EFFECT OF COMBS AGE ON HONEY PRODUCTION AND ITS PHYSICAL AND CHEMICAL PROPERTIES

EL-KAZAFY A. TAHA[1], AND SAMIR Y. A. EL-SANAT[2]

[1]Econ. Entomol. and [2]Food Tech. Dept., Fac. Agric. Kafr El-Sheikh Univ., Kafr El-Sheikh, Egypt.

ABSTRACT

This study was carried out at the apiary of the College of Agriculture. Kafr El-Sheikh University, to study the effect of combs age on honey production, physical and chemical properties. Sixteen honey bee colonies were divided into four groups according to age of combs (1, 2, 3 and 4-years old). The highest amount of honey yield was harvested from colonies contained 1-year old combs. Specific gravity, viscosity, color and electrical conductivity of clover honey samples showed significant variations in values according to combs age. Protein, Phenols and ash contents were increased in parallel with age of combs. Organoleptic properties indicated that honey sampls produced on combs with one-year old had the best qualities compared to the others. The mean values of color and flavor (taste & odor) of it were higher than honeys produced in old combs.

INTRODUCTION

Honey is one of the most complex mixtures of sugars and other minor components produced in nature. The physical properties and chemical composition of honey from different sources have been published by many authors (Badei & Shawer, 1986 & 1990; Nour, 1988 & 1998; Swallow & Low, 1990; Sporns *et al.*, 1992; Perez-Arquillue *et al.*, 1995; Esti *et al*, 1997; Singh & Kaur, 1997; Anklam, 1998; Andrade *et al.* 1999; Costa *et al.*, 1999; Garcia-Alvarez *et al.*, 2000; Coronel & Monti, 2001; Popek, 2002; Rateb, 2005; Mohamed, 2006 and Sheref, 2007). The composition depends mainly on nectar sources as well as climatic conditions (Abu-Tarboush *et al.*, 1993).

The color of comb when first built is near-white. Wax comb consists primarily of hydrocarbons and ester components (Tulloch, 1980) which easily absorb many types of materials. Comb used for food storage takes on a yellowish hue over time due to the accumulation of pollen (Free & Williams, 1974). As comb used for brood rearing ages it becomes darker and almost black (Hepburn, 1998) and the cells tend to become smaller (Winston, 1987) because of accumulated cocoons and fecal

Proc. 2nd Inter. Conf. Ent. Soc. Egypt, Vol. II, 2007 (9-18)

material that are deposited by the larval and pupal instars developing within the cell (Jay, 1963), propolis and pollen (Free & Williams, 1974). The darker color may also be resulted from numerous unidentified contaminants that are collected and absorbed in the wax over time. The cell walls thicken with an accumulation of debris from larval cocoons and other detritus (Coggshall & Morse, 1984).

Since there are very little data available on the effect of combs age on honey production (El-Dakhakhni, 1995) and composition, this work aimed to find the effect of combs age on honey production and its physical and chemical properties.

MATERIAL AND METHODS

This study was carried out at the apiary of the College of Agriculture, Kafr El-Sheikh University. One week before starting of clover season 2006, sixteen colonies (each five combs) of hybrid Carniolan honey bee headed by young sisters open-mated queens were equalized to be about the same strength (brood and bees). The colonies were divided into four groups according to age of combs (1, 2, 3 and 4-years old). Combs in all groups had been replaced by empty 1, 2, 3 and 4-years old combs according to their groups. Food supply was stopped to avoid the presence of honey from feeding in combs.

1- Honey yield

By the end of May, honey yield was calculated from the difference between weight of honey combs before and after extraction. Samples of each group were taken directly from the combs to measure the physical and chemical properties of honey in Food Technology lab.

2- Physical characteristics.

The analytical procedures followed were: specific gravity by method of Wedmore (1955), viscosity was measured by using viscometer at 29°C according to Munro (1943), color was measured as optical density at 400 nm using spectrophotometer (White, 1967), electrical conductivity value (EC) using "HANNA" Electro-conductivity of 20°C and expressed in mS/cm (Vorwohl, 1964), Hydroxy methyl furfural was determined using a colorimetric method developed by Meyav & Berk (1978), granulation was calculated as glucose to water, and also glucose to fructose ratios.

3- Chemical characteristics.

Moisture content was estimated using Abbe refractometer. The readings of refractive index, after correction for temperature were converted to moisture, using the

table of White *et al.* (1962). Crude protein, by using the Micro-Kjeldahl method to determine the total nitrogen and multiply its value by the factor of 6.25. Ash content by ashing in an electric muffle at 550°C until constant weight; total soluble solid and total charbohydrates, all were determined according to the methods described in A.O.A.C. (2000). Free acidity, lacton and total acidity content were determined as described by White *et al.* (1962). The pH value was examined using glass rod "Hanna" pH-meter, model H 19321, according to A.O.A.C. (2000).

4- Sugar determinations

Reducing sugars were estimated according to the methods described in A.O.A.C. (2000). Sugars (fructose, glucose, sucrose and maltose) were determined using an HPLC chromatographic method according to A.O.A.C. (2000). All analyses of the experiment were carried out in triplicate.

5- Organoliptic evaluation of honey

Organoliptic properties of the investigated honeys were carried out according to the method reported by Molander (1976) by twenty panalests. Organoleptic score were recorded according to the following judging scale, very good (8-9), good (6-7), fair (4-5), poor (2-3) and very poor (0-1).

Statistical analysis

Data obtained were statistically analysed according to Steel & Torrie (1980). Treatment means were compared by Duncan's Multiple Range Test (Duncan, 1955). Simple correlation was made by using "SPSS 10.0 for windows".

RESULTS AND DISCUSSION

1- Honey production

Data illustrated in Fig. (1) showed clearly that, the highest amount of honey was harvested from colonies contained combs of 1-year old (4.77 kg/colony), while the lowest yield was in colonies contained combs aged 4-years (4.05 kg/ colony). The high honey yield in colonies with new combs in compared to those with old ones may be due to the higher brood production in new combs (Berry & Delaplane, 2001) resulted in increasing bee populations who gathered high honey yield. These results are in agree with those obtained by El-Dakhakhni (1995) as she found that the highest honey area was obtained from colonies containing combs of one-year-old, while the lowest area was found in colonies had combs of 4-years-old.

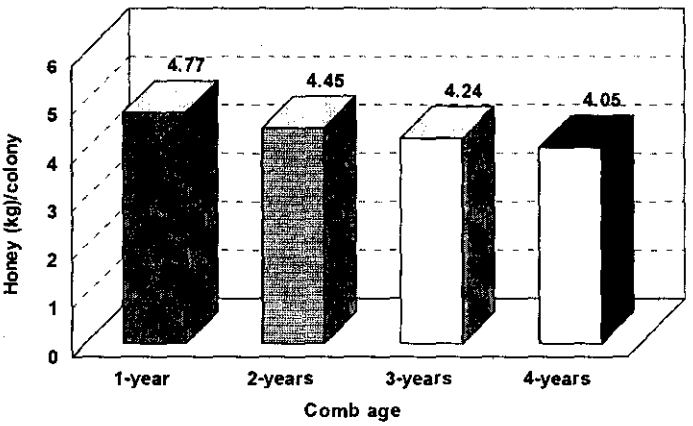

Fig. (1): Effect of combs age on honey yield.

2- Physical properties

The results given in Table (1) indicated that specific gravity, viscosity, color intensity and electrical conductivity of clover honey samples were ranged from 1.408 to 1.414, 34.80 to 34.92 poise, 0.28 to 0.39 O.D. and 2.24 to 4.18 mS/cm with averages of 1.413, 34.85 poise, 0.33 O.D. and 3.52 mS/cm, respectively, significantly depending on age of combs. The highest values of specific gravity, viscosity, color intensity and electrical conductivity of clover honey were obtained from combs aged 4-years. These results may be due to high contents of ash, protein and total phenols resulted from using the combs for food storage ages, and subsequently accumulation of pollen (Free & Williams, 1974) and for brood rearing ages (Hepburn, 1998) as well as reduction of moisture content.

TABLE (I)

Effect of combs age on physical properties of clover honey.

Age of combs	Specific gravity	Viscosity (poise)	Color (O.D.)	EC mS/cm	HMF (mg/kg)	Granulation	
						G/W	G/F
1-year	1.408^{d}	34.80^{d}	0.28^{a}	2.42^{d}	6.78^{d}	1.72^{d}	0.791^{a}
2-years	1.409^{c}	34.83^{c}	0.32^{b}	3.61^{c}	8.42^{a}	1.73^{c}	0.790^{a}
3-years	1.411^{b}	34.85^{b}	0.35^{c}	4.03^{b}	8.27^{ab}	1.75^{b}	0.788^{b}
4-years	1.414^{a}	34.92^{a}	0.39^{d}	4.18^{a}	7.40^{c}	1.76^{a}	0.783^{b}
Mean	1.412	34.85	0.33	3.52	7.72	1.74	0.788

Means of each column followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

Significant positive correlations between specific gravity, viscosity and color intensity from one side and each of ash %, protein % and total phenols from other side were detected, while significant negative correlations with moisture were noticed (Table 2).

The hydroxy methyl furfural (HMF) was ranged from 6.78 to 8.42 mg/kg and averaged 7.72 mg/kg, independent on combs age.

Granulation of honey expressed as glucose to water ratio (G/W) was ranged from 1.72 to 1.76 depending significantly on age of combs, and as glucose to fructose ratio (G/F) was ranged from 0.783 to 0.791. The actual proportion of fructose to glucose, in any particular honey, depends largely on the source of the nectar (Anklam, 1998).

TABLE (II)

Correlation coefficient values for some characteristics of clover honey produced in combs with different ages.

Comparison	r-values
Specific gravity × Moisture %	- 0.99**
Specific gravity × Ash %	0.95*
Specific gravity × Protein %	0.99**
Specific gravity × Total phenols	0.98*
Viscosity × Moisture %	- 0.98*
Viscosity × Ash %	0.96*
Viscosity × Protein %	0.98*
Viscosity × Total phenols	0.95*
Color × Moisture %	- 0.99**
Color × Ash %	0.99**
Color × Total phenols	0.96*
Color × Protein %	0.97*
Moisture % × Ash %	- 0.98*
Moisture % × Protein %	- 0.99**
Moisture % × Total phenols	- 0.98*
Protein % × Total phenols	0.99**
Honey's ash × Wax's ash	0.95*
Honey's phenols × Wax's phenols	0.99**

* and ** indicate P<0.05 and P<0.01, respectively.

3- Chemical properties

As shown in Table (3) results indicated that moisture % of clover honey samples was ranged from 18.15 to 18.70% with an average of 18.44 % in opposite trend to age of combs. The percentage of total soluble solid (T.S.S.) was increased from 81.30 to 81.85% in parallel to age of combs, and averaged 81.56%. These may be due to high contents of ash, protein and total phenols resulted from using the combs for food storage and brood rearing ages. Significant negative correlation between moisture content and each of ash %, protein % and total phenols was obtained (Table 2).

Reducing sugars; fructose and sucrose showed ascending orders according to age of combs. In contrast, non-reducing sugars, glucose and maltose showed descending orders according to age of combs. Significant differences among honey samples produced in combs with different ages were detected.

TABLE (III)

Effect of combs age on chemical properties of clover honey.

Chemical properties	Age of combs (years)				Mean
	1	2	3	4	
Moisture %	18.70^{a}	18.54^{Pb}	18.38^{c}	18.15^{d}	18.44
T.S.S (%)	81.30^{d}	81.46^{c}	81.62^{b}	81.85^{a}	81.56
Total carbohydrates (%)	79.21^{a}	79.04^{b}	78.93^{bc}	78.86^{c}	79.01
Reducing sugars	72.83^{bc}	72.84^{b}	72.92^{ab}	72.98^{a}	72.89
Non-reducing sugars	6.38^{a}	6.20^{b}	6.01^{c}	5.88^{cd}	6.12
Glucose %	32.17^{a}	32.15^{b}	32.14^{c}	32.04^{d}	32.12
Fructose %	40.66^{d}	40.69^{c}	40.78^{b}	40.94^{a}	40.77
Maltose %	4.40^{a}	4.35^{ab}	4.20^{b}	4.15^{c}	4.27
Sucrose %	2.50^{c}	2.55^{b}	2.68^{ab}	2.78^{a}	2.63
Ash %	0.18^{d}	0.28^{c}	0.32^{b}	0.41^{a}	0.31
Protein %	0.59^{c}	0.62^{b}	0.69^{b}	0.78^{a}	0.67
Undetermined (%)	1.32^{d}	1.53^{c}	1.68^{b}	1.80^{a}	1.57
pH	3.30	3.24	3.25	3.30	3.27
Free acidity mq/kg	32.75^{a}	32.50^{b}	30.75^{c}	30.25^{d}	31.56
Lacton mq/kg	10.50^{a}	10.00^{b}	10.50^{a}	10.50^{a}	10.38
Total acidity mq/kg	43.25^{a}	42.50^{b}	41.25^{c}	40.75^{d}	41.94
Total phenols (mg/100g)	1.40^{d}	1.50^{c}	1.90^{b}	2.20^{a}	1.75

Means of each column followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

The pH of honey was ranged from 3.24 to 3.30, and averaged 3.27. Free acidity of clover honey was ranged from 30.25 to 32.75 mq/kg with an average of 31.56 mq/kg. The total acidity was ranged from 40.75 to 43.25 mq/kg with a mean of 41.94 mq/kg. The pH of a honey is not directly related to the free acidity because of the buffering action of the various acids and minerals present (Abu-Tarboush *et al.*, 1993). The variation in acidity among different honey types may be attributed to variation due to harvest season (Perez-Arquillue *et al.*, 1995).

Ash, protein and total phenols contents in clover honey and comb's wax were detected to be in ascending orders according to age of combs (Table 3&4). Ash, protein and total phenols contents in honey or wax of old combs were significantly higher than those of the newest ones. The high values with old combs compared to the newest ones may be due to frequent using of combs for food storage resulted in accumulation of propolis and pollen (Free & Williams, 1974).

Also, repeated brood cycle resulted in accumulation of cocoons and fecal material that are deposited by the larval and pupal instars developing within the cell (Jay, 1963). Honey normally has low ash content and it depends on the material collected by the bees during foraging on the flora (Abu-Tarboush *et al.*, 1993).

Highly significant positive correlation between protein % and total phenols was noticed. Significant positive correlation between honey's ash and wax's ash as well as between honey's phenols and wax's phenols were noticed (Table 2).

TABLE (IV)

Effect of combs age on ash, protein and phenols contents in comb's wax.

Age of combs	Ash content (%)	Protein content (%)	Phenol content (mg/100g)
1-year	2.14^{d}	0.72^{d}	2.2^{d}
2-years	2.67^{c}	0.81^{bc}	3.0^{c}
3-years	3.13^{b}	0.82^{b}	4.5^{b}
4-years	4.24^{a}	0.85^{a}	6.3^{a}

Means of each column followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

4- Organoleptic properties of honey

The intrinsic changes in honey quality were classified into five categories according to total scores of their organoleptic qualities. Investigated honey samples showed that all honeys were free from any insect fragments, sand particles, undesirable flavours and any fermentation. As shown in Table (5) the panel test judgment of honey refereed to their color, taste, odour, viscosity, sourness and overall acceptability. The results revealed that the age of combs affected the organoleptic properties of honey. The honey produced in combs aged one-year had the best qualities compared to all tested honey samples. The mean values of color and flavor (taste & odor) of it was higher than all of the studies honeys.

TABLE (V)

Effect of combs age on organoleptic properties of clover honey.

Age of combs	Color	Odor	Taste	Viscosity	Sourness	Overall acceptability
1-year	9^{a}	8^{a}	9^{a}	8^{a}	9^{a}	8.6^{a}
2-years	8^{b}	8^{a}	9^{a}	7^{b}	8^{b}	8.0^{b}
3-years	7^{c}	8^{a}	8^{b}	7^{b}	7^{c}	7.4^{c}
4-years	7^{c}	7^{b}	8^{b}	7^{b}	6^{d}	7.0^{d}
Significance	*	*	*	*	*	*

Means of each column followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

-* indicate $P<0.05$

Lastly, it could be recommended to use new combs during flow seasons for obtaining higher honey yield.

REFERENCES

ABU-TARBOUSH, H.; H. AL-KAHTANI and M. EL-SARRAGE (1993): Floral-type identification and quality evaluation of some honey types. *(Food Chem., 46: 13–17).*

ANDRADE, P.; M. AMARAL; P. ISABEL; J. CARVALHO; R. SEABRA and A. CUNHA (1999): Physicochemical attributes and pollen spectrum of Portuguese heather honeys. *(Food Chem., 66: 503–510).*

ANKLAM, E.A. (1998): Review of the analytical methods to determine the geographical and botanical origin of honey. *(Food Chem., 63: 549–562)..*

A.O.A.C. (2000): Official methods of analysis (17th ed.) Washington, DC., USA: Association of Official Analytical Chemists.

BADEI, A.Z. and M.B. SHAWER (1986): Effect of long storage on the chemical composition and flavor constituents of the Egyptian clover honey. *(J. Agric. Res. Tanta Univ., 12(1): 166-175).*

BADEI, A.Z. and M.B. SHAWER (1990): Retardation of honey granulation by heat treatment. *(Egyptian J. Food Sci., 18(1-3):221-231).*

BERRY, J.A. and K.S. DELAPLANE (2001): Effect of comb age on honey bee colony growth and brood survivorship. *(J. apic. Res. 40, 3–8).*

COGGSHALL, W.L. and R.A. MORSE (1984): Beeswax. Production, Harvesting. Processing and Products. *(Wicwas Press, Ithaca, New York, p. 192).*

CORONEL, B.B. and H. MONTI (2001): Characterization of *Eucalyptus* sp. Honey of Argentinean production. *(Proc. 37th Apic. Cong., 28 Oct. -1 Nov. 2001, Durban, South Africa).*

COSTA, L.; M. ALBUQUERQUE; L. TRUGO; L. QUINTEIRO; O. BARTH; M. RIBEIRO and C. DE MARIA (1999): Determination of non-volatile compounds of different botanical origin Brazilian honeys. *(Food Chem., 65: 347–352).*

DUNCAN, B.D. (1955): Multiple Range and Multiple F. Test. *(Biometrics, 11: 1- 42).*

EL-DAKHAKHNI, T. N. (1995): Effect of comb age on some economic characteristics of honeybee colony. 1- Brood rearing activity and honey production. *(J. Agric. Sci. Mansoura Univ., 20(6): 3107-3111).*

ESTI, M.; G. PANFILI; E. MARCONI and M. TRIVISOMO (1997): Valorization of the honeys from the Molise region through physico-chemical, organoleptic

and nutritional assessment. *(Food Chem., 58: 125–128).*

FREE, J.B. and I.H. WILLIAMS (1974): Factors determining food storage and brood rearing in honeybee (*Apis mellifera* L.) comb. *(J. Entomol. Series A, 49: 47–63).*

GARCIA-ALVAREZ, M.; J.F. HUIDOBRO; M. HERMIDA and J.L. RODRIQUEZO-TERO (2000): Major components of honey analysis by near-infrared transflectance spectroscopy. *(J. Agric. Food Chem., 48(11): 54–58).*

HEPBURN, H.R. (1998): Reciprocal interactions between honeybees and combs in the integration of some colony functions in *Apis mellifera* L. *(Apidologie, 29: 47–66).*

JAY, C.S. (1963): The development of honeybees in their cells. *(J. apic. Res., 2: 117–134).*

MEYAV, S. and M. BERK (1978): Carbohydrate analysis. *(J. Agric. Food Chem., 26 (1): 282).*

MOHAMED, A.D. (2006): The physico-chemical properties and composition of honeys resulted from different botanical origin. *(J. Agric. Sci. Mansoura Univ., 31(3): 1695-1704).*

MOLANDER, A.L. (1976): Discernment of primary taste substance and probable ability to Jude Food. *(Lowa State Univ. Press, Ames, Lowa).*

MUNRO, J.A. (1943): The viscosity and thixotrophy of honey. *(J. Econ. Entomol., 36(5): 769-777).*

NOUR, M.E. (1988): Some factors affecting quality of Egyptian honey. *(Unpublished Ph.D. Thesis Fac. Agric.Cairo Univ).*

NOUR, M.E. (1998): Physico-chemical of some Egyptian honey. *(J. Agric. Sci. Mansoura Univ., 23(4): 1749-1756).*

PÉREZ-ARQUILLUÉ, C.; P. CONCHELLO; A. ARIÑO; T. JUAN and A. HERRERA (1995): Physicochemical attributes and pollen spectrum of some unifloral Spanish honeys. *(Food Chem., 54: 167–172).*

POPEK, S. (2002): A procedure to identify a honey type. *(Food Chem., 79: 401–406).*

RATEB, S.H. (2005): Studies on pollen spectrum, chemical and physical characters of some types of honeys. *(Unpublished Ph.D. Thesis Fac. Agric. Assiut Univ., 335pp).*

SHEREF, A.F. (2007): Studies on Egyptian honey. *(Unpublished Ph.D. Thesis Fac. Agric. Kafr El-Sheikh Univ., 179pp.)*

SINGH, N. and P. KAUR (1997): Quality evaluation of different types of Indian honey. *(Food Chem., 58: 129–133).*

SPORNS, P.; L. PLHAK and J. FRIEDRICH (1992): Alberta honey composition. *(Food Res. Int., 25: 93–100).*

STEEL, R.G. and J.H. TORRIE (1980): Principles and procedures of statistics. *(2 nd ed. pp 120. Mc Graw-Hill, New York, USA.).*

SWALLOW, K. AND N. LOW (1990): Analysis & quantitation of the carbohydrates in honey using high-performance liquid chromatography. *(J. Agric. Food Chem., 38: 1828–1832).*

TULLOCH, A.P. (1980): Beeswax – composition and analysis. *(Bee World, 61: 47–2).*

VORWOHL, G. (1964): Die Beziehungen zwischen der elektrischen Leitfähigkeit der Honige und ihrer trachtmässigen Herkunft. *(Ann. Abeille, 7: 301–309).*

WEADMORE, E.B. (1955): The accurate determination of the water content of honeys. 1- Introduction and results. *(Bee world, 36(11): 197-202).*

WHITE, J.W. (1967): Measuring in honey quality a rational approach. *(Amer. Bee J., 107(10): 374-375).*

WHITE, J.W.; M.L. REITHOF; M.H. SUCERS and J. KUSHNIR (1962): Composition of American honeys. *(Tech. Bull. USDA, 1261).*

WINSTON, M.L. (1987): The biology of the honeybee. Harvard University Press, Cambridge, Massachusetts, *(London, England, p. 281).*