Ass. Univ. Bull. Environ. Res. Vol. 7 No. 1, March 2004

AUCES

ISOLATION AND CHARACTERIZATION OF FUNGI CONTAMINATING PACKAGED HONEY COMMONLY CONSUMED IN SAUDI ARABIA

Laila A. Nasser

Girl's College of Education, Riyadh, Saudi Arabia

`

ABSTRACT :

Forty-five packaged honey samples, gathered from different retail markets in Saudi Arabia, were mycologically studied. The direct baiting-technique on 10% sucrose-Czapek's agar at 28°C was employed. Of the 45 samples tested, 40 (88.9%) were contaminated with fungi. A total of 358 mould colonies/ 360 pieces representing 14 species related to 9 genera were isolated and identified. So it could be concluded that microbial contamination level in honey is generally low. The most prevalent moulds isolated were *Aspergillus flavus, A. niger, A. fumigatus, A. versicolor*. Also, other saprophytic species were isolated in rare occurrence. Some 30 colonies/ 360 pieces of unidentified species of yeasts were also isolated but in low frequently of occurrence.

INTRODUCTION:

Honey is an interesting food that can be used as an ingredient or as a final product (Snowdon and Cliver 1996). It is a highly-energy natural carbohydrate produced when the nectar and sweet deposits from plants, gathered, modified and stored in the honey comb by honey bees (White and Rudyj 1978, White 1980, 1992). Honey is mainly composed of sugars, particularly the monosaccharides fructose and glucose, though it contains a large variety of di- and trisaccharides. Enzymes that bees produce turn di- and trisaccharides into monosaccharides (White 1983, Martins *et al.* 2003).

The antimicrobial activity of honey is important factor that inhibits the development of many saprophytic fungi in stored food and that could destroy some pathogenic microorganisms (Burgett 1978, Fleche *et al.* 1997, Vardi *et al.* 1998). Also, honey as a hypersomatic medium may kill many living cells except those of osmophilic fungi and bacteria (Glinski and Buczek 2003). Honey has been described in ancient and modern medicine as being effective in the healing of various infected wounds. Also, it is useful in the treatment of post-surgical wounds that are infected and do not respond to conventional systemic and local antibiotic treatment (Vardi *et al.* 1998). On the other hand, honey may undergo various changes during storage and one of the most significant of these changes is the spontaneous fermentation induced by yeasts, moulds and bacteria (Jimenez *et al.* 1994). These microorganisms may be involved in spoilage of provisions. So

that microbiological characteristics of honey are inherent to quality and safety (Goerzen 1991).

Consumption of honey has remarkably increased in the last years all over the world. However, the safety of these products is not regularly assessed. The aim of the present study is to give a preliminary evaluation of microbial (moulds and yeasts) contaminating packaged honey commonly consumed in Saudi Arabia.

MATERIALS AND METHODS:

Forty-five packaged honey samples (honey bees products) were randomly collected from retail markets in the city of Riyadh, Saudi Arabia (Table 1). All packaged samples were transferred to the Mycological Laboratory and stored at room temperature till fungal analysis.

Mycological examination:

For enumeration and identification of moulds and yeasts in honey samples, 8 pieces (about 0.5 gm each) of each sample were spread over the surface of two plates of 10% sucrose-Czapek's agar (g/L: sucrose, 100; $NaNO_3$, 3; KH_2PO_4, 1; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; agar, 15; King *et al.* 1984). Rose-bengal (0.003%) and chloramphenicol (0.025%) were added as bacteriostatic agents (Smith and Dawson 1944). The plates, were incubated at 28°C for 7 days. The developing fungi were counted and calculated per 8 pieces for each sample. Each isolated mould colony was examined microscopically for morphological characterization and identification according to the keys of Booth 1971, Ellis 1971, Raper & Fennell 1977, Domsch *et al*. 1980, Pitt 1979, 1985, Samson and Pitt 1989, Moubasher 1993, Samson *et al*. 1995.

Table (1): Trade name and origin of honey samples investigated.

No	Trade Name	Origin	No	Trade Name	Origin
1	Sedre honey hadramy	Yemen	24	Jasty honey	U.S.A.
2	Sedre honey hadramy (shabibe)	Yemen	25	Shefa honey and black forey	Saudi Arabia
3	Hadramy flower honey	Yemen	26	Golden shefa honey	Saudi Arabia
4	Hadramy talh honey	Yemen	27	Russian honey	Russia
5	Taef (lavander)	Saudi Arabia	28	Turkish spring flower honey	Turkey
6	Taef (summer flower) honey	Saudi Arabia	29	Turkish mountain honey	Turkey
7	Taef sedre honey	Saudi Arabia	30	Turkish saater honey	Turkey
8	Taef spiny sedre honey	Saudi Arabia	31	Dragon Lwezian honey	U.S.A.
9	Hadramy sieved honey	Yemen	32	Biophar black forest honey	Germany
10	Abha honey	Saudi Arabia	33	Biophar *Acassia* honey	Germany
11	Egyptian Honey	Egypt	34	Biophar needel honey	Germany
12	Hadramy red sedre honey	Yemen	35	Sary honey	Australia
13	Hadramy white sedre honey	Yemen	36	Sary honey	Australia
14	Nagran honey	Saudi Arabia	37	Forest honey	Germany
15	Egyptian honey	Egypt	38	Black forest honey	Germany
16	Halawany brothers (white) honey	Saudi Arabia	39	Natural honey	Germany
17	Halawyny brothers (red)	Saudi Arabia	40	*Acassia* honey	Germany
18	Honey raw wild (sue bee)	U.S.A.	41	Miel carlota honey	Mexico
19	*Acassia* honey (El-Shifa-Jeddah)	Saudi Arabia	42	El-Taieb natural honey	Egypt
20	Honey pasmah honey	Saudi Arabia	43	Isis-honey bees wax	Egypt
21	Natural honey	Argentina	44	Rania-pure honey	Egypt
22	Honey (sue bee)	U.S.A.	45	Rania-pure honey	Egypt

23	Neeta flower (mountain honey)	Swissland			

RESULTS AND DISCUSSION:

The mycological analysis revealed that 40 honey samples (88.9%) out of 45 investigated were contaminated with fungi. Of these samples, 41 were polluted with moulds, and 9 samples showed both moulds and yeasts. Representative of the filamentous fungi corresponding to a total of 358 colonies/360 pieces, assigned to 14 species of 9 genera were identified. Samples No. 1, 5, 8, 12, 13, 15, 18, 23, 32, 34 and 42 were relatively highly contamin-ated with fungi containing 12-16 colonies/8 pieces. On the other hand, samples No. 16, 19, 26, 30 and 31 were free from fungi (Table 2). From the present study, it could be concluded that microbial contamination level in honey is generally low (Tables 2, 3). In this respect, these results were greatly identical to those obtained by Martins *et al*. (2003) who made an extensive survey of fungi contaminating honey, and reported that from the 80 honey samples analyzed, 71 (88.8%) were contaminated with fungi. Fleche *et al*. (1997) reported that honey contains very little contamination, due to both the ability of colonies to eliminate pathogenic and non-pathogenic micro-organisms present in their environment and to the physico-chemical properties of these products, as well as the role of bees in filtering chemical pollutants. Also, Hilldrup *et al*. (1977) studied fungal growth on aspiarian substrates (unprocessed honey, pollen, brod comb, whole larvae and whole bees) and varified that fungi grew and sporulated in all substrates except the unprocessed honey.

The genus of the highest incidence and its respective numbers of species was *Aspergillus*. It was represented in all positive samples contributing 91.6% of total moulds. From the genus, 5 species were identified of which *A. flavus* and *A. niger* were the most prevalent species. They occurred in 91.6% and 77.8% of the samples comprising 60.4% and 32.6% of total *Aspergillus* and 55.3% and 29.9% of total moulds, respectively. *A. candidus* (1.8% of total *Aspergillus*), *A. fumigatus* (3.7%) and *A. versicolor* (1.5%) were also identified from the examined samples in low frequency of occurrence (Table 3). These results were nearly similar to those obtained by Martins *et al*. (2003). They noticed that species of *Aspergillus* were the most prevalent fungi in honey samples tested with the most predominant species being *A. flavus* (57.5%), followed by *A. niger* (51.3%), *A. fumigatus* (45.0%) and *A. candidus* (28.7%). Also, Jimenez *et al*. (1994), studying raw honey, refferred that the dominant *Aspergillus* was *A. flavus, A. niger, A. candidus* and *A. terreus*. Wellford *et al*. (1978) inoculated unprocessed honey with strains of *A. flavus* and *A. parasiticus* and the fungal growth was observed. The previous *Aspergillus* species and others were also, isolated from honey or honey products as reported by Gilliam and Prest (1972), Gilliam *et al*. (1974), Hilldrup *et al*. (1977), Wellford *et al*. (1978), Jimenez *et al*. (1994), Costa & Oliveira (1998) and several others.

***Acremonium strictum, Botryotrichum atrogriseum, Cladosporium cladosporioides, Emericella nidulans, Fusarium oxysporum, Humicola grisea, Penicillium corylophilum, P. funiculosum* and *Trichoderma hamatum* were isolated in rare frequency of occurrence, emerging collectively about 8.4% of the total moulds (Table 3). These species were also, isolated from different insects (including honey bees), bees comb, honey products, pollen grains or soil that is used by insects for population (Gilliam and Prest 1972, Gilliam *et al*. 1974, 1983, Kaaya and Okech 1990, Ismail and Abdel Sater 1993, Sarquis and Oliveira 1996, Snowdon**

and Cliver 1996, Costa and Oliveira 1998, 2001, 2003).

Madeira 1998, Sales *et al.* 2002, Martins *et al.*

Table (2): Total counts (calculated per 8 pieces for each sample), number of genera and species isolated from 45 honey samples on 10% sucrose-Czapek's agar at 28°C.

Sample No.	Total counts	No. of genera	No. of species	Sample No.	Total counts	No. of genera	No. of species
1	12	2	6	24	8	1	3
2	11	2	3	25	7	2	2
3	11	1	2	26	-ve	-ve	-ve
4	6	1	2	27	9	2	3
5	14	2	3	28	9	2	4
6	6	1	2	29	12	2	3
7	10	1	3	30	-ve	-ve	-ve
8	15	1	3	31	-ve	-ve	-ve
9	6	1	1	32	13	2	4
10	11	1	3	33	9	4	5
11	6	1	2	34	14	1	2
12	12	2	4	35	9	2	3
13	16	2	3	36	8	1	4
14	11	1	3	37	9	1	2
15	16	3	5	38	7	2	2
16	-ve	-ve	-ve	39	10	3	3
17	4	2	2	40	4	1	2
18	12	1	3	41	10	1	2
19	-ve	-ve	-ve	42	13	1	2
20	9	1	3	43	11	1	2
21	7	2	4	44	11	1	3
22	3	1	2	45	8	1	2
23	9	4	5				

Table (3):Total counts (TC, calculated per 360 pieces in all samples), number of cases of isolation (NCI, out of 45 samples) and occurrence remarks (OR) of fungal genera and species recovered from honey on 10% sucrose-Czapek's agar at 28°C.

Genera & species	TC	NCI & OR
Acremonium strictum	**1**	**1 R**
Aspergillus	**328**	**40 H**
A. candidus	**6**	**4 L**
A. flavus	**198**	**40 H**
A. fumigatus	**12**	**8 L**
A. niger	**107**	**35 H**
A. versicolor	**5**	**4 L**
Botryotrichum atrogriseum	**6**	**2 R**
Cladosporium cladosporioides	**5**	**3 R**
Emericella nidulans	**2**	**2 R**
Fusarium oxysporum	**3**	**2 R**
Humicola grisea	**7**	**2 R**
Penicillium	**3**	**3 R**
P. corylophilum	**2**	**2 R**
P. funiculosum	**1**	**1 R**
Trichoderma hamatum	**3**	**1 R**
Yeasts	**30**	**9 L**
Total counts	**358**	
Number of genera = 9		
Number of species = 14		

Occurrence remarks (OR): H= high occurrence, 21-45. M= moderate occurrence, 10-20.

L= low occurrence, 4-9. **R= rare occurrence, 1-3 samples.**

A total of 30 yeast colonies/360 pieces of honey samples were recovered. They occurred in 20% of the samples constituting 7.7% of total fungi isolated in the present study. In this respect, Martins *et al*. (2003), reported that the yeast species identified (*Candida humicola* and *Saccharomyces* sp.) were detected in a very high frequency and at high levels of contamination. These osmophilic yeasts are probably good indicators for microbiological quality of honey. Also, numerous yeasts were isolated from foods, food products, or soft drinks as indicated by Sand *et al*. (1976), Van Easch (1992), Abdel-Sater and Saber (1999), Abdel- Sater *et al.* (2001) and several others.

REFERENCES:

1-Abdel- Sater M. A. and S. M. Saber (1999): Mycoflora and mycotoxins of some Egyptian dried fruits. Bull. Fac. Sci., Assiut Univ. 28: 91-107.

2-Abdel –Sater M. A., Zohri A. A. and Ismail M. A. (2001): Natural contamination of some Egyptian fruits and beverages by mycoflora and mycotoxins. J. Food Sci. Technol. 38 (4): 407- 411.

3-Booth C. (1971): The genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, UK.

4-Burgett D.M. (1978): Antibiotic systems in honey, nectar and pollen. In Morse R.A. (ed.). Honey Bee Pests, Predators, and Diseases. Comstock Publ. Ass. Ithaca and London, pp. 297-308.

5-Costa G.L. and Oliveira P.C. (1998): *Penicillium* species in mosquitoes from two Brazilian regions. J. Basic. Microbial. 38: 343-347.

6-Domsch K.H., Gams W. and Anderson T.H. (1980): Compendium of Soil Fungi. Academic Press, New York, USA, pp. 1156.

7-Ellis M.B. (1971): Dematiaceous Hyphomycetes. 2nd ed. Commonwealth Mycological Institute, Kew, Surrey, England.

8-Fleche C., Clement M.C., Zeggane S. and Faucon J.P. (1997): Contamination of bee products and risk for human health. Rev. Sci. Tech. 16: 609-619.

9-Gilliam M. and Prest D.B. (1972): Fungi isolated from the intestinal contents of foraging worker honey bees, *Apis mellifera*. J. Invert. Pathol. 20: 101-103.

10-Gilliam M., Prest D.B. and Morton H.L. (1974): Fungi isolated from honey bees *Apis mellifera*, Fed 2,4D and antibiotic. J. Invert. Pathol. 24: 213-217.

11-Gilliam M., Motisn J.O. and Kauffield N.M. (1983): Examination of floral nectar of citrus, cotton and Arizona desert plants for microbes. Apidologie 14: 299-302.

12-Glinski Z. and Buczek K. (2003): Response of the Apoidea to fungal infections. Apiacta 38: 183-189.

13-Goerzen D.W. (1991): Microflora associated with the alfalfa leaf cuting bee, *Egachile rotundata* (Fab) (Hymenoptera:Megachilidae) in Saskatchewan, Canada. Apidologie 22: 553-561.

14-Hilldrup J.A., Eadie T. and Liewellyn G.C. (1977): Fungal growth and aflatoxin production on apiarian substrates. J.Ass. Off. Anal. Chem. 60: 96-99.

15-Ismail M. A. and Abdel- Sater M. A. (1993): Fungi associated with the Egyptian leafworm *Sepodoptera littoralis* Boisdoval. Mycopathologia 124: 79- 86.

16-Jimenez M., Mateo J.J., Huerta T. and Mateo R. (1994): Influence of the storage conditions on some physicochemical and

mycological parameters of honey. J. Sci. Food Agric. 64: 67-74.

17-Kaaya G.P. and Okech M.A. (1990): Microorganisms associated with teste in nature: Preliminary results on isolation, identification and pathogenicity. Insect Sci. Applic. 11: 443-448.

18-King A.D.Jr., Pitt J. I., Beuachat L.R. and Coory J.E.I. (1984): Methods for the Mycological Examination of Food. London, UK.

19-Madeira N.G. (1998): Persistence of conidia of *Entomophthora muscae* in relation to age, temperature and humidity. Biol. Control 43: 87-95.

20-Martins H.M., Martins M. L., Lanca A. and Bernardo F. (2001): Microbial safety assessment of honey bees (*Apis mellifera*) p. 146. In Proceeding Book, Micro 2001, Congresso Nacional de Microbiologia. Povoa de Varzim, Portugal.

21-Martins H.M., Martins M.L. and Bernardo F.M.A. (2003): Bacillaceae spores, fungi and aflatoxins determination in honey. Revista Portuguesa de Ciencias Veterinarias 98 (546): 85-88.

22-Moubasher A.H. (1993): Soil Fungi in Qatar and other Arab Countries. The Scientific and Applied Research Centre. University of Qatar, Doha, Qatar (pp. 566).

23-Pitt J.I. (1979): The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press, London.

24-Pitt J.I. (1985): A laboratory guide to common *Penicillium* species. Academic Press, Australia, 182 pp

25-Raper K.B. and Fennell D.I. (1977): The genus *Aspergillus*. R.E. Krieger Publishing Company, Huntington, NY, USA.

26-Sales M. S.N., Costa G. L., Elias V. R. and Bittencourt P. (2002): Isolation of fungi in *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae) captured at two natural breeding grounds in the Municipality of Seropedica, Rio de Janeiro, Brazil. Mem. Inst. Oswaldo Curz. Rio de Janeiro 97: 1107- 1110.

27-Samson R.A. and Pitt J.I. (1989): Modern concepts in *Penicillium* and *Aspergillus* classification. Ed. by Robert A. Samson and John I. Pitt. NATO ASI Series. Series A: Vol. 185. Plenum Press, New York, USA.

28-Samson R.A., Hoekstra E.S., Frisvad J.S. and Filtenborg O. (1995): Introduction to food-borne fungi (fourth edition): Centraalbureau voor Schimmelcultures Baarn Delft.

29-Sand F. E. M. J., Kolfschoten G.A. and Van Grinsven A. M. (1976): Yeast isolated from proportioning pumps employed in soft drink plants.Brauwissenschaft 29: 294- 298.

30-Sarquis M.I.M. and Oliveira P.C. (1996): Diversity of microfungi in the sandy soil of Ipanema Beach, Rio de Janeiro, Brazil. J. Basic Microbiol. 36: 51-58.

31-Smith N.R. and Dawson V.I. (1944): The bacteriostatic action of rose-bengal in media used for the plate count of soil fungi. Soil Sci. 58: 467-471.

32-Snowdon J.A. and Cliver D.O. (1996): Microorganisms in honey. Review article. Int. J. Food Microbiol. 31: 1-26.

33-Van Esch F. (1992): Yeast in soft driks and concentrated fruit juices. Brygmesteren 4:9-29.

34-Vardi A., Barzilay Z., Linder N., Cohen H.A., Paret G. and Barilai A. (1998): Local application of honey for treatment of neonatal postoperative wound infection. Acta Paediatr. 87: 429-432.

35-White J.W.Jr. (1980): Detection of honey adulteration by carbohydrate analysis. J. Ass. Off. Anal. Chem. 63: 11-18.

36-White J.W. Jr. (1983): Honey. Adv. Food Res. 24: 287-374.

37-White J.W. Jr. (1992): Quality evaluation of honey. Role of HMF and diastase assays. Am. Bee J. 132: 737-792.

38-White J.W.Jr. and Rudyj O.N. (1978): The protein content of honey. J. Apicul. Res. 17: 234-238.

39-Wellford T.E., Eadie T. and Liewellyn G.C. (1978): Evaluation the inhibitory action of honey on fungal growth sporulation. In aflatoxin production. Z. Lebensm Unters Forsch. 28: 280-283.

عزل وتشخيص الفطريات الملوثة لعسل النحل المعبأ شائع الاستخدام بالمملكة العربية السعودية

ليلى أحمد ناصر

كلية التربية للبنات– الرياض– المملكة العربية السعودية

يهدف هذا البحث إلى التعرف على الفطريات الملوثة لعسل النحل المعبأ وشائع الاستعمال بالمملكة العربية السعودية، تمت هذه الدراسة على ٤٥ عينة عسل نحل جمعت عشوائياً من السوبر ماركت المختلفة بالمملكة العربية السعودية، وذلك باستخدام طريقة وضع أجزاء من العسل على سطح الوسط الغذائى ١٠% سكروز– تشابكس أجار والتحضين عند ٢٨°م، وتم عزل وتعريف الفطريات الملوثة للعسل.

ومن النتائج لوحظ أن ٤٠ عينة (٨٨.٩% من العينات المختبرة) ملوثة بالفطريات، ولكن بمستويات منخفضة جداً. وجد أن العينات ١، ٥، ٨، ١٢، ١٣، ١٥، ١٨، ٢٣، ٣٢، ٣٤، ٤٢ هى أكثر العينات تلوثاً بالفطريات. بينما العينات ١٦، ١٩، ٢٦، ٣٠، ٣١ خالية تماماً من الفطريات.

تم عزل وتعريف ٣٥٨ مستعمرة لكل ٦٠ قطعة من العسل تمثل ١٤ نوعاً تنتمى إلى ٩ أجناس فطرية. وكانت أكثر الفطريات تعداداً وانتشاراً فى العينات قيد الدراسة هى أسبرجيللس أنواع فلافس، نيجر، فيوميجاتس، فيرسيكلر، أيضاً تم عزل بعض الأنواع الأخرى ولكن بترددات نادرة. أمكن أيضا عزل ٣٠ مستعمرة لكل ٣٦٠ قطعة من العسل من الخمائر غير المعرفة، ولكن بترددات منخفضة.