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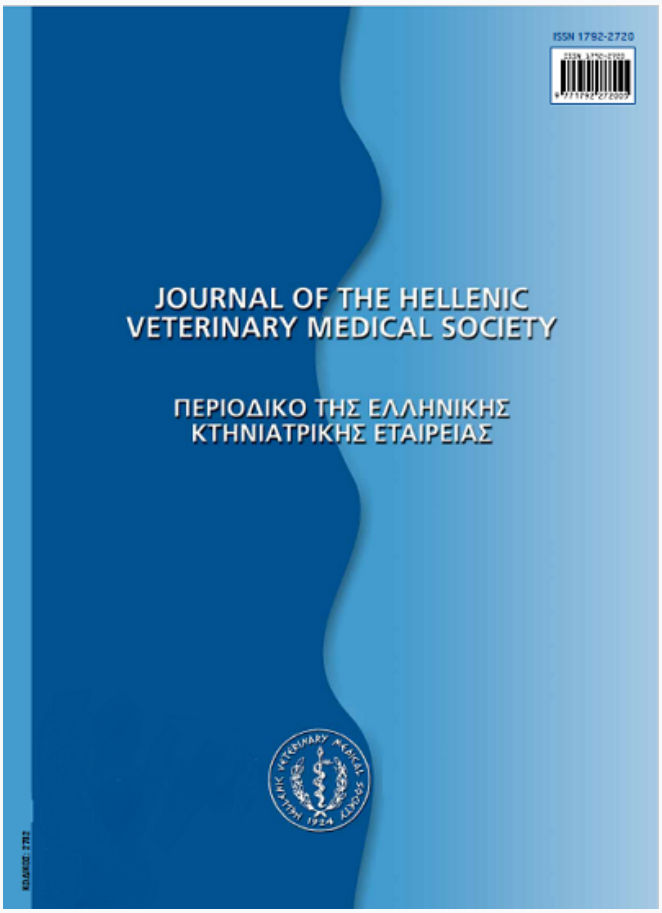

Microbiological investigation of honey collected from Şırnak province of Turkey

M. E. ERKAN, A. VURAL, H. S. GURAN, H. DURMUSOGLU

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Research article
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Microbiological investigation of honey collected from Şırnak province of Turkey

Erkan M.E., Vural A., Guran H.S., Durmusoglu H.

University of Dicle, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, 21280 Diyarbakır, Turkey

ABSTRACT. In this study, 50 randomly selected honey samples purchased from different retail markets in Şırnak, Turkey, were investigated for microbiological quality and probable public health risks. The honey samples were tested for the presence and enumeration of vegetative and spore forms of total mesophilic aerobic bacteria, total mesophilic anaerobic bacteria, sulfite reducing anaerobic bacteria, *Clostridium botulinum, Clostridium perfringens* and *Bacillus cereus* according to standard techniques. *C. botulinum, C. perfringens* and sulfite reducing anaerobic bacteria spores were not detected in the analyzed honey samples. The contamination rate of vegetative form of *B. cereus*, sulfite reducing anaerobic bacteria, total mesophilic aerobic bacteria and total mesophilic anaerobic bacteria were found to be 4%, 4%, 86% and 44%, respectively. The spore form of *B. cereus*, total mesophilic aerobic bacteria and total mesophilic anaerobic bacteria contamination rate were detected as 2%, 84% and 42%, respectively. It is concluded that, during the production, storage, and sales, honey samples may be contaminated with some microorganisms from different sources, causing important public health risks.

Keywords: honey, microbiological quality, spore forming bacteria, *Clostridium, Bacillus cereus*

Correspondence: M.E. Erkan.
University of Dicle, Faculty of Veterinary Medicine,
Department of Food Hygiene and Technology, 21280 Diyarbakır, Turkey.
E-mail: eminerkan@dicle.edu.tr

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INTRODUCTION

Honey is a sweet and flavorful product which has been consumed as a high nutritive value food. It is essentially composed of a complex mixture of carbohydrates (of which fructose and glucose accounts for nearly 85-95%) and other minor substances, such as organic acids, amino acids, proteins, minerals, vitamins, and lipids (White, 1975). Honey is a global food that is known also for its healing, antiseptic, antioxidant and antibacterial properties (Aboud et al., 2011).

The quality of honey is mainly determined by its sensorial, chemical, physical and microbiological characteristics (Finola et al., 2007). Microorganisms in honey may influence the stability of the products and its hygienic quality. Honey has several sources of microbial contamination. Primary sources include pollen, the digestive tracts of honey bees, dust, air, soil and nectar, and are somewhat difficult to eliminate. On the other hand, secondary sources, due to honey handlers and processing, are easier to control by the application of good manufacturing practices (Snowdon and Cliver, 1996).

Bacillus, *Clostridium*, *Penicillium*, *Mucor*, *Saccharomyces*, *Schizosaccharomyces* and *Torula* are the microorganisms of concern in honey (Collins et al., 1999; Migdal et al., 2000; Finola et al., 2007). Sulfite reducing Clostridia is an indicator organism, whose presence in honey provides evidence of contamination or pollution (Collins et al., 1999). The presence of spores of Clostridium is specially dangerous for infants and small children (Centorbi et al., 1999). Infant botulism is mainly caused by the consumption of honey contaminated with *C. botulinum* (Finola et al., 2007).

This study was conducted with the aim of evaluating the microbiological quality of honey produced in, Turkey.

MATERIALS AND METHODS

Sampling

A total of fifty (50) honey samples were collected from various retailers of Şırnak, Turkey, during different seasons in the years 2011 and 2012. The samples were transferred to laboratory under appropriate conditions for performing microbial analysis, and were stored at 4 °C until analysis time. All tests were conducted in triplicate.

Microbiological analysis

Ten grams of each honey sample were homogenized into 90 mL of peptone water solvent (Gomes et al., 2010) in room temperature (25 °C). Preparing decimal dilutions, the appropriate medium was inoculated by standard analysis methods. After vegetative microorganism analysis, main dilution was applied at 80 °C for 10 min. and the tubes were left to rapidly cool down for microorganism spores. Microbial counts were expressed as colony forming units per gram of honey (cfu/g). The results are shown as mean values and standard deviation.

For the count of total mesophilic aerobic bacteria (TMAB), Plate Count Agar (Merck 1.05463) was inoculated, using pour plate method; the reproduced colonies were counted after 48 h of incubation at 37 °C (BAM, 2001). For total mesophilic anaerobic bacteria (TMAnB) also the same method was used but the incubation was applied under anaerobic conditions. Count of yeasts was accomplished by surface plating dilutions on Potato Dextrose Agar (Oxoid CM139) and incubating at 25 °C for 72 h. Moulds were enumerated on Sabouraud agar (Oxoid CM41). The plates were incubated at 25 °C for 7 days (Malika et al., 2005). Sulfite Polymyxin Sulphadiazine Agar (Merck 1.10235) was used for counting the sulfite reducing anaerobic bacteria. After the culturing by using roll tube method, 24 hours incubation at 37°C was applied. The black colonies that were formed with irregular edges in the tubes were taken into examination (Harrigan, 1998). Two enrichment media were used, i.e. meat extract broth (Cooked Meat Medium, Merck) and TPGY broth (trypticase-peptone-glucose-yeast, Merck) for detection of *C. botulinum*. Two 2.0 mL aliquots of honey were inoculated into tubes containing 15 mL of meat extract broth and two additional aliquots were inoculated into tubes containing the same volume of TPGY broth. The two tubes of meat extract broth were incubated at 35 °C and the two TPGY tubes were incubated at 28 ºC. After an incubation period of 7 days, each culture was examined for turbidity, gas production, digestion of meat particles, odor, and morphology (presence and location of the spores inside the cell). Cultures that did not present growth within 7 days were re-incubated for 10 additional days in order to allow a possible late germination of spores. If no growth was

detected after this period, the culture was considered to be negative (Rall et al., 2003). For detection of *C. perfringens*, 0.1 mL of the sample dilutions were spread on Tryptose Sulfite Cycloserine (TSC) Agar (Oxoid CM587) containing egg yolk emulsion (Oxoid SR047). After the inocula had been absorbed (i.e. after about 5 min), the plates were overlaid with 10 mL of TSC agar without egg yolk emulsion. When the agar had solidified, the plates were placed in an upright position and incubated for 20-24 h at 35 °C under anaerobic conditions (BAM, 2001). To enumerate *B. cereus*, predried Mannitol Egg Yolk Polymyxin Agar (Oxoid CM929) plates containing egg yolk emulsion (Oxoid SR047) were inoculated by evenly spreading 0.1 mL of the sample dilutions onto the surface. Incubation was for 24 h at 30°C. Typical colonies were sub-cultured on Tryptone Soya Agar (Oxoid CM131), and further confirmed by their morphological (Gram staining) and biochemical properties (Voges Proskauer reaction, gelatine hydrolysis, nitrate reduction, tyrosine degradation, lysozyme test) as described by BAM (BAM, 2001).

RESULTS

In this study, the numbers of the average total vegetative mesophilic aerobic and anaerobic bacteria were found to be 9.6×10^6 cfu/g and 5.2×10^4 cfu/g, respectively; the numbers of the spore forms were found as 8.8×10^6 cfu/g and 3.8×10^5 cfu/g, respectively.

The vegetative mold and yeast contamination levels in the honey samples analyzed were found as 26% and 46%, respectively; the contamination levels of spore forms were found as 20% and 24%, respectively. The microbiologically analysis and microorganism spores of honey samples are reported in Table 1 and Table 2, respectively.

DISCUSSION

Gomes et al. (2010) reported aerobic mezophilic bacteria existence at the level of 2×10^1 cfu/g in one of 5 commercial honey samples in Portuqal. Iurlina and Fritz (2005) determined the number of aerobic mesophil bacteria as 30-1200 cfu/g (mean: 244 cfu/g) in commercial honey samples in Argentina. Tysset and Rousseau (1981) found a mean value for viable counts to be 227 cfu/g, with values that varied from 3 to 9500 cfu/g from different geographical regions of France. In a study conducted in Poland, Rozanska and Osek (2012) found the number of total aerobic bacteria as 4.6×10^3 cfu/g in different botanic originated honeys before and after one year of storage period at room temperature. Our results are fairly higher than reported by other researchers. The variation in bacterial counts may be due to the type of sample (raw, finished or retailed), the freshness of the honey, the time of harvest and the analytical techniques used (Snowdon and Cliver 1996).

Iurlina and Fritz (2005) determined the number of mold and yeast in commercial honey samples as 0-300 (mean: 34 cfu/g) in Argentina. Gomes et al. (2010) found the number of mold and yeast at the

Table 1. Microbiologically analysis of honey samples collected in Şırnak, Turkey.

Microorganism (vegetative)	Positive samples		Microorganism counts (cfu/g)		
	n	%	minimum	maximum	mean ± SD[e]
TMAB[a]	43/50	86	1.0×10^1	9.6×10^6	$1.9\times10^6\pm3.1\times10^6$
TMAnB[b]	22/50	44	1.0×10^1	5.2×10^4	$1.5\times10^4\pm1.9\times10^4$
B. cereus	2/50	4	1.0×10^1	1.0×10^2	$5.5\times10^1\pm6.3\times10^1$
SRAB[c]	2/50	4	1.0×10^1	1.0×10^1	$1.0\times10^1\pm0.00$
C. perfringens	0/50	0	ND[d]	ND	ND
C. botulinum	0/50	0	ND	ND	ND
Mold	13/50	26	1.0×10^2	1.2×10^3	$3.5\times10^2\pm3.3\times10^2$
Yeast	23/50	46	7.4×10^3	1.4×10^5	$5.4\times10^4\pm3.1\times10^4$

TMAB[a]: total mesophilic aerobic bacteria, TMAnB[b]: total mesophilic anaerobic bacteria,
SRAB[c]: sülfite reducing anaerobic bacteria, ND[d]: not detected, SD[e]: standart deviation

Table 2. Microorganism spores found in honey samples collected in Şırnak, Turkey.

Microorganism (spore forming)	Positive sample		Microorganism counts (cfu/g)		
	n	%	minimum	maximum	average ± SD[e]
SFTMAB[a]	42/50	84	1.0×10^{1}	8.8×10^{6}	$2.4\times10^{5}\pm1.4\times10^{6}$
STMAnB[b]	21/50	42	1.0×10^{1}	3.8×10^{5}	$3.1\times10^{4}\pm8.2\times10^{4}$
B. cereus	1/50	2	1.0×10^{2}	1.0×10^{2}	$1.0\times10^{2}\pm0.00$
SRAB[c]	0/50	0	ND[d]	ND	ND
C. perfringens	0/50	0	ND	ND	ND
C. botulinum	0/50	0	ND	ND	ND
Mold	10/50	20	1.0×10^{2}	2.7×10^{3}	$8.7\times10^{2}\pm1.0\times10^{3}$
Yeast	12/50	24	3.8×10^{3}	6.8×10^{4}	$3.7\times10^{4}\pm2.4\times10^{4}$

SFTMAB[a]: spore forming total mesophilic aerobic bacteria, SFTMAnB[b]: spore forming total mesophilic anaerobic bacteria spores, SRAB[c]: sülfite reducing anaerobic bacteria, ND[d]: not detected, SD[e]: standart deviation

level of 1.1×10^{1}-2.1×10^{1} cfu/g in 3 of 5 commercial honey samples in Portuqual. In France, Tysset and Rousseau (1981) found that counts of moulds and yeasts also varied from 0 to 2500 cfu/g, whereas the mean count was 90 cfu/g. Rall et al. (2003) found an incidence of 64% of mould and yeasts in industrial and domestic production honeys with counts ranging from absence to 1.5×10^{5} cfu/g in Brazil. Duman Aydın et al. (2008) reported the range of mold and yeast existence as 10^{2}-10^{3} cfu/g in 8 of 20 honey samples in Kars, Turkey. Rozanska and Osek (2012) found the number of mold and yeast as 1.9×10^{2} to 9.8×10^{1} cfu/g in different botanic originated honeys before and after one year storage period at room temperature in Poland. Mahmoudi et al. (2012) reported yeast existence only in 7 of the 263 (2.66%) honey samples they had examined in Iran. In this study, contamination levels are lower than those reported by Rall et al. (2003) and Gomes et al. (2010).

Monetto et al. (1999) isolated 7% *C. botulinum* in 45 honey samples. In Brazil, 6/85 honey samples analyzed were found positive for *C. botulinum* (7.06%) and identified as producers of type A, B, and D toxins (Schocken-Iturrino et al.,1999). According to Küplülü et al. (2006) *C. botulinum* was isolated from 12.5% of honey samples from retail market in Ankara, Turkey. In Finland, spores of *C. botulinum* were detected in 8 (7%) of the 114 Finnish and in 12 (16%) of the 76 imported honey samples Nevas et al. (2002). Our results agree with those obtained by Tysset et al (1970), Piana et al. (1991) and Delmas, Vidon and Sebald (1994), in which *C. perfringes* or *C. botulinum* was not found in any sample tested. The reason for different findings in the studies may be due to contamination variety or antimicrobial substances in the honey samples.

In their studies, Iurlina and Fritz (2005) and Gomes et al. (2010) could not determine sulfite reducing Clostridia existence in any of the honey samples. Finola et al. (2007) on the other hand, reported sulfite reducing Clostridia existence in 70% of 23 honey samples they had examined in Argentina. In our study, only 2 of 50 honey samples (4%) was determined to be in vegetative form of sulfite reducing anaerob bacteria existence.

Iurlina and Fritz (2005) found the existence of *Bacillus* spp. (*B. cereus, B. pumilus and B. laterosporus*) in 23% of 70 honey samples in Argentina. On the other hand, in Argentinean commercial honeys, *B. cereus* was detected in 78% of the samples with values lower than 10.000 spores/kg (Monetto et al., 1999). In our study, *B. cereus* vegetative form and spore forms contamination rate were detected as 4% and 2%, respectively.These results are lower than those reported by earlier researchers.

CONCLUDING REMARKS

The microbiological characteristics of honey from the Şırnak Region of Turkey were determined. Honey samples is contaminated with fungi and bacterial organisms indicating inadequate hygiene con-

dition during harvesting, handling, processing and/or storage. It is concluded that the hygienic quality of the honey samples is low, and this situation pose a significant risk on public health.

CONFLICT OF INTEREST STATEMENT

None of the authors of this article has any conflict of interest.

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