

Influence of Honey on the Suppression of Human Low Density Lipoprotein(LDL) Peroxidation (In –vitro)

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Summary:

The antioxidant activity of four honey samples from different floral sources (Acacia, Coriander, Sider and Palm) were evaluated with three different assays; DPPH free radical scavenging assay, superoxide anion generated in xanthine –xanthine oxidase (XOD) system and low density lipoprotein (LDL) peroxidation assay. The dark Palm and Sider honeys had the highest antioxidant activity in the DPPH assay. But all the honey samples exhibited more or less the same highly significant antioxidant activity within the concentration of 1mg honey / 1 ml in XOD system and LDL peroxidation assays.

The chemical composition of these samples was investigated by GC/MS and HPLC analysis, 11 compounds being new to honey. The GC/MS revealed the presence of 90 compounds, mainly aliphatic acids (37 compounds), which represent 54.73, 8.72, 22.87 and 64.10 % for Acacia, Coriander, Sider and Palm honeys respectively. In HPLC analysis, 19 flavonoids were identified. Coriander and Sider honeys were characterized by the presence of large amounts of flavonoids.

The data support the concept that honey is bioavailable and it can increase the antioxidant activity in-vitro. It can be speculated that as honey dramatically slowed the rate of LDL peroxidation, it might be able to protect human LDL from oxidative stress.

Introduction:

The oxidative modification hypothesis of atherosclerosis predicts that low-density lipoprotein (LDL) oxidation is an early event in atherosclerosis (Stocker, et al. 2004). Therefore, inhibition of LDL oxidation might be an important step in preventing atherogenesis (Kamiya, et al., 2004).

Humans protect themselves from reactive oxygen species, in part, by absorbing dietary antioxidants. During the past decade, the use of honey as a therapeutic substance has been reevaluated in a more scientific setting. Studies have shown that honey has both antibacterial (Hegazi et al.,----?) and anti-inflammatory properties (Postmes et al., 1993), useful in stimulation of burn and wound healing (Molan,1999) and in the treatment of gastric ulcer and gastritis (Ali et al.,1997).

The aim of this study was to evaluate honey from four different floral sources to find out the highly effective antioxidant to protect the human low density lipoprotein (LDL) against copper-induced oxidation in-vitro, in correlation with studying their chemical composition by GC/ MS and HPLC.

Materials and Methods

Reagents and Honey:

All reagents are of analytical purity grade. Distilled water was used for all dilution steps. Acacia, Coriander and Palm honeys were collected as market samples of Egyptian origin, while Sider honey was kindly provided by El-Yahia Company, Saudi Arabia (2004, flowering season).

Extraction of honey for GC/MS:

50 g. of each honey sample was extracted with diethyl ether according to Tan et al.(1990), and concentrated by evaporation under vacuum at 40°C. 5mg of the ether extract was dissolved in 0.05ml pyridine + 0.1 ml BSTFA (N,O-bis(trimethylsilyl)trifluoro-acetamide(BSTFA), from Sigma) and heated for 30 min. at 60 °C and injected in the GC/MS according to (Abd El Hady & Hegazi ,2002)

Flavonoid extraction for HPLC:

200 g. of each honey sample was passed through a column (25 x 2 cm) of Amberlite XAD-2 (Supelco; pore size 9 nm, particle size 0.3-1.2 mm). The phenolic fraction was dissolved in methanol and filtered through 0.45- μ m filter before direct HPLC analysis (Ferreeres et al, 1994).

Determination of DPPH free radical Scavenging activity:

The DPPH (1,1-diphenyle-2-picryl-hydrazyl) radical scavenging activity was determined according to the method of Hegazi and Abd El-Hady (2002). The absorbance was measured at 520 nm. Honey samples were dissolved in distilled water and 6 M DPPH was dissolved in Methanol. Mean of 3 measurements of each sample were calculated.

Determination of superoxide anion radical scavenging activity:

The superoxide anion radical scavenging activity by generating superoxide anion free radical in xanthine-xanthine oxidase system was measured following the method of Matsushige, et al. (1996). The color obtained was measured at 560 nm. Mean of 3 measurements of each sample were calculated.

Measurement of Copper-Induced Low Density Lipoprotein (LDL) oxidation in-Vitro: Isolation of LDL:

LDL was isolated according to the method of Gugliucci, and Menini (2002). LDL (1.019-1.055 g/ml) was separated by sequential ultra -centrifugation using TL-100 Ultracentrifuge(Beckman , U.S.A.) from plasma. LDL then extensively dialyzed against phosphate- buffered saline (PBS), pH 7.2, containing 0.01% EDTA at 4 °C. Samples were stored at 4°C in the dark and used within 24hr. Protein content was determined according to Lowry's method (1951).

Thiobarbituric Acid Reactive Substances (TBARS) assay:

LDL was oxidized using 5 μ M/ml CuSO₄ (Masaki, et al., 1989). Oxidation of LDL was monitored in the presence or absence of honey sample by measuring the thiobarbituric acid reactive substances (TBARS). The absorbance was measured at 534 nm using UV Spectrophotometer [UNICAM UV300], malondialdehyde-bis-(dimethylacetal) which yields malondialdehyde (MDA) by acid treatment, was used as a standard.

GC/MS analysis:

A finnigan MAT SSQ 7000 mass spectrometer was coupled with a Varian 3400 gas chromatograph. DB-5 column, 30m x 0.32 mm (internal diameter), was employed with helium as carrier gas and the temperature programmed from 40 to 260°C at 5°C / min. (3-min. initial hold, 10-min. final hold). The mass spectra were recorded in electron ionization (EI) mode at 70 eV, ion source temperature 150°C. The scan repetition rate was 0.5 s

Identification of compounds:

Peaks were identified by computer search of user-generated reference libraries, incorporating mass spectra. Peaks were examined by single-ion chromatographic reconstruction to confirm their homogeneity; mixed peaks were resolved by computer program aimed at resolving the mass spectral data of one compound from overlapping mass spectra of another.

HPLC analysis of honey flavonoids:

The HPLC analysis was achieved with Agilent 1100 series liquid chromatograph with UV detector and an auto-sampler. The column used was a Lichrochart RP-18 (Merck, Darmstadt, Germany; 25 x 0.4 cm, 5- μ m particle size). Elution was with water : formic acid (19:1 v:v; solvent A) and acetonitrile (solvent B), and the flow rate was 1ml/min. Gradient elution started with 20% B, reaches 25 % B at 25 min and 30% B at 35 min, and then the system became isocratic until 50 min, reaches 50 % B at 60 min and 70 % B at 67 min. The flavonoids were detected with UV detector and the chromatograms were recorded at 340 and 290 nm.

Flavonoid identification and quantification:

The different flavonoids were identified by chromatographic comparisons with authentic flavonoids, some of them are commercial and the most were kindly provided by Prof. Wollenweber, (Institut of Botanik Schittspahnstr. TU Darmstadt, Germany). The flavanones were detected at 290 nm and the flavones at 340 nm. Flavonoid identification was carried out by direct HPLC comparison of authentic flavonoids and was based on co-chromatography in 290 and 340 nm. Response factors for the authentic flavonoids and the concentration of flavonoids in each honey sample were calculated according to Ogan & Katz (1981) and Annual book of ASTM Standards (1983).

Results:

The antioxidant activity of four honey samples from different floral sources (acacia, coriander, sider and palm) were evaluated with three different assays, DPPH free radical scavenging assay, superoxide anion generated in xanthine –xanthine oxidase (XOD) system and low density lipoprotein (LDL) peroxidation assay. Their chemical

composition was investigated by gas chromatography-mass spectrometry (GC / MS) and high performance liquid chromatography (HPLC).

Assessment of the antioxidant activity of honey:

The DPPH free radical scavenging activity of honey:

The results of the DPPH free radical scavenging activity of honey samples were summarized in Table (1). The highly antioxidant activity in DPPH scavenging assay was about (64.7% in conc.10mg honey/ ml). The concentration (1mg honey/ ml) showed a lower activity ranged from (24.11% -14.11%), while the lowest activity (13.83%-9.00%) appeared with the honey concentration (0.1mg honey/ml). It is clear that Palm and Sider honeys had the highest antioxidant activity (64%) in DPPH scavenging assay while low antioxidant activity was observed in Acacia and Coriander honeys (30.5 & 23.9 % respectively) at the concentration (10mg honey/ ml).

Effect of honey samples on superoxide anion radical generated in the Xanthine–Xanthine Oxidase system:

The free radical scavenging activity on superoxide anion radical generated by an enzymatic method was evaluated. The results are shown in Table (2). All the honey samples used exhibited more or less the same high antioxidant activity within the concentration 1mg honey/1 ml, which ranged from (91.58% - 89.22 %). In contrast, the concentration (10mg honey/ ml) showed a lower activity than that of (1mg honey/ ml) which ranged from (87.2% - 79.80 %).

Antioxidant activity of honey samples on Copper-Induced human LDL peroxidation in-Vitro:

Pre-incubation of LDL with honey samples resulted in significant inhibition of TBARS accumulation. From the data shown in Table (3), It is clear that in the LDL peroxidation assays, all the honey types under this experiment exhibited more or less the same high antioxidant activity within the concentration of 1mg honey/1ml (0.095- 0.099, i.e., it has the same result of the control), while the concentrations (100mg and 10mg honey/ ml) showed lower activity. Thiobarbituric acid Reactive Substance (TBARS), as an index of lipid peroxidation, were undetectable in control LDL, slightly rising only after 3hrs of incubation. Incubation with the oxidant resulted in a marked elevation of TBARS. After 24hrs of incubation in the presence of the oxidant, TBARS level did not further increase significantly [data not shown].

Chemical composition of honey:

GC/MS analysis:

The investigation of the ether extract of the four honey samples by GC/MS revealed the presence of 90 compounds, 9 of which are new to honey. The main compounds are aliphatic acids (37 compounds), which represent 54.73, 8.72, 22.87 and 64.10 % for Acacia, Coriander, Sider and Palm honeys respectively (Table4). The presence of ten aliphatic dioic acids represents 5.91, 0.16, 1.17 and 37.66 % for acacia, coriander, sider and palm honeys respectively. Succinic acid as a dioic acid was only present in Palm honey with a very high concentration (28.72%), 3-hydroxy-sebacic acid was only present in Acacia honey. Decandioic acid was present with a large amount in Palm and Acacia honeys. Palm honey contained most of these dioic acids while Coriander honey contained a little number with very small amounts of these dioic acids. Methyl butandioic acid was the only dioic acid sheared in all honey samples. Octandioic and

nonandioic acids were present in Acacia honey in high amount (0.66 and 0.94 respectively), they were also present in Coriander and Sider honeys.

Ether extract contained some aromatic acids, esters (Table 4). Five anthraquinones were present in Acacia, Coriander and Palm honeys (1.53, 1.37, and 1.03% respectively). Sider honey did not contain any anthraquinones. These anthraquinones are new to honey (Table 4). Acacia honey was characterized by the presence of high percentage of 2-hydroxypropanoic acid, 5-hydroxy-n-valeric acid and 2-hexenoic acid, benzoic acid, cinnamic acids, 2,3-butanediol., 2,3-butanediol(isomer), 3-methyl-1,3-dihydroxybutane and 2,4-bis(dimethyl benzyl)-6-t- butyl phenol.

Coriander honey was the only sample showed the presence of 3,4-dimethoxybenzoic acid and 3,4-dimethoxybenzene acetic acid, monoethylsuccinate, ethyl palmitate, ethyloleate, ethylstearate, 12-hydroxy stearic acid methyl ester, palmitic acid decyl ester, docosanoic acid ethyl ester, oleic acid octyl ester and tetracosanoic acid ethyl ester, 2,3-dimethoxybenzaldehyde and vanillyl alcohol.

Sider honey was characterized by the presence of 2-oxo-3-hydroxypropanoic acid, 2,3,4,5-tetrahydroxypentanoic acid-1,4-lactone, p-hydroxy-dihydrocinnamic acid and 1,2-benzenediol-3,5-bis(1,1-dimethylethyl). 2-aminobenzoic acid, furyl acrylic acids were present for the first time in honey.

Palm honey was characterized by the presence of a very high significant amount of succinic acid (28.72%). Also it contained 3,4- dimethoxy-cinnamic acid , 2,5-dimethoxy-cinnamic acid , caffeic acid, 1-methyl pentanol and 4H-pyran-4-one-5-hydroxy-2-hydroxymethyl isomer. 3-hydroxypyridine and picolinic acid (pyridine carboxylic acid) were present for the first time in honey.

HPLC analysis:

The flavonoids present in four honey samples were studied by HPLC analysis. 23 flavonoids were detected in the four honey samples, from which 19 were completely identified. The difference in the flavonoid composition between the four honey samples is clear in Table (5). Coriander honey has the highest content of myricetin, eriodictyol, naringenin, 8-methoxy kaempferol, apigenin, kaempferol, quercetin and quercetin - 3,32-dimethylether, Liquiriteginin, luteolin and quercetin -7-methylether were present only in Coriander honey. Pinobankasin and formonontin were present only in Sider honey. Acacia and Palm honeys were characterized by the presence of lesser amounts of flavonoids. Liquiriteginin and formonontin were identified for first time in honey.

Discussion :

LDL peroxidation is considered to be essential in the pathogenesis of atherosclerosis (Stocker, et al. 2004). Although data concerning the mechanisms by which lipid peroxidation occurs in-vivo are scarce, several lines of evidence suggest that some endogenous and exogenous compounds with antioxidant activity could have some beneficial effects in the prevention of the disease (Fuhrman & Aviram, 2001). Many plant phenols and flavonoids may be important dietary antioxidants (Craig, 1999 and Giugliano, 2000).Honey, also rich in phenolic compounds (Tomás-Barberán, et al., 2001).

In this study, we set out to demonstrate the antioxidant properties of 4 different honeys (Acacia, Coriander, Sider and Palm honeys) employing three different assays. The DPPH radical scavenging assay, superoxide generated in Xanthine–Xanthine oxidase system and the LDL oxidation assay.

In the DPPH radical system, antioxidant directly reacts with DPPH radical. In Xanthine-Xanthine oxidase system, superoxide anion radical is enzymatically generated. The harmful effect of superoxide is reduced by superoxide dismutase enzyme (SOD) present in the animal body, honey also showed similar activities to that of the SOD enzyme. So in the present study, all honey types produced high antioxidant activity either in DPPH free radical (chemically) or in Xanthine-XOD (enzymatically) systems.

Transition metals are powerful initiators of lipid peroxidation. It was observed that several aldehyds are formed mainly the 4-hydroxy-2-nonenal and malondialdehyde (MDA) (Uchida, et al.,1994). The formation of MDA was monitored through measuring the TBARS. From the data shown in (Table 3), it was observed that all honey samples had highly significant antioxidant activity through inhibition of LDL oxidation when compared to the control oxidized LDL, a mechanism suggested that these honey samples also act by metal chelation. Frankel et. .al., (1998) found that honey from various floral sources exhibit a wide range of antioxidant activity and a linear correlation with honey color has been observed, our data are in agreement with theirs only in the DPPH radical scavenging assay. But in Superoxide anion generated in Xanthine –Xanthine Oxidase system and LDL peroxidation assays, all honey types exhibited more or less the same highly significant antioxidant activity within the concentration of 1mg honey / 1 ml. McKibben & Engeseth (2002) found that honey, as a source of antioxidants, has been proven to be effective against deteriorative oxidation reactions in food, such as lipid oxidation.

From all the above mentioned data, Tables 1-3, it could be concluded that the dark Palm and Sider honeys had the highest antioxidant activity in the DPPH assay. But in Superoxide anion generated in Xanthine –Xanthine Oxidase system and LDL peroxidation assays, all the honey samples exhibited more or less the same high antioxidant activity within the concentration of 1mg honey / 1 ml. So it was suggested that these honeys could play an important role in the inhibition of lipid peroxidation in biological systems through their antioxidant, metal chelating, and free radical scavenging ctivities.

The phenolic compounds present in honey can originate from flower nectar, propolis (and / or beeswax), and pollen (Gill et al, 1995). Flavonoid glycosides present in nectar are hydrolyzed to give the corresponding aglycons by glycosidases of bee salivary glands (Sabatier et al., 1992) and therefore only the aglycons are detected in honey, as shown in a study on citrus nectar and honey (Ferrerres et al., 1993). Honey is a natural product that can be a rich source of phenolic antioxidants. Honey is a supersaturated solution of sugars of which fructose (38%) and glucose (31%) are the main carbohydrates. A wide range of minor constituents is also present in honey, many of which are known to have antioxidant properties. These include phenolic acids and flavonoids (Tomás-Barberán, et al., 2001), certain enzymes (glucose oxidase, catalase) (White, 1975), ascorbic acid (White, 1975), carotenoid-like substances (Tan et al., 1989), organic acids (Cherchi et al., 1994), Mailard reaction products (White, 1975),

and amino acids ((White & Rudyj, 1978). Frankel,et al., (1998) found that honey had significant antioxidant activity .

To illustrate the differences in honey phenolics due to the geographical origin (propolis-derived phenolics) and the similarities between floral derived phenolics of monofloral honey samples, it is clear that the profiles are quite different. The GC/MS and HPLC analysis of Palm honey revealed the similarities with palm propolis in hydroxyacetic acid, 3-hydroxypropanoic acid, malic acid, palmitic acid, 4-hydroxybenzoic acid, 3,4-dimethoxy-cinnamic acid, Caffeic acid, glycerol and phosphoric acid (Hegazi & Abd El Hady, 2002) and quercetin, quercetin-3-methylether, , quercetin-3,3'-dimethylether and apigenin (Hegazi and Abd El Hady, 2005). Also Palm honey revealed the similarities with date palm in 4-OH benzoic, vanillic, caffeic, p-coumaric acids and quercetin, quercetin-3-methyl ether (Regnault-Roger et al, 1987 and Ziouti et al., 1994). Acacia honey revealed the similarities with acacia wood only in vanillic acid (Miyazawa et al., 1995). The hydroxycinnamates, caffeic, p-coumaric and ferulic acids were found in European Acacia honeys (Tomás-Barberán et al ,2001). In our investigation cinnamic and cis-p-coumaric acids were only identified in Acacia honey.

Conclusion:

From this study, the data support the concept that honey is bioavailable and it can increase the antioxidant activity in-vitro. It can be speculated that as honey dramatically slowed the rate of LDL peroxidation, it might be able to protect human LDL from oxidative stress. These results are very interesting to be studied in-vivo in the future.

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Table (1): The Free Radical Scavenging activity of honey samples against DPPH radical.

	Honey color	10mg		1mg		0.1mg	
		Absorbance	% activity	Absorbance	% activity	Absorbance	% activity
* DPPH		0.255 ± 0.0016	0.00 %	0.255 ± 0.0016	0.00 %	0.255 ± 0.0016	0.00 %
Acacia	Light brown	0.177 ± 0.003**	30.59 %	0.219 ± 0.003	14.11 %	0.221 ± 0.002	13.33 %
Coriander	Light brown	0.194 ± 0.005**	23.92 %	0.210 ± 0.002	17.65 %	0.224 ± 0.002	12.15 %
Sider	Dark brown	0.090 ± 0.003**	64.3 1%	0.212 ± 0.003	16.86 %	0.225 ± 0.003	11.76 %

Palm	Very dark brown	$0.091 \pm 0.002^{**}$	64.70 %	0.192 ± 0.002	24.70%	0.215 ± 0.003	15.68%
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*The DPPH free radical scavenging effect was measured by the absorbance of DPPH radical at 520 nm in a reaction containing the test sample and 60 μ M DPPH (Results are expressed as mean \pm S. D. , **P < 0.01).

Table (2): The Free Radical Scavenging activity of honey samples in Xanthine – XOD System

	10mg		1mg		0.1mg	
	Absorbance	% activity	Absorbance	% activity	Absorbance	% activity
Control	0.297 ± 0.002	0.00 %	0.297 ± 0.002	0.00 %	0.297 ± 0.002	0.00 %
Acacia	$0.038 \pm 0.002^{**}$	87.20%	$0.028 \pm 0.003^{**}$	90.57%	$0.029 \pm 0.003^{**}$	88.32%
Coriander	$0.047 \pm 0.001^{**}$	84.17%	$0.032 \pm 0.001^{**}$	89.22%	$0.076 \pm 0.004^{**}$	74.41%
Sider	$0.055 \pm 0.003^{**}$	81.48%	$0.025 \pm 0.004^{**}$	91.58%	$0.034 \pm 0.001^{**}$	88.55 %
Palm	$0.060 \pm 0.004^{**}$	79.80%	$0.028 \pm 0.002^{**}$	90.57%	$0.032 \pm 0.002^{**}$	89.22%

The free radical scavenging activity on superoxide anion radical generated in XOD system, measured at 560 nm (Results are expressed as mean \pm S. D. , **P < 0.01).

Table (3): Antioxidant activity of honey samples on Copper-Induced human LDL peroxidation in-Vitro

Honey sample	100 mg	10 mg	1mg	0.1mg
control	0.096 ± 0.006	0.096 ± 0.006	0.096 ± 0.006	0.096 ± 0.006
control oxidized	0.202 ± 0.002	0.202 ± 0.002	0.202 ± 0.002	0.202 ± 0.002
Acacia honey	$0.106 \pm 0.003^{**}$	$0.102 \pm 0.002^{**}$	$0.095 \pm 0.005^{**}$	$0.107 \pm 0.002^{**}$
Coriander honey	$0.108 \pm 0.001^{**}$	$0.101 \pm 0.001^{**}$	$0.099 \pm 0.003^{**}$	$0.105 \pm 0.004^{**}$
Sider honey	$0.108 \pm 0.002^{**}$	$0.101 \pm 0.001^{**}$	$0.097 \pm 0.002^{**}$	$0.107 \pm 0.002^{**}$
Palm honey	$0.119 \pm 0.004^{**}$	$0.099 \pm 0.003^{**}$	$0.095 \pm 0.001^{**}$	$0.108 \pm 0.002^{**}$

Oxidation of LDL was monitored in the presence or absence of honey sample by measuring the thiobarbituric acid reactive substances (TBARS) at 534 nm. (Results are expressed as mean \pm S. D., **P < 0.01).

Table 4. Chemical composition assessed by GC/MS of ether extracts of Honey Samples.

Compound	Acacia	Coriander	Sider	Palm
	% TIC ^a			
<i>Aliphatic Acids</i>				
Hydroxyacetic acid	--	0.03	0.06	0.17
2-Hydroxypropanoic acid	18.70	0.21	0.04	0.56
3-Hydroxypropanoic acid	0.40	0.07	0.01	0.13
2-Methyl-3-hydroxypropanoic acid	0.01			
2,3-Dihydroxypropanoic acid	0.32	0.04	2.04	0.82
2-Oxo-3-hydroxypropanoic acid	---	---	0.04	---
Lactic acid dimmer	---	---	---	0.05
2-Methyl-2-hydroxybutanoic acid	0.05	---	---	---
3-Methyl-3-hydroxybutanoic acid	0.04	---	---	---
5-Hydroxy-n-valeric acid	8.27	3.62	0.21	---
4-Oxo-pentanoic acid	0.11	---	---	---
Pentanoic acid-2-deoxy-3,5-dihydroxy	---	0.30	---	---
Pentanoic acid-5-deoxy-2,3-dihydroxy-©-lactone	---	0.05	---	---
Pentanoic acid-5-deoxy-2,3-dihydroxy-©-lactone (isomer)	---	0.30	---	---
2,3,4,5-Tetrahydroxypentanoic acid-1,4-lactone	---	---	2.07	---
2,3,4,5-Tetrahydroxypentanoic acid-1,4-lactone(isomer)	--	---	0.09	---
Succinic acid ***	---	---	---	<u>28.72</u>
Malic acid (hydroxyl-succinic acid)***	<u>0.30</u>			<u>1.16</u>
2-butenedioic acid (E) ***	<u>0.07</u>	---	---	<u>1.25</u>
Methyl butandioic acid***	<u>0.14</u>	<u>0.02</u>	<u>0.01</u>	<u>0.30</u>
2-Hexenoic acid	10.50	---	0.08	---
Pentanedioic acid ***	---	<u>0.01</u>	---	<u>0.37</u>
7-Methyl- pentanedioic acid ***	---	---	---	<u>0.12</u>
3-Hydroxy caproic acid	---	0.30	0.15	---
7-Hydroxy-octanoic acid	---	---	0.03	0.20
Octandioic acid ***	<u>0.66</u>	<u>0.04</u>	<u>0.13</u>	---
2,3,5-Trihydroxyxylonic acid-©-lactone	---	0.04	---	---
Nonandioic acid(azelic acid)***	<u>0.94</u>	<u>0.09</u>	<u>0.35</u>	---
Decandioic acid(sebacic acid) ***	<u>3.29</u>	---	<u>0.68</u>	<u>5.74</u>
3-Hydroxy-sebacic acid ***	<u>0.51</u>	---	---	---
Tetradecanoic acid	0.17	0.06	0.20	---
Pentadecanoic acid	---	----	0.19	---
Palmitic acid	4.75	0.9	3.39	1.50
Oleic acid	5.41	1.53	3.34	---
Stearic acid	---	0.79	1.04	---
Ecosanoic acid	0.09	0.18	---	0.14
Docosanoic acid	---	0.14	---	---
<i>Total Aliphatic Acids</i>	54.73	8.72	22.87	64.10

<i>Aromatic acids</i>				
Benzoic acid	<u>0.07</u>	-	-	-
2-Aminobenzoic acid ^b	-	-	<u>0.03</u>	-
3,4-Dimethoxybenzoic acid	---	<u>0.11</u>	---	---
4-Hydroxy benzoic acid	0.32	0.57	1.67	4.15
Vanillic acid	0.36	0.14	0.27	3.05
3,4-Dihydroxybenzoic acid	---	0.04	0.07	---
3,4-Dimethoxybenzene acetic acid	-	<u>0.02</u>	-	-
γ-Hydroxybenzene propanoic acid	0.05	-	-	0.30
2-Furancarboxylic acid	0.12	0.02	-	-
2-Furancarboxylic acid-5-hydroxymethyl	1.14	0.07	-	0.44
Furyl acrylic acid ^b	-	-	<u>0.03</u>	-
Cinnamic acid	0.09	-	-	-
p-Hydroxydihydro-cinnamic acid	-	-	<u>0.56</u>	-
3,4- Dimethoxy-cinnamic acid	-	-	-	<u>3.00</u>
2,5- Dimethoxy-cinnamic acid	-	-	-	<u>0.17</u>
Cis- p-Coumaric acid	0.15	0.05	0.07	0.21
Caffeic acid	-	-	-	<u>0.36</u>
<i>Esters</i>				
Monoethylsuccinate	-	0.05	-	-
Ethyl palmitate	-	0.18	-	-
Methyloleate	0.37	-	-	-
Ethyloleate	-	0.7	0.13	-
Ethylstearate	-	0.03	-	-
12-hydroxy stearic acid methyl ester	-	0.08	-	-
Palmitic acid decyl ester	-	0.22	-	-
Oleic acid octyl ester	-	0.38	-	-
Docosanoic acid ethyl ester	-	0.08	0.04	-
Tetracosanoic acid ethyl ester	-	0.15	-	-
<i>Diterpenes</i>				
Dehydroabietic acid	0.8	0.08	0.04	-
<i>Others</i>				
1-Methyl pentanol	-	-	-	0.06
2,3-Butane diol	<u>0.52</u>	-	-	-
2,3-Butane diol(isomer)	<u>0.8</u>	-	-	-
3-Methyl-1,3-dihydroxy butane	<u>0.01</u>	-	-	-
Glycerol	-	-	-	0.8
Phosphoric acid	-	0.05	0.02	0.04
3-Hydroxypyridine ^b	-	-	-	0.03
Picolinic acid(pyridine carboxylic acid) ^b	-	-	-	0.03
1,2- cyclohexane dicarboxylic acid	-	0.09	-	0.89

1,4-Dihydroxy benzene	-	0.07	-	1.06
2,3-Dimethoxy benzaldehyde	-	<u>0.02</u>	-	-
4-Hydroxy phenyl ethanol	-	0.02	-	0.11
Vanillyl alcohol	-	<u>0.04</u>	-	-
1,2-Benzenediol-3,5-bis(1,1-dimethylethyl)	-	-	<u>0.20</u>	-
2,4-bis(dimethyl benzyl)-6-t- butyl phenol	<u>0.52</u>	-	-	-
2(3H)-Furanne-dihydro-3,4-dihydroxy-(trans)	-	0.08	-	0.55
4H-pyran-4-one-5-hydroxy-2-hydroxymethyl	-	0.04	-	0.39
4H-pyran-4-one-5-hydroxy-2-hydroxymethyl (isomer)	-	-	-	1.11
Octadecanyl glycerol ether	0.06	1.4	-	-
Eicosanyl glycerol ether	0.14	1.6	-	-
Dihydroxy-methyl anthraquinones ^b	0.06	-	-	0.05
Dihydroxy-trimethyl anthraquinones ^b	0.26	0.3	-	-
Dihydroxy-trimethyl anthraquinones ^b	0.53	0.2	-	0.58
Dihydroxy-pentamethyl anthraquinones ^b	0.26	0.17	-	0.08
Dihydroxy-pentamethyl anthraquinones ^b	0.42	0.7	-	0.32

^a The ion current generated depends on the characteristics of the compound concerned and it is not a true quantitation.

^b For the first time in honey

*** : dioic acid

Table 5. Flavonoids detected in honey samples by HPLC technique
(µg/100g honey).

No	Name	Structure	Origin	Acacia	Coriander	Sider	Palm
1	Major unknown (Mu)	-----		-	(Mu)	-	-
2	Major unknown (Mu)	-----		-	(Mu)	-	--
3	Myricetin	3,5,7,3',4',5'-hexahydroxyflavone	Pollen-nectar	-	188.15	-	4.37
4	Liquiritigenin	7,4'-dihydroxyflavanone	Pollen-nectar	-	75.23	-	-
5	Eriodictyol	5,7,3',4'-tetrahydroxyflavanone	Pollen-nectar	-	170.06	80.23	21.15
6	Luteolin	5,7,3',4'-tetrahydroxyflavone	Pollen-nectar	-	22.22	-	-
7	Quercetin	3,5,7,3',4'-pentahydroxyflavone	Pollen-nectar	17.58	28.47	-	3.41
8	Naringenin	5,7,4'-trihydroxyflavanone	Pollen-nectar	-	154.78	90.08	8.01
9	Pinobankasin	3,5,7-trihydroxyflavanone	propolis	-	-	24.65	-
10	Quercetin-3-methylether	5,7,3',4'-tetrahydroxy-3-methoxyflavone	propolis	-	9.87	18.32	0.90
11	-----	5,7,4'-trihydroxyisoflavone		1.04	-	-	9.88
12	Hesperetin	5,7,3'-trihydroxy-4-methoxyflavanone	Pollen-nectar	-	21.22	159.33	-
13	8-Methoxykaempferol	3,5,7,4'-tetrahydroxy-8-methoxyflavone	Pollen-nectar	1.63	23.67	-	0.20
14	Apigenin	5,7,4'-trihydroxyflavone	Pollen-nectar	0.12	19.81	-	0.20
15	Major unknown (Mu)	-----	Pollen-nectar	-	-	-	Mu
16	Kaempferol	3,5,7,4'-tetrahydroxyflavone	Pollen-nectar	2.36	27.80	20.07	4.36
17	Luteolin-3'-methylether	5,7,4'-trihydroxy-3'-methoxyflavone	Pollen-nectar	-	34.29	43.27	-
18	Kaempferol-3-methylether	5,7,4'-trihydroxy-3-methoxyflavone	propolis	-	37.34	38.14	14.62
19	Quercetin-3,3'-dimethylether	5,7,4'-trihydroxy-3,3'-dimethoxyflavone	propolis	-	14.26	12.36	10.38
20	Formonontin	7-hydroxy-4'-methoxyisoflavone		-	-	12.43	-
21	Quercetin-7-methylether	3,5,3',4'-tetrahydroxy-7-methoxyflavone	propolis	-	3.08	-	-
22	Major unknown (Mu)	-----		Mu	-	-	-
23	-----	5,4'-dihydroxy-7-methoxyisoflavone		10.16	-	-	-