Acacia caven pollen from South America. An useful bio-indicator of environmental pollution. A healthy food supplement?

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Abstract

The aim of this work is to evaluate the mineral and heavy metal contents of *Acacia caven* (Mol.) Molina pollen, a South American plant which grows in the Northwest of Argentina, for studying its behavior as bio-indicator of environmental pollution and their consequences as dietary supplement. Mineral and heavy metal contents were determined by flame atomic absorption / emission spectrometry (FAAS and FAES, respectively), inductively coupled plasma optical emission spectrometry (ICP-OES) and hydride generation atomic absorption spectrometry (HG-AAS). The obtained results demonstrated that some mineral contents (K, Ca, P, Fe, Mg, Na, Zn and Se) of *Acacia caven* pollen met the requirements of both, the Food and Nutritional Board (FNB) and the Argentinean Alimentarius Codex (AAC) to be used as a dietary supplement. Nevertheless, some heavy metals analyzed such as Al, Cu, Ni, V, Cr and Cd, exceeded the upper levels (UL) established by FNB and AAC, probably due to environmental contamination. Beyond the high level of some heavy metals found in the *Acacia caven* pollen, this could be an important dietary supplement by their high Fe and low Na contents. Considering that the suggested doses of commercial pollen by pharmacists and companies advice is 13.5 g/day, an only doses of *Acacia caven* pollen would satisfy the recommended daily intake (RDI) of Fe given by FNB for an adult individual. In spite of this promising date, further studies on the Fe bioavailability and heavy metal toxicity from *Acacia caven* pollen should be carried out for being used this pollen as dietary supplement.

Keywords - *Acacia caven, pollen, bio-indicator, environmental contamination, dietary supplement.*

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Abbreviations

SDI: intake estimations of each mineral calculated according to suggested doses by pharmacists and companies advice (13.5g/day).

RDI: the average daily mineral intake level to meet the mineral requirement of nearly all (97 to 98%) individuals between 18 – 50 years old.

UL: the highest average daily mineral intake level that is likely to pose no risk of adverse health effects to individuals between 18 – 50 years old.

I. Introduction

Pollen is food stuff due to its significant content of aminoacids, carbohydrates, proteins, lipids, vitamins and minerals [1]. Nevertheless, pollen is frequently used as bio-indicator of environmental contamination by heavy metal [2, 3]. Argentina has an important apicultural activity, occupying the first places of sale in the world market of multiflower honey. Pollen frequency in honey samples reflects the major crops or plant types where the hives were located. Honeys from Northwest of Argentina have a high content of *Acacia caven* pollen, which determines the relevance of this research work. The aim of this work is to evaluate the mineral and heavy metal contents of *Acacia caven* (Mol.) Molina pollen, a South American plant which grows in the Northwest of Argentina, for studying its behavior as bio-indicator of environmental pollution and their consequences as dietary supplement. The obtained results will also contribute to qualify the pollen and honey from Argentina and they will have practical implications in international labelling, supervision and authorisation requirements for honey bee products.

II. Experimental

A. Reagents and chemicals

18MΩ cm ultra pure water (Barnstead Easy Pure RF compact ultrapure water system) was used in all studies. Ultra pure quality HNO_3, $HClO_4$, H_2SO_4, H_3BO_3, NH_4OH and

HF (Merck, Argentina) were used. Metal standard solutions were prepared immediately before of being used by appropriate dilutions of their respective 1000 mg/l stock solutions. A 0.5 % (w/v) sodium tetrahydroborate solution (Aldrich Chemical Co. > 98%) was prepared in 0.5 % (w/v) sodium hydroxide solution and then filtered through a Whatman No. 42 filter paper to remove undissolved solids. This solution was prepared daily.

B. Apparatus

The AAS measurements were performed with Shimadzu Model AA-6800 atomic absorption spectrometer (Tokyo, Japan) equipped with a deuterium background corrector, and the measurements were based on the peak height. Hallow-cathode lamps (Hamamatsu Photonics K.K., Tokyo - Japan) were employed as radiation source for FAAS determination. A sequential inductively coupled plasma spectrometer [BAIRD (Bedford, M.A, USA) ICP 2070] with 1m Czerny Turner monochromator (holographic grating with 1800 grooves/mm) was also used. Tygon-type pumps tubing (Ismatec, Cole-Parmer, and Vernon Hills, IL. USA) was employed to carry the sample. The hydride unit used was a hydride generator (PS Analytical Ltd.) and the flow rate of the reagents was controlled by a Watson-Marlow 303X peristaltic pump pH-meter model 215, with glass membrane pH/ATC electrodes, was used for pH measurements.

C. Sample preparation

Pollen of *Acacia caven* (Mol.) Molina Fabaceae (folk name: “espinillo”) was hand-collected in the hill of San Luis, Argentina. The sample was protected from light, dried to room temperature and stored in desiccators. Hand-collected pollen was treated before of the mineral content determination. 750 mg of the sample was digested by heating in a Kjeldhal flask with 5 ml perchloric acid and 10 ml nitric acid to oxidize the carbonaceous matter. Precautions were taken during heating so that no excess foaming took place. 20 ml concentrated nitric acid was added until the organic matter was totally oxidized. This point was reached when no further darkening of solution occurred on continuous heating and a clear solution was obtained. After that, the solution was cooled and transferred to a 25 ml volumetric flask and the volume was made up with ultra pure water. The sample treatment took approximately 40 min, but many samples were processed at the same time.

D. Minerals and heavy metals determination

Sodium, potassium and calcium were analyzed by FAES. Magnesium, manganese, zinc and iron were determined by FAAS. Phosphorous, copper, chromium, nickel, lithium, cadmium, lead, vanadium and aluminium were determined by ICP-OES. In addition, selenium was determined by HG-AAS.

E. Recovery study

Finally, in order to demonstrate the validity of the digestion procedure a recovery study was performed. 7.5 g pollen sample was divided into 10 portions of 0.75 g each one. The proposed method was applied to six portions and the obtained average quantities of potassium, sodium and magnesium (which were considered as representative elements) were taken as base values. Then, increasing quantities of these elements were added to the other aliquots of sample and the analytes were determined by the same method.

III. Results and discussion

Table 1 shows the comparative mineral and heavy metal contents of *Acacia caven* (Mol.) Molina pollen (measured by FAAS, FAES, ICP-OES and HG-ASS) and honey-bee collected pollen. The presence of seventeen mineral elements of *Acacia caven* pollen was determined and K was predominant (54.8 % of the total), followed by P, Ca, Fe, Al and Mg (34.3 %). The mean values of the main mineral contents of twenty honey-bee collected pollen are reported in Table 1. There were significant differences in the mineral content in those pollens. *Acacia caven* (Mol.) Molina pollen has 2 or 3 times more K, Ca, P, Mg , Mn; 33 times more Fe and 7,65 lesser of Na than honey bee-collected pollen. Table 2 shows comparative values of suggested daily intake (SDI) of *Acacia caven* (Mol.) Molina pollen with the recommended daily intake (RDI) and upper level (UL) from FNB and AAC [4 – 8]. Table 3 shows a recovery study for demonstrating the validity of the digestion procedure of the sample. Similar results were found for the other elements studied.

According to Table 2, *Acacia caven* (Mol) Molina pollen does not provide 100 % of the mineral RDI by FNB and AAC. Then, other mineral sources should be provides by the diet in order to satisfy the requirements of adult individuals (18-50 years old). Taking into account that the suggested daily intake (SDI) of commercial pollen is about 13.5 g/day [9], the studied pollen could provides the following percentages of the mineral RDI by FNB and AAC: 3% K; 1.6 – 2 % Ca; 1.7 - 3 % P; 97.5 - 219 % Fe; 3 - 3.9 % Mg; 1.5 % Na; 17.4 - 22 % Mn; 1.5 - 2.9 % Zn and 28.6 - 36.4% Se. Pb was not detected.

According to our results *Acacia caven* (Mol) Molina pollen is a very good source of Fe and Se. A dose (13.5 g/ day) of this natural product would provide the total amount of Fe that an adults needs per day. Fe is the micronutrient more investigated in dietary food stuff. The deficit of this mineral is the most frequent in the diet of both, developed and developing countries [10]. The no hemo form of Fe in plants represents 85% of the dietary Fe. Se plays an important role in the regulation of the immune system and the inflammatory response [11].

The values reported in Table 1 also show that K/Na ratio of *Acacia caven* (Mol.) Molina pollen is nearly 60- fold. This date agrees with others reported in the literature for vegetal foods and it demonstrates that *Acacia caven* (Mol) Molina pollen could be used as dietary supplement in cases of arterial hypertension, cardiovascular diseases and gastric cancer [12]. By other hand, Al, Cu, Ni, V, Cr, Li and Cd show a limited nutritional relevance. FNB established a probably RDI for those elements which was based in animal tests and, at the same time, suggested further studies to encourage their essential character [7]. According to FNB, average contents of these elements in *Acacia caven* pollen largely exceeded the maximum level of the daily mineral intake that is likely to pose no risk of adverse health effects (UL). As a consequence, extra caution should be taken in consuming the recommended pollen daily doses (13.5 g/day) by pharmacists and companies advice.

IV. Conclusions

In this work, the mineral and heavy metal contents of *Acacia caven* (Mol.) Molina pollen were studied. K, Ca, P, Fe, Mg, Na, Zn and Se are in good agreement with the requirements of the Argentinean Alimentarius Codex and Food and Nutrition Board for being used as a dietary supplement. Particularly, this pollen is a very good source of Fe and Se; it has a low Na content and Pb was not detected.

However, several heavy metals such as Al, Cu, Ni, V, Cr, Li and Cd exceeded the upper levels established by the above mentioned regulations, which would indicates potential environmental contamination. Then, extra caution should be taken in the labelling, supervision and authorization requirements for be used this pollen as food.

Since pollen is a natural component of honey it could escape any safety checks even though it might carry significant quantities of heavy metals risky for human health. Then, further studies in order to investigate on the bioavailability and absorption mechanisms of Fe and Se should be carried out. Besides, heavy metal toxicity from *Acacia caven* pollen should be investigated before that can be used as dietary supplement.

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TABLE 1. COMPARATIVE MINERAL CONTENT OF *ACACIA CAVEN* (MOL) MOLINA POLLEN (MEASURED BY FAES, FAAS, ICP-OES AND HG-AAS) AND HONEY BEE-COLLECTED POLLEN.

Mineral	Concentration (mg/g)		Wavelength (nm)	Technique
	A. caven pollen	*Honey bee-pollen* (*)		
K	10.44	4.153	766.5	FAES
Ca	1.55	0.490	422.7	FAES
P	1.55	0.580	213.62	ICP-OES
Fe	1.30	0.039	248.3	FAAS
Al	1.21	_	396.15	ICP-OES
Mg	0.93	0.432	285.2	FAAS
Cu	0.70	0.0087	324.75	ICP-OES
Ni	0.47	_	221.65	ICP-OES
V	0.33	_	309.26	ICP-OES
Cr	0.20	_	283.53	ICP-OES
Na	0.17	1.301	589.0	FAES
Li	0.15	_	670.78	ICP-OES
Mn	0.03	0.011	279.50	FAAS
Zn	0.017	0.034	213.90	FAAS
Se	0.0012	_	196.03	HG-AAS
Cd	0.0011	_	228.80	ICP-OES

(*) Mean values from 20 commercial samples (Serrá Bonheví and Escola Jordá, 1997).

TABLE 2. COMPARATIVE VALUES OF SUGGESTED DAILY INTAKE (SDI) OF ACACIA CAVEN´S POLLEN WITH RECOMMENDED DAILY INTAKES (RDI) AND THE UPPER LEVELS (UL) FROM FOOD & NUTRITIONAL BOARD (FNB) AND ARGENTINEAN ALIMENTARIUS CODEX (AAC).

Mineral	SDI (mg/day)	RDI (mg/day)		UL (mg/day)	
	A. caven pollen	*AAC*	*FNB*	*AAC*	*FNB*
K	141.00	---	4700	---	No Set
Ca	20.92	1000	1200-1300	1000	2500
P	20.92	1000	700-1250	1500	4000
Fe	17.55	18	8-18	65	45
Al	16.33	---	2-10	---	---
Mg	12.55	400	320-420	700	350
Cu	9.45	2	0.900	9	10
Ni	6.34	---	< 0.100	---	1
V	4.45	---	0.010	---	1.8
Cr	2.70	0.12	0.025-0.035	0.20	No Set
Na	2.29	---	150	---	2300
Li	2.02	---	0.2-0.6	---	No Set
Mn	0.40	2	1.8-2.3	10	11
Zn	0.23	15	8-11	30	40
Se	0.02	0.70	0.055	0.20	0.4
Cd	0.15	---	0.010-0.020	---	---
Pb	No detected	---	0.015-0.1	---	---

TABLE 3. RECOVERY STUDY.

Analyte	Aliquots	Base value (µg/g)	Quantity of metal added (µg/g)	Quantity of metal found (µg/g)	Recovery (*) (%)
K	1-5	---	0.0	10392.8	---
	6	10392.8	1000.0	11342.8	95.0
	7	10392.8	1500.0	11862.3	98.0
	8	10392.8	2000.0	12394.8	100.1
	9	10392.8	2500.0	12897.5	100.2
	10	10392.8	3000.0	13380.0	99.6
Na	1-5	---	0.0	173.0	---
	6	173.0	10.0	182.8	98.0
	7	173.0	20.0	193.0	100.0
	8	173.0	50.0	222.9	99.8
	9	173.0	80.0	251.9	98.6
	10	173.0	100.0	272.0	99.0
Mg	1-5	---	0.0	929.1	---
	6	929.1	50.0	978.1	98.0
	7	929.1	100.0	1025.0	96.0
	8	929.1	200.0	1129.3	100.1
	9	929.1	250.0	1179.1	100.0
	10	929.1	300.0	1228.8	99.9

(*) 100 x [(Found-Base)/Added.