

Influence of Storage on Chemical Composition and Antimicrobial Activity of Coriander (*Coriandrum sativum* L.) Honey

*A. G. Hegazi , Faten K. Abd El-Hady, M. A. El-Ansari, Fayrouz A. Mahmoud and N. A. M. Saleh

National Research Center, Cairo, Egypt.

THE INFLUENCE of storage on the antimicrobial activity and chemical composition of coriander honey was investigated. *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* were used for antimicrobial assay of the fresh and stored coriander honey. It was clear that fresh or stored crude coriander honey showed a higher antibacterial activity if compared with n-hexane extract. It was obvious that fresh coriander honey and its n-hexane extract were effective against different examined pathogens. *Staphylococcus aureus* was the most affected bacteria by both fresh and stored coriander honey as well as their n-hexane extracts. The stored honey extracted with n-hexane showed the highest antifungal activity against *Candida albicans*. Comparative gas chromatography - mass spectrometry (GC/MS) study of the fresh and stored Coriander honey revealed that the storage produced a significant decrease in the amounts of mono-, sesqui- and diterpens, fatty acid octyl esters, antibacterial, antifungal activity and produced a significant increase in the amounts of fatty acid ethyl esters. 1-hydroxylinalool, benzenemethanol - 3,4-dimethoxy and oleic acid showed the highest significant concentration. Few fatty acids, fatty acid esters and alkanols, alkanals and alkanones are also present.

Honeys differ widely in aroma and taste, each region producing its own typical varieties. The composition of honey has been studied extensively, and most of these studies have been directed towards the non-volatile components. A comprehensive survey was published by Tan *et al.* (1988, 1989, 1990). In honey there are two sorts of antimicrobial agents. One of them is heat and light

* E. Mail: ahmedgaffer@frcu.eun.eg

sensitive (inhibine) and has its origin in the H₂O₂ produced by honey glucose oxidase (White, 1963). The other consists of thermo-stable substances, (Bogdanov, 1984; Tan *et al.*, 1988, 1989, 1990; Russel, 1990;) . Studies of extractable substances present in New Zealand honeys have revealed a range of compounds that appear characteristic to the floral source (Tan *et al.*, 1988, 1989, 1990) and with variable antimicrobial activities (Brady, 1997).The coriander honey is one of the unifloral honeys present in Egypt , this directed the authors to study the biological activity and chemical composition as well as the effect of storage on its constituents and antimicrobial activity of both fresh and stored honey.

Material and Methods

Honey sample

Coriander honey is a unifloral honey produced from the nectar of *Coriandrum sativum* L., collected in April 1998 and 1995. This honey was kindly collected by Prof. Dr. M. S. Nour, Professor of Apiculture, Faculty of Agriculture, Cairo University. He identified this honey by microscopic pollen analysis (Mellisspalynology) method. The coriander honey was stored at room temperature in a dark glass jars until used.

Bacterial and fungal strains

Staphylococcus aureus, *Escherichia coli* and *Candida albicans* were used . These strains were isolated and identified in the section of Microbiology and Immunology, Dept. of Parasitology and Animal Diseases, National Research Center , Egypt.

Extraction

Two equal weight samples of Coriander honey were extracted according to Graddon *et al.* (1979), where 50 g honey were thoroughly mixed with 25 ml n-hexane and then decanted. This was repeated with seven further 25 ml portions. Aliquots of solvent and the combined extract was concentrated by evaporating the solvent under vacuum at 25°C. This led to an extract with a strong overall honey-like aroma similar to that of the original sample .

GC/MS analysis

A finnigan MAT SSQ 7000 mass spectrometer 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 was 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 and 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.

Antibacterial assay

Two bacterial strains were used: *Staphylococcus aureus* and *Escherichia coli*. The bacterial suspension was prepared and adjusted by comparison against 0.5 Mc-Farland turbidity standard (5×10^7 organisms / ml) tubes. It was further diluted to obtain a final of 5×10^6 organisms / ml. *Staphylococcus aureus* was enriched on polymyxin agar (Finegold & Sweeney, 1961) as a selective media while *Escherichia coli* was enriched on MacConkey. Both bacteria were subcultured on nutrient broth for further bacterial propagation (Cruickshank *et al.*, 1979). The broth was inoculated by the 0.20 ul/10 ml broth either with *Staphylococcus aureus* or *Escherichia coli* . Then added 20 % honey or their extract. The tubes were incubated at 37°C for 24 hr. The growth of control bacterial strains as well as inhibition of the bacterial growth due to honey were measured by Spectrophotometric assay as a turbidity at 420 nm wavelength. The mean values of inhibition were calculated from triple reading in each test. The minimal inhibitory concentration (MIC) of honey and its n-hexane extracts were determined by ten-fold dilution method against bacterial strains in in-vitro (Hegazi *et al.*, 1996 a). Data were analyzed statistically using student "T" test according to senedcor (1961).

Antifungal assay

The antifungal activity of tested honey and its n-hexane extracts was carried out against *Candida albicans* as described in British Pharmacopoeia (1968). Sabouraud's glucose agar and broth inoculated by the spore suspension (0.20 ul/10 ml). Then added 20 % honey or its extract. The tubes were incubated at 28°C for 48hr. The growth as well as inhibition were measured by Spectrophotometric assay as a turbidity at 420 nm wave length .The mean value of inhibition were calculated from triple reading in each test. Data were analyzed statistically using student "T" test according to Senedcor (1961).

Results and Discussion

The results of antimicrobial activity of the fresh and stored coriander honey and their n-hexane extracts against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* are illustrated in (Table 1). It was clear that the crude coriander honey (concentration 20% v/v) showed a higher antimicrobial activity than its n-hexane extract. Furthermore, it was obvious that fresh coriander honey and its n-hexane extract were effective against examined pathogens. *Staphylococcus aureus* was the most affected bacteria by fresh or stored coriander honey as well as their n-hexane extracts. These results revealed that there is a variation in the antibacterial activity against all tested pathogens. The variation in the antibacterial activity is attributed to the chemical composition, pH value, osmotic pressure, inhibine concentration and enzymatic constituents of the honey. Also the storage has an effect on some volatile or enzymatic constituents of honey. Similar results were obtained by other authors (Molan, 1992; Meresta & Meresta, 1983; Jeddar *et al.*, 1985; Toth, 1986; Hegazi *et al.*, 1996 b and Hegazi, 1997). This discrepancy in the results between different pathogenic bacteria may be related to the hexane extract of coriander honey used, also they varied according to the plant origin and the condition under which they are produced (Mousa, 1997).

The minimal inhibitory concentration of examined honey and its n-hexane extracts against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* varied according to the type of the pathogenic microorganisms. The fresh honey or its n-hexane extract was lower in its values if compared with the stored honey or its extract (Table 2).

The antifungal activity depends on the aroma producing compounds of the coriander honey which will act as antifungal. The volatile fraction showed a marked inhibitory effect of fungal growth (Caccioni *et al.*, 1995) as well as non peroxide antimicrobial activity (Brady, 1997). It has been reported that terpene hydrocarbones and sesquiterpenes showed antimicrobial activity against *Asprgillus niger*, *Asprgillus ochraceus*, *Penicillium, Rubrum* and *P. spinulosum* (Kalodera *et al.*, 1994). Toth(1986) suggested that there is a connection between aromatic content of honey and beneficial effects of honey consumption in some cases of diseases of upper respiratory tract (caused by *Candida albicans*, *Klebsiella pneumoniae* and *E. coli*).

TABLE 1. Antibacterial and antifungal activity of Coriander honey and its different extracts measured by optical density .

Organisms	Normal growth	Honey		n-Hexane extract	
		Fresh	Stored	Fresh	Stored
<i>Staphylococcus aureus</i>	0.740± 0.0064	0.005± 0.0004	0.018± 0.0008	0.007± 0.0002	0.021± 0.0002
<i>Escherichia coli</i>	0.907± 0.0036	0.534± 0.0004	0.652± 0.0006	0.321± 0.0005	0.496± 0.0014
<i>Candida albicans</i>	0.970± 0.0013	0.407± 0.0021	0.601± 0.0012	0.407± 0.0071	0.301± 0.0064

TABLE 2. The minimal inhibitory concentration (MIC) of coriander honey and n-hexane extracts measured by inhibition zones (diameter) .

Tested materials	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
Tetracycline (50ug)	+++++	+++	0
Cefotaxime (50 ug)	++++	+++	0
Mecillinam (25 ug)	++++	0	0
Gentamicine (10 ug)	++++	++	0
Ketoconazole (50 ug)	0	0	++
Clotrimazole (50 ug)	0	0	++
Nystatin (100 ug)	0	0	+
Fresh Coriander honey 20%	+++++	++++	++++
Stored Coriander honey 20%	++++	+++	+++
n- hexane extract of Fresh Coriander honey 20%	+++	+++	+++
n- hexane extract of Stored Coriander honey 20%	++	++	++
MIC (ug/ml) Fresh Coriander honey 20%	10	22	16
MIC (ug/ml) stored Coriander honey 20%	15	27	20
MIC (ug/ml) n- hexane extract of Fresh Coriander honey 20%	21	40	40
MIC (ug/ml) stored Coriander honey 20%	25	45	34

The results of GC/MS analysis of fresh and stored n-hexane extracts of coriander honey are summarized in Table (3); which represents different groups of compounds.

Hydroxylinalool represented the dominant compound in coriander honey, even in the fresh or the stored sample. Linalool oxide-cis (furan) or (pyran) was found in both samples and in a significant amount in the fresh one, while linalool oxide-trans (furan) was found only in the fresh sample. Coriander honey was also characterized by the presence of many oxygenated monoterpenes like, terpineol isomers, hydroxycineole isomers, octadienol and octadiendiol isomers in high concentration (Table 3). 2-H-pyran-2-one-5,6-dihydro-6-pentyl-(R) was the dominant pyran derivative in the two samples. There were 26 sesquiterpenes and diterpenes tentatively identified from the similarity of most of their fragment ions with that of authentic spectra as well as their retention times. Most of these compounds were present as traces and mainly in the fresh sample.

The hydrocarbons typically consisted of alkanes and alkenes. The alkanes ranged from C8 - C36 (22 compounds), tricosane, pentacosane, noancosane and hexadecane were the dominant ones respectively. The alkenes ranged from C12:1 - C36:1 (20 compounds), hexatriacontene was present in a significant amount, while the other alkenes presented in minor amounts. The hydrocarbons presented in higher numbers and amounts in the fresh sample.

Hexanol was the only alcohol identified in the two samples, all the remaining alkanol appeared in the fresh sample .

It is clear that most of the alkanols, alkanals and alkanones appeared in the fresh sample.

The fatty acids ethyl esters were mainly present in the stored honey, ethyl oleate was dominant in the stored honey, while it was absent in the fresh sample.

Benzenemethanol-4-methoxy, benzenemethanol-3,4-dimethoxy, Benzene methanol-3,5-dimethoxy,benzenemethanol-3,4,5-trimethoxy,benzenepropanol - 4 - methoxy, benzaldehyde-4-methoxy and benzaldehyde-3,4-dimethoxy were characteristic for both samples. Benzenemethanol-3,4-dimethoxy was the dominant aromatic alcohol in coriander honey.

The GC/MS study of the n-hexane extract of the fresh and stored coriander honey revealed that these extracts are very complicated mixtures, containing various classes of compounds.

TABLE 3 . Compound identified by GC/MS analyses in Coriander Honey n-hexane extract .

Compound	TIC % *	
	Stored	Fresh
Monoterpenes, sesquiterpenes		
Cyclohexene,4-methyl-1-(1-methyl ethyl) ^b	-----	0.02
Linalool oxide I (pyran)	tr.	0.01
Linalool oxide-cis (furan)	0.02	0.07
Linalool oxide-trans (furan)	----	0.02
1,6-Octadien-3-ol-3,7-dimethyl,(+ / -)	-----	0.09
β -Terpineol (E)	0.01	0.17
β -Terpineol (Z)	0.02	0.26
4-Terpineol	0.02	0.10
2-H-Pyran-3-ol-6-ethenyl tetrahydro-2,2,6- trimethyl	0.02	0.05
2-H-Pyran-3-ol-6-ethenyl tetrahydro-2,2,6-trimethyl (isomer)	0.18	0.44
5-Hepten-3-one-2-(5-ethenyl tetrahydro-5-methyl-2-furanyl)-6-methyl ^a	0.02	-----
5-Hepten-3-one-2-(5-ethenyl tetrahydro-5-methyl- 2-furanyl)-6-methyl (isomer) ^a	0.02	-----
3-Cyclohexene-1-acetaldehyde, α -4-dimethyl	-----	0.02
cis-Hydroxy-1,4 -cineole ^c	-----	0.11
cis-Hydroxy-1,8 -cineole ^c	0.01	0.13
trans-Hydroxy-1,4 -cineole ^c	0.16	1.24
trans-Hydroxy-1,8 -cineole ^c	0.35	2.47
1,9-Octadecadien-1-methoxy ^b	0.46	0.77
2,7-Octadiene,1,6-diol,2,6-dimethyl (Z)	0.15	0.84
1-Hydroxylinalool	1.20	8.62
7-Oxabicyclo [4.1.0.]heptane,1-methyl-4-(2- methyl oxiranyl) ^a	-----	0.02
2,7-Octadiene,1,6-diol,2,6-dimethyl (E)	-----	0.07
2-H-Pyran-2-one,5,6-dihydro-6-pentyl-,(R)	0.16	1.94
4-Oxo- β -isodamascol	0.01	0.45
2-Cyclohexene1-one-4-hydroxy-3,5,6-trimethyl-4- (3-oxo-1-butenyl)	0.01	0.25
Thunbergol	-----	0.01
Ketons, Aldehyds and alcohols		
3-Hexanone	0.03	0.24
2-Hexanone	0.05	0.37
3-Pentanol-2-methyl	0.02	-----
2-Hexanol	0.03	0.22
1-Propanol-2-ethoxy	-----	0.16
2-Pentanone,4-hydroxy-4-methyl	-----	0.15
Pentanal,2,2-dimethyl ^a	0.01	-----
Ethanone,1-(3-ethyl-oxiranyl) ^a	0.14	-----
Ethanone,1-(3-ethyl-oxiranyl) isomer ^a	0.13	-----
3-Hexene-2,5-diol ^a	0.01	-----

TABLE 3. Cont .

1-methyl-cycloheptanol ^a	----	0.05
6-Hepten-2-ol-4-methylene ^a	-----	0.14
5-Hepten-2-one-6-methyl	-----	0.06
2-Isoronenal	-----	0.01
Nonanal	-----	0.06
Tetradecanal	-----	0.14
Hexadecanal	-----	-----
Hexadecanol	-----	0.09
Heptadecanol	-----	0.27
Cyclobutanone,2-(2,6-dimethyl pentadecyl) ^b	0.01	0.01
Cyclobutanone,2-(2,6-dimethyl hexadecyl) ^b	-----	0.06
Hexacosanol	-----	0.06
Cyclobutanone,2-(2,6-dimethyl heptadecyl) ^b	-----	0.03
Heptacosenol	-----	0.21
Fatty acids		
Hexadecanoic acid	0.13	1.87
Oleic acid	0.19	6.25
Octadecanoic acid	-----	0.52
Methyl ester		
2,4-Dimethyl tetradecanoic acid methyl ester	0.01	-----
Hexadecanoic acid methyl ester	-----	0.03
Ethyl ester		
Tetradecanoic acid ethyl ester	0.13	0.13
Hexadecanoic acid ethyl ester	0.31	0.32
Linoleic acid ethyl ester	0.04	-----
Ethyl oleate	1.32	-----
Heptadecanoic acid -15-methyl ethyl ester	0.04	-----
Eicosanoic acid ethyl ester	0.01	0.01
Lignoceric acid ethyl ester	0.03	-----
Octyl ester		
Tetradecanoic acid octyl ester	0.03	1.52
Hexadecanoic acid octyl ester	0.08	2.28
Octadecenoic acid octyl ester	0.04	0.90
Eicosanoic acid octyl ester	-----	0.21
Docosanoic acid octyl ester	-----	0.27
Aromatic and nitrogen compounds		
Pyrazine,2,6-dimethyl	0.02	0.02
Trimethylpyrazine	0.05	0.50
[1,1'-Biphenyl]-3-amine	0.01	-----
Phenol-3-methyl	0.01	0.04
Phenol-2,6-dimethoxy-4-(2-propenyl)	0.03	-----
Phenol-4-(1,1,3,3,tetramethylbutyl)	-----	0.06
Phenyl ethyl alcohol	0.03	0.04
Benzaldehyde-4-methoxy	0.07	0.02
Benzaldehyde-3,4-dimethoxy	0.37	0.85
Bezenemethanol-4-methoxy	0.12	0.32

TABLE 3. Cont .

Benzenemethanol,3,4-dimethoxy	0.91	7.40
Benzenemethanol,3,5-dimethoxy	0.01	0.12
Benzenemethanol,3,4,5-trimethoxy	0.25	1.38
Benzenepropanol-4-methoxy	0.07	0.04
3,4,5-Trimethoxy-benzylmethyl ether	0.30	1.90
Vanilline	0.02	0.01
Butylated hydroxy toluene	0.05	0.03
Bis(2-ethylhexyl) phthalate	4.41	-----
Cyclohexane carboxylic acid,1-phenylmethyl ester	-----	0.37
1,2-Benzene dicarboxylic acid di-isooctyl ester	0.01	1.26

^a Compound was identified from the similarity of its mass spectrum with the library, we do not aware of its retention time .

^b These compound were identified by similarity of most of their spectra fragment ions with that of authentic spectra and also from the retention times.

^c Tentatively identified from mass spectra and retention times (new compounds). tr : traces < 0.01.

The monoterpenes, sesquiterpenes and diterpenes represented a high concentration (20.33%) in the extracted fresh honey, while it was very low in the stored extracted one (2.93%).

In this study 1-hydroxylinalool was the main component in coriander honey and was reported for the first time in honey. Linalool oxide was found in cis - (furan) and (pyran) and trans (furan) only. Linalool oxide was reported before in Australian and Piedmont (Italy) honeys (Graddon *et al.*,1979; Bicchi *et al.*,1983). In coriander fruit carbon dioxide extract, oxygenated monoterpenes were found to comprise 80% of it, linalool was the main compound, this is beside the presence of cis-linalool oxide (Kerrola & Kallio,1993). 3,7 - octadiene-2,6-diol-2,6-dimethyl was identified in leather wood honey (Rowland *et al.*,1995) while in the present study 2,7-octadiene-1,6-diol-2,6-dimethyl (Z) and (E) isomer were detected for the first time in honey. Terpinen - 4-ol and α -terpineol were detected in coriander fruit (Kerrola & Kallio, 1993) and trans- α -dihydroterpineol in Sidr honey (Abd El-Hady *et al.* 1998). In this study β -terpineol (E), β - terpineol(Z) and 4-terpineol were identified. 1,8-cineole was reported in Piedmont honeys (Bicchi *et al.*, 1983) and in coriander seeds (Boselah, 1995), while in this work hydroxycineole isomers were tentatively identified. Some furan derivatives were identified in different Belgium unifloral honeys (Bouseta *et al.*, 1992) and Russian coriander, lime and sunflower honeys.

Some furan and pyran derivatives were identified in the present study. None of the above mentioned terpenes were reported in the examination of Russian coriander honey (Artem'ev and Chepurnoi, 1984).

In Russian coriander, lime and sunflower honeys 3-hexen-1-ol, undecanol, n-octanol were identified (Artem'ev and Chepurnoi, 1984), while hexanal, heptanal and diketones identified in lavender and eucalyptus honeys (Bouseta *et al.*, 1992). Alkenals in the C9-C16 range, C7-C17 alkanals, C10-C12 primary alkenols alkanols and nonane were detected in coriander leaf (Potter & Fagerson, 1990). In the present study some different alkanol, alkenol, alkanal, alkanone and alkenone were identified.

In contrast to Sidr honey, white clover, manuka & kanuka honeys and thyme & willow honeys, where free fatty acids represented the majority of the n-hexane or ether extracts (Abd El-Hady *et al.* 1998 ; Tan *et al.*, 1988, 1989, 1990), only 3 free fatty acids were identified in the present study for coriander honey.

It was noticed that the fatty acid ethyl esters had significant amounts and numbers of esters increased in the stored coriander honey if compared to the fresh sample. This could be explained by the increased rate of acid catalyzed esterification of these acids with ethanol, which had probably arisen from the fermentation of glucose and fructose by yeast cells in the stored honey sample (Tan *et al.*, 1988). This could be confirmed in the present work , where oleic acid had significant amounts in the fresh sample than that of the stored one (6.25% , 0.19%), while ethyl oleate showed a significant amount in the stored sample (1.32%) and it was absent in the fresh sample.

Cinnamaldehyde and cinnamyl alcohol were identified in Russian coriander, lime and sunflower honeys (Artem'ev and Chepurnoi, 1984) 2- methoxyacetophenone, benzaldehyde, benzyl alcohol, methoxybenzadehyde, trimethyl-phenol and phenylacetaldehyde were identified in some unifloral honeys (Tan *et al.*, 1988,1989,1990). o-Methoxybenzyl alcohol, p-methoxybenzyl alcohol and α -hydroxyacetophenone were identified in unifloral Australian honeys (Graddon *et al.*, 1979). The present study of coriander honey revealed the presence of another benzenemethanol and benzaldehyde derivatives.

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تأثير التخزين على التركيب الكيميائي والكفاءة المضادة للميكروبات لعسل الكسبرة

أحمد جعفر حجازي - فاتن كمال عبد الهادي - محمد أمين
الانصاري - فيروز عبد الله محمود - نبيل عبد الحميد صالح

المركز القومي للبحوث - القاهرة - مصر .

درس تأثير التخزين على التركيب الكيميائي والكفاءة المضادة للميكروبات لعسل الكسبرة حيث استخدم الميكروب العنقودي الذهبي والميكروب القولوني وخميرة الكنديدا ألبيكانز لقياس الكفاءة المضادة للميكروبات لعسل الكسبرة المخزن وغير المخزن . لقد ظهر واضحا أن عسل الكسبرة المخزن وغير المخزن له كفاءة مضادة للميكروبات عالية إذا ما قورن بمستخلص العسل بالهكسان . كما أن الميكروب العنقودي الذهبي كان أكثر الميكروبات تأثرا بعسل الكسبرة المخزن وغير المخزن وخلص الهكسان لكلا منها . كما أن مستخلص الهكسان للعسل المخزن له أعلى تأثير مضاد لنمو الكنديدا البيكانز .

أسفرت الدراسة المقارنة لكوروماتوجرافيا الغاز وطيف الكتلة للعسل المخزن وغير المخزن عن تناقص في كمية التربينات الأحادية والسيكوتربينات وأسترات الأحماض الدهنية وأيضا الكفاءة المضادة للميكروبات والفطريات في العسل المخزن ؛ كما أسفرت النتائج عن زيادة جوهريّة في كمية أسترات الأثيلية للأحماض الدهنية ١- الهيدروكسيلينانول والبنزينميثانول ٣- و ٤- دميثوكسي وحمض الأوليك . كما وجد قليل من الأحماض الدهنية وأستراتها والكانول والألكانال والألكانونات في العسل المخزن .