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ORIGINAL RESEARCH ARTICLE

The relationship between comb age and the amounts of mineral elements in honey and wax

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Summary

This study was carried out at the Faculty of Agriculture, Kafrelsheikh University, Egypt, to study the relation between honey bee comb age and the amount of different minerals present in honey and wax. Mineral element traces in honey and wax were significantly correlated with comb age. The highest mineral values were obtained from honey and wax from combs greater than four years old. The elements copper (Cu), cadmium (Cd), nickel (Ni) and chromium (Cr) were not detected in all honey and wax samples, but Ni was only detected in wax of combs over four years old, at a content of 1.00 mg / kg. It was concluded that mineral traces in honey can be correlated with comb age, and it is possible to differentiate between honeys harvested from combs of different ages.

Relación entre la edad de los cuadros y la cantidad de elementos minerales en la miel y la cera

Resumen

Este estudio se llevó a cabo en la Facultad de Agricultura de la Universidad de Kafrelsheikh en Egipto, para estudiar la relación entre la edad de los cuadros de abejas y la cantidad de diferentes minerales presente en la miel y en la cera. Trazas de elementos minerales en miel y cera fueron significativamente correlacionadas con la edad de los cuadros. Los valores más altos de minerales se obtuvieron de miel y cera de cuadros con más de cuatro años. Los elementos cobre, cadmio, níquel y cromo no se detectaron en todas las muestras de miel y cera, el níquel se detectó solamente en cera de cuadros de cuatro años son un contenido de 1,00 mg/kg. Se concluye que las trazas de minerales en la miel puedes estar correlacionadas con la edad de los cuadro, y que es posible diferenciar entre mieles recolectadas de cuadros con diferentes edades.

Keywords: honey, minerals, trace elements, risk elements, wax

Introduction

Honey contains varying amounts of mineral elements, ranging from 0.02 to 1.03% (White, 1975). Several investigators have shown that the mineral element content of honey depends on its botanical origin (Abu-Tarboush *et al.*, 1993; Nour, 1998; AL-Khalifa and Al-Arify, 1999; Conti, 2000; Nanda *et al.*, 2003; Fernández-Torres *et al.*, 2005; Gonzalez-Miret *et al.*, 2005; Hernández *et al.*, 2005; Rateb, 2005; Mohamed, 2006; Bogdanov *et al.*, 2007; Küçük, *et al.*, 2007; Sheref, 2007), geographical origin and environment (Uren *et al.*, 1998; AL-Khalifa and Al-Arify, 1999; Latorre *et al.*, 1999; González *et al.*, 2000; Terrab *et al.*, 2004; Bratu and Georgescu, 2005; Erbilir and Erdoğrul, 2005; Hernández *et al.*, 2005; Muñoz and Palmero**,** 2005; Bogdanov, 2006; Bogdanov *et al.*, 2007; Küçük *et al.*, 2007; Lachman *et al.*, 2007; Sheref, 2007; Rehman *et al.*, 2008) and length of storage (Badei and Shawer, 1986). Taha and El-Sanat (2007) found that colour intensity and electrical conductivity of honey significantly correlated to the age of comb used. Most elements correlate significantly with electrical conductivity (Bogdanov *et al.*, 2007). Dark honeys have a higher mineral content than pale ones (Feller-Demalsy *et al.*, 1989; Ankalm, 1998; Gonzalez-Miret *et al.*, 2005).

Very few data are available on the effect of comb age on the mineral content of honey, so we investigated the relation between different comb ages and the content of mineral elements which are considered to be essential for human nutrition [potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), copper (Cu), Iron (Fe), manganese (Mn)

and zinc (Zn)] and harmful elements [lead (Pb), cadmium (Cd), nickel (Ni) and chromium (Cr)] in honey and wax.

Materials and methods

The study was carried out at the apiary of the Faculty of Agriculture, Kafrelsheikh University, Egypt. One week before the start of the clover season in 2008, twelve colonies (each of six combs) of hybrid Carniolan honey bees headed by young open-mated sister queens were equalized for brood and bee strength. The central four combs in each colony had been replaced by empty combs which were 1, 2, 3 or 4 years old. These colonies were divided into four groups of three with comb arrangements as follows: Group 1. 1, 2, 3 and 4 years old; Group 2. 2, 3, 4 and 1 year old; Group 3. 3, 4, 1 and 2 years old; and Group 4 4, 1, 2 and 3 years old. Feeding of the colonies was stopped to avoid the presence of honey from feeding in the experimental combs. By the end of May, samples of honey and wax were taken directly from the combs to determine the mineral content at the central laboratory of Kafrelsheikh University.

Wet digestion with nitric acid following the method of AOAC (2000) was used to prepare honey and wax samples for determination of mineral content. One gram of honey or wax was digested in a Kjeldahl flask with 10 ml of 75% HNO_3 for oxidation of carbonaceous matter. The contents of the flask were heated 100-120ºC, to evaporate the acid. Drops of perchloric acid were added until all the organic matter was oxidized. This point was reached when no further darkening of the solution occurred on continuous heating and a clear solution was obtained. It was cooled and gauged to 50 ml with distilled water. A blank experiment was carried out by adding same amount of nitric acid to one ml distilled water. An Atomic Absorption GBC AVANTA Σ GF 3000 was used for detection of iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), lead (Pb), cadmium (Cd), nickel (Ni) and chromium (Cr) concentrations. Potassium (K), sodium (Na), calcium (Ca) and magnesium (Mg) concentrations were determined using a flame photometer (Sherwood 410, UK). The instrument was calibrated by using standard solutions of various metal salts**.** All determinations were made in triplicate.

Data were statistically analyzed by the analysis of variance according to Gomez and Gomez (1984). Treatment means were compared by Duncan's Multiple Range Test (Duncan, 1955). Simple correlation was made by using "SPSS 10.0 for windows".

Results

Data illustrated in Fig. 1 and Table 1 show that the levels of K, Na, Ca, Mg, Fe, Zn, Pb and Mn in clover honey were variable and significantly depended on the age of combs. The highest values (480.00, 468.00, 460.00, 254.00, 30.00, 2.55, 1.15 and 0.97 mg / kg, respectively) were obtained from combs aged 4 years. On the other hand, Cu and harmful elements (Cd, Ni and Cr) were not detected in all samples. Significant ($P < 0.01$) positive correlations between all determined mineral elements of clover honey were observed (Table 2).

The content of K, Na, Ca, Mg, Fe, Zn, Pb and Mn in comb wax of was found to occur in ascending order with comb age. The largest values (2600.00, 1036.00, 1360.00, 986.00, 36.00, 3.50, 2.18 and 2.55 mg / kg, respectively) were obtained from combs aged 4-years (Fig. 2 and Table 3). There were highly significant ($P < 0.01$) positive correlations between all determined mineral elements in comb wax of combs apart from that between Mn and Pb which was significant ($P < 0.05$) only (Table 2).

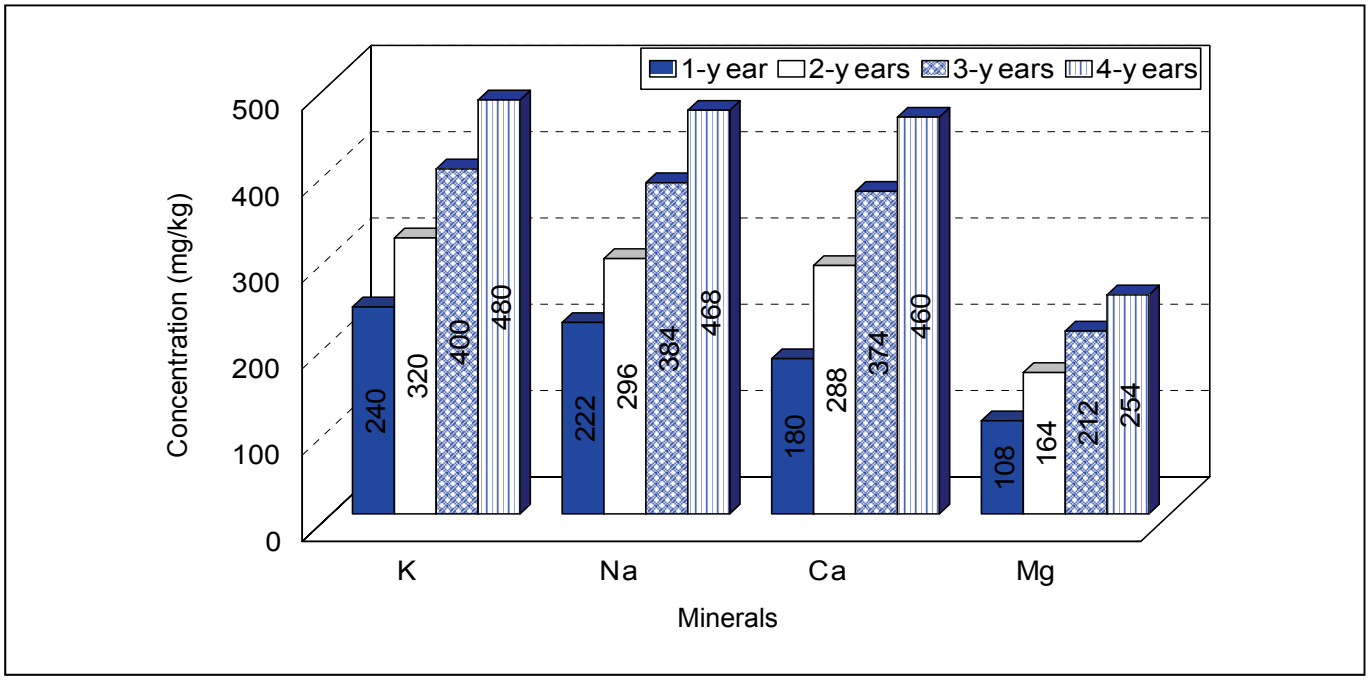

Fig. 1. Mineral content (mg / kg) of clover honey in relation to comb age.

Table 1. Trace element content (mg / kg) in clover honey in relation to comb age. ND, below detection limit (not detected). Means of each row followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test. ** and NS indicate P < 0.01 and non-significant, respectively.

Elements (mg / kg)	**Age of comb (years)**				**Significance**
	1	2	3	3	
Fe	23.00^{c}	24.00^{c}	26.00^{b}	30.00^{a}	**
Zn	1.35^{c}	1.36^{c}	1.85^{b}	2.55^{a}	**
Mn	0.17^{c}	0.17^{c}	0.32^{b}	0.97^{a}	**
Pb	0.75^{c}	0.85^{b}	0.85^{b}	1.15^{a}	**
Cu	ND	ND	ND	ND	NS
Cd	ND	ND	ND	ND	NS
Ni	ND	ND	ND	ND	NS
Cr	ND	ND	ND	ND	NS

Table 2. Pearson correlation coefficients for minerals in clover honey and comb wax. Normal and italic letters indicate minerals in honey and wax, respectively. *Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

	K	**Na**	**Ca**	**Mg**	**Fe**	**Zn**	**Pb**	**Mn**	***K***	***Na***	***Ca***	***Mg***	***Fe***	***Zn***	***Pb***	***Mn***
K																
Na	0.99**															
Ca	0.99**	0.99**														
Mg	0.99**	0.99**	0.99**													
Fe	0.90**	0.91**	0.88**	0.89**												
Zn	0.93**	0.94**	0.90**	0.90**	0.94**											
Pb	0.86**	0.87**	0.83**	0.82**	0.90**	0.96**										
Mn	0.89**	0.89**	0.88**	0.87**	0.91**	0.93**	0.97**									
K	0.97**	0.98**	0.97**	0.96**	0.92**	0.96**	0.94**	0.96**								
Na	0.98**	0.98**	0.98**	0.98**	0.86**	0.90**	0.80**	0.81**	0.94**							
Ca	0.98**	0.98**	0.99**	0.99**	0.85**	0.86**	0.78**	0.84**	0.94**	0.98**						
Mg	0.99**	0.99**	0.98**	0.97**	0.92**	0.96**	0.92**	0.94**	0.99**	0.96**	0.96**					
Fe	0.94**	0.95**	0.93**	0.94**	0.98**	0.94**	0.88**	0.89**	0.94**	0.92**	0.91**	0.95**				
Zn	0.98**	0.98**	0.98**	0.98**	0.94**	0.93**	0.85**	0.88**	0.96**	0.97**	0.97**	0.97**	0.97**			
Pb	0.88**	0.89**	0.85**	0.85**	0.89**	0.98**	0.95**	0.87**	0.91**	0.87**	0.80**	0.91**	0.90**	0.88**		
Mn	0.93**	0.92**	0.95**	0.94**	0.80**	0.77**	0.72**	0.83**	0.90**	0.89**	0.80**	0.91**	0.84**	0.90**	0.67*	

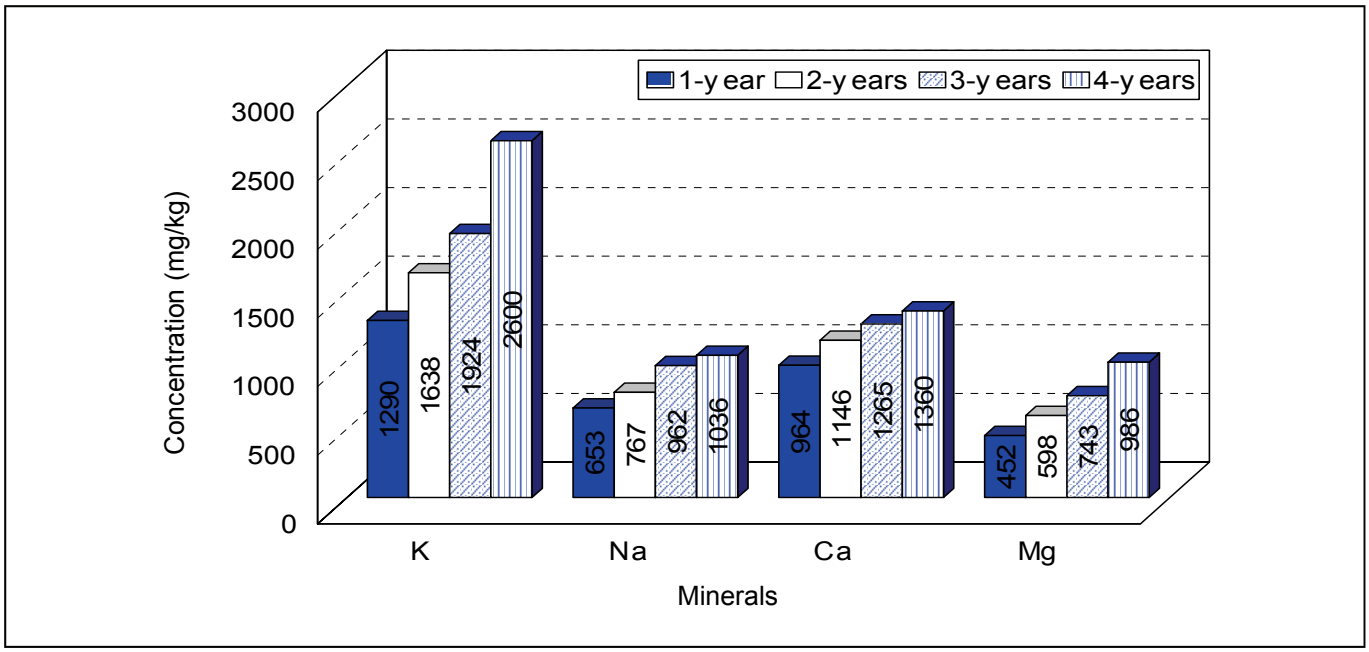

Fig. 2. Mineral content (mg / kg) in comb wax in relation to comb age.

Table 3. Effect of comb age on trace element content (mg / kg) in comb wax. ND, below detection limit (not detected). Means of each column followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test. ** and NS indicate $P < 0.01$ and non-significant, respectively.

Age of combs	Fe	Zn	Mn	Pb	Cu	Cd	Ni	Cr
1-year	25.00^{d}	2.00^{d}	1.38^{c}	1.12^{c}	ND	ND	ND	ND
2-years	27.00^{c}	2.45^{c}	1.16^{d}	1.80^{b}	ND	ND	ND	ND
3-years	31.00^{b}	3.00^{b}	1.85^{b}	1.85^{b}	ND	ND	ND	ND
4-years	36.00^{a}	3.50^{a}	2.55^{a}	2.18^{a}	ND	ND	1.00	ND
Significance	**	**	**	**	NS	NS	NS	NS

Discussion

Minerals in honey

The levels of K, Na, Ca, Mg, Fe, Zn, Pb and Mn were significantly varied from 240.00-480.00, 222.00-468.00, 180.00-460.00, 108.00-254.00, 23.00-30.00, 1.35-2.55, 0.75-1.15 and 0.17-0.97 mg / kg, respectively depending on comb age. These ranges are similar to those obtained by Serra (1989), Terrab *et al.* (2004), Fernández-Torres *et al.* (2005), Hernández *et al.* (2005) and Rateb (2005). Potassium was the most prevalent mineral in clover honey and estimated as 29.00% of the total mineral quantified, followed by Na (27.60%) and Ca (26.22%), then Mg (14.86%) and Fe (2.08%), while, Zn (0.14%), Pb (0.07%) and Mn (0.03%) were traces. These results are confirmed by the findings of Terrab *et al.* (2004) who found that K is the most important mineral, and accounts for 48% of the total; Na and Ca were present in moderate amounts and accounted for 27% and 13% of the minerals, respectively, while Mg comprised only 5.44%. The high mineral concentrations (K, Na, Ca and Mg) detected in honey produced in combs aged 4-years may be due to accumulation on the wax of old combs. Significant ($P < 0.01$) positive correlations between mineral elements of honey and mineral elements in comb wax were observed (Table 2). These results are similar to those of Rateb (2005) and Bogdanov *et al.* (2007) who found significant positive correlations between Fe, Zn, Mn and pb in honey.

Minerals in wax of combs

Large amounts of minerals were found in comb wax compared to honey, and amounts increased with comb age. The high mineral contents of wax in old combs may result from the use of combs for

food storage especially pollen grains as well as accumulation of propolis and pollen (Free and Williams, 1974) and for brood rearing (Hepburn, 1998). Repeated brood cycles result in accumulation of cocoons and faecal material deposited by the larval and pupal instars developing within the cell (Jay, 1963). Taha and El-Sanat (2007) found in contrast that ash content in clover honey and comb wax was in ascending order according to comb age. The elements (Cu, Cd, Ni and Cr) were not detected in all honey and wax samples, except for Ni in wax of combs aged 4-years which recorded 1.00 mg / kg. These results are in agreement with those obtained by Badei and Shawer (1986) for Cu, Nour (1998), Rateb (2005) and Sheref (2007) for Cd in honey.

Generally, the relatively large amounts of trace elements (Fe, Zn, Pb and Mn) in honey and wax might be due to atmosperic polluntant deposition on plants and flowers, which might be absorbed together with flower nectar, might pollute combs through bees' contaminated bodies, through contamination of water collected by bees, or by deposition on pollen which is then collected on the hairy bodies of bees brought to the hive (Porrini *et al.*, 2003). From this study, it can be concluded that the mineral content of honey significantly depended on the age of combs from which it was harvested, so it is possible to differentiate between honeys harvested from combs of different ages.

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