey's test ($P < 0.05$) if denoted by these letters.
Phenolic content is expressed as the mg equivalent of gallic acid/kg of sample. FRAP (*): the values are expressed as mM equivalents of Fe^{+2}/g of sample. DPPH (**): the values are expressed as μg of ascorbic acid equivalents/g of sample.
DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; FRAP, ferric reducing/antioxidant power; SD, standard deviation.

bee pollen may be explained by the nature of the organic matrices. Bee pollen may be more suitable for inorganic accumulation, given its chemical composition. Furthermore, bee pollen is more exposed to air pollutants, such as Pb, Cd and Cr, when it is carried by bees from flowers to beehives (Roulston and Cane 2000; Kroyer and Hegedus 2001; Gergen *et al*. 2006).

Phenolic Compounds: Antiradical and Antioxidant Activities in Bee Products

The values obtained for total phenolic concentration were similar in all of the honey samples analyzed (1,000.0–1,255.35 mg gallic acid eq/kg honey) and this concentration was lower than in the control. Even though the values for total phenolic content were low compared with other reports on honey samples from around the world, for example, Manuka (Stephens *et al*. 2010) and Czech honey (Lachman *et al*. 2010), there were samples with similar total phenolic compound content (Silici *et al*. 2010).

The results associated with the antioxidant activity suggest that phenolic compounds are primarily responsible for this property of honey and the presence of metals affects such activities by decreasing either their strength or effectiveness. Although the interaction between phenolic and metal content remains unclear for those bee products studied, experimental evidence suggests that heavy metal complexes are formed by a simple phenolic ligation with certain metals (Ambrosi *et al*. 2003). This interaction would explain the low values detected for the activities in honey and bee pollen that contain metals compared with the control samples. If this ligation occurs, the metals complexed to the phenolic compounds in bee products will decrease any biological or chemical property that the phenolic compounds may have.

Although no previous studies have been directed toward characterizing honey and bee pollen from *L. pedunculatus* and *E. rubra*, in this work, the bee products from those species were chemically characterized and a slight antioxidant activity in honey was detected in the control samples (Table 3). The values from the FRAP (0.22–0.29 mM Fe^{+2} eq/g honey) and DPPH (0.36–3.42 μg ascorbic acid eq/g honey) assays were low compared with the reported results for other unifloral honey from different regions in the world (Bertoncelj *et al*. 2007; Silici *et al*. 2010). With regard to the results from the antioxidant activity assays, we determined changes based on the metals present in the selected samples.

The honey samples with high metal content had a decreasing trend in antioxidant activity; the bee pollen samples showed the same trend. Such differences may be attributed to the metal content. For establishing quantitatively correlation between higher metal content and decreased antioxidant activity, we assessed the correlation matrix for the corresponding variables. Tables 4–6 describe the correlation matrix for the samples of honey and bee pollen samples of *L. pedunculatus* and *E. rubra*, respectively.

Table 4 shows a positive correlation between phenolic content and antioxidant activity in honey samples ($\rho > 0.80$). In the same way, the antioxidant activity correlation (for all the variables) and metal content were negative

TABLE 4. CORRELATION MATRIX FOR HONEY SAMPLES

	Cu	Mn	Fe	Zn	Phenolic	FRAP	DPPH
Cu	1.00	0.28	0.74	–0.66	–0.38	–0.34	–0.06
Mn	0.28	1.00	0.60	0.07	–0.95	–0.73	–0.90
Fe	0.74	0.6	1.00	–0.12	–0.55	–0.59	–0.33
Zn	–0.66	0.07	–0.12	1.00	0.12	0.02	0.02
Phenolic	–0.38	–0.95	–0.55	0.12	1.00	0.85	0.81
FRAP	–0.34	–0.73	–0.59	0.02	0.85	1.00	0.49
DPPH	–0.06	–0.9	–0.33	0.02	0.81	0.49	1.00

DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; FRAP, ferric reducing/antioxidant power.

TABLE 5. CORRELATION MATRIX FOR BEE POLLEN SAMPLES OF *LOTUS PEDUNCULATUS*

	Cu	Mn	Fe	Zn	Pb	Cd	Cr	Phenolic	FRAP	DPPH
Cu	1.00	–0.76	0.80	0.20	0.66	0.63	–0.55	–0.86	–0.96	–0.59
Mn	–0.76	1.00	–0.59	–0.33	–0.21	–0.35	0.48	0.73	0.66	0.04
Fe	0.80	–0.59	1.00	–0.13	0.77	0.90	–0.92	–0.75	–0.90	–0.79
Zn	0.20	–0.33	–0.13	1.00	–0.43	–0.43	0.12	–0.38	–0.05	0.28
Pb	0.66	–0.21	0.77	–0.43	1.00	0.91	–0.53	–0.33	–0.71	–0.83
Cd	0.63	–0.35	0.90	–0.43	0.91	1.00	–0.79	–0.41	–0.71	–0.79
Cr	–0.55	0.48	–0.92	0.12	–0.53	–0.79	1.00	0.66	0.70	0.69
Phenolic	–0.86	0.73	–0.75	–0.38	–0.33	–0.41	0.66	1.00	0.90	0.52
FRAP	–0.96	0.66	–0.90	–0.05	–0.71	–0.71	0.70	0.90	1.00	0.75
DPPH	–0.59	0.04	–0.79	0.28	–0.83	–0.79	0.69	0.52	0.75	1.00

DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; FRAP, ferric reducing/antioxidant power.

(except for Zn), but only the coefficients of correlation between Mn and antioxidant activity were statistically significant ($P < 0.05$).

For bee pollen samples, Tables 5 and 6 show a similar behavior compared with honey.

Similarly, we were able to establish differences between honey and bee pollen compared with the total phenolic content and antioxidant activity. A multivariate analysis of variance (MANOVA) was carried out to compare the antioxidant activity between honey samples and both types of bee pollen samples. The multivariate response matrix was set by binding antioxidant-related variables: phenolic content, FRAP and DPPH.

The MANOVA allowed us to confirm the presence of significant differences between honey and both types of pollen. It considers the variables related to the antioxidant activity jointly and besides it shows the differences between zones (Llaima's bee products samples and control). The antioxidant activity is higher in bee pollen samples used as control. This finding is consistent with our hypothesis. In a parallel line, for checking whether these differences are significant, the univariate ANOVA was made for each variable related to the antioxidant activity including geographical area as a blocking variable. A Tukey's test for evaluating the differences between honey and pollen was applied (Tables 2 and 3).

The most important differences were found among honeys and both bee pollen samples. Those statistical tests supported our proposal that bee products and geographic zone are involved in the obtained differences about the antioxidant activity.

By considering that honey is an organic matrix with a different composition compared with bee pollen, the presence of other compounds, such as carotenoids in the bee pollen, may be related to the increased antioxidant properties in these samples. Several studies have reported results suggesting that the matrix in bee pollen alone may play an important role in the strength of this property, in addition to the botanical origin (Saric *et al.* 2009). This fact could explain the differences detected between total phenolic content for bee pollen samples from *L. pedunculatus* and *E. rubra*. However, the values obtained for FRAP and DPPH were similar for both species (LeBlanc *et al.* 2009; Leja *et al.* 2007).

According to other studies, our findings suggest that phenolic compounds are primarily responsible for the antioxidant properties in these bee products, despite the metal content.

Further analysis in chemical isolation and identification of the phenolic compounds will aid in elucidating the interaction of such molecules with the metals and minerals present in bee products that are produced in metal-polluted environments.

TABLE 6. CORRELATION MATRIX FOR BEE POLLEN SAMPLES OF *ESCALLONIA RUBRA*

	Cu	Mn	Fe	Zn	Pb	Cd	Cr	Phenolic	FRAP	DPPH
Cu	1.00	–0.49	0.83	0.31	0.36	0.98	–0.16	–0.94	–0.97	–0.95
Mn	–0.49	1.00	–0.77	0.38	0.03	–0.48	–0.46	0.71	0.66	0.43
Fe	0.83	–0.77	1.00	0.04	0.22	0.83	–0.16	–0.92	–0.88	–0.80
Zn	0.31	0.38	0.04	1.00	0.91	0.44	–0.51	–0.17	–0.22	–0.53
Pb	0.36	0.03	0.22	0.91	1.00	0.53	–0.23	–0.33	–0.37	–0.61
Cd	0.98	–0.48	0.83	0.44	0.53	1.00	–0.19	–0.95	–0.96	–0.98
Cr	–0.16	–0.46	–0.16	–0.51	–0.23	–0.19	1.00	0.00	–0.04	0.25
Phenolic	–0.94	0.71	–0.92	–0.17	–0.33	–0.95	0.00	1.00	0.97	0.89
FRAP	–0.97	0.66	–0.88	–0.22	–0.37	–0.96	–0.04	0.97	1.00	0.94
DPPH	–0.95	0.43	–0.80	–0.53	–0.61	–0.98	0.25	0.89	0.94	1.00

DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; FRAP, ferric reducing/antioxidant power.

The reported activity of the Llaima Volcano affects the final composition of metals in bee products that originate in the surrounding area. However, the metal profiles for honey and bee pollen differ, which suggests that the chemical nature of these pollutants and the organic matrix for the bee products are involved. Both honey and bee pollen had low concentrations of phenolic compounds. Furthermore, decreased antioxidant activity in bee products was observed in comparison with control samples in this study. Such differences could be attributed to the metals present. The results from this work suggest that honey and bee pollen may be used as markers for environmental pollution by metals where pollution sources and apicultural activity coexist.

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