RESEARCH ARTICLE

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Survey of Hygienic Quality of Honey Samples Collected form Qazvin Province during 2011-2012

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Background & Aims of the Study: Consumption of honey has remarkably increased in the last years all over the world. Factors such as plant species, environmental, processing, and storing condition are affecting honey quality. The purpose of this study was to evaluation of Hygienic quality of honey samples produced from Qazvin province.

Materials and Methods: 34 fresh honey Samples were obtained from beekeepers from different regions of the alamut area in the period between June and November 2011. The microbial contamination (bacteria and fungi) was determined using conventional microbiological methods and the total aflatoxin was detected by "high performance liquid chromatography" (HPLC).

Results: The results of microbial analysis showed that the aerobic mesophil bacteria count (50 cfu/g) and fungal count ($1.5*10^2$ cfu/g) were in low levels. However, coliforms were not detected in any of the honey sample. The most prevalent bacteria and fungi were Bacillus cereus and Aspergillus flavus respectively. Based on the HPLC method analysis, all of honey samples were contaminated whit aflatoxin and the mean concentration of aflatoxin was 3.67 ppb. Also the aflatoxin levels in 35% honey samples were higher than the maximum allowable amount of Europe Union (4 µg/kg).

Conclusion: According to the results, should have more control on the Hygienic quality of honey over the production, storage and supply periods in this area.

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Background

Honey is the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants and it consists essentially of carbohydrates, predominantly fructose and glucose, organic acids, enzymes and solid particles, a very complex mixture of minerals, aroma compounds, flavonoids, vitamins, pigments and other phyto-chemicals (1,2).

The composition of honey depends on the plant species visited by the honeybees and the environmental processing and storage conditions (3).

The quality of honey is mainly determined by its sensorial, chemical, physical and microbiological characteristics (4).

The various factors such as nectar and pollen, quality of processing equipment (machines and container) are affecting honey microbial quality. The most common microorganisms found in honey are spores, such as Bacillus. Microorganisms such as fungi due to the dispersion in the environment, process equipment and flowers possibility of their contamination exists in honey (2). Important contaminants in honey are fungi and spore-forming bacteria bacteria, respectively. These microorganisms cause spoilage, aflatoxin production and the loss of nutrients in the honey (5).

Mycotoxins are secondary metabolites of molds specially produced by two closely related fungi, Aspergillus favus and Aspergillus parasiticus (6-9). Under favorable conditions of temperature and humidity, fungi grow on certain foods and feeds, resulting in the production of toxins. Aflatoxins are among the most important mycotoxins cause a significant threat to human and animal health (10). Among four common aflatoxins available (aflatoxin B1, B2, G1, and G2), aflatoxin B1 (AFB1) is the most prevalent and poisonous molecule and it is categorized as group 1 human carcinogen by International Agency for Research on Cancer (IARC) (11). Aflatoxins are highly toxic, mutagenic, teratogenic, and carcinogenic compounds, a group of difuranocoumarin derivatives, consisted of a coumarin and a double-furan-ring of molecule usually (12). Consumption of honey has remarkably increased in the last years all over the world. However, the safety of these products is not regularly assessed. In this study, we examine the microbial quality and total aflatoxin contamination on Iranian honey samples from alamut area, Qazvin province. Honey export from Iran is an interesting case due to Iranian's important roles in global production and trade.

Materials & Methods

Honey samples: Samples of fresh honey (n=34) were obtained randomly from beekeepers from different regions of the Alamut area in the period between June and November 2011. The collected samples for microbial analysis were put in sterile glass and were kept at 4°C. Other Samples were kept in glass containers in the laboratory under room temperature for aflatoxins analysis.

Microbiological analysis: 10 g of each honey sample were homogenized into 90 ml of sterile peptone water and appropriately diluted suspensions of samples (100 ul) were cultured by the spread plate method. The standard plate count method was used for culturing and isolating the different micro-organisms. MacConkey agar was used as the medium for enterobacterial isolation and culture, while potato dextrose agar and Sabouraud dextrose agar were used for growing yeasts. Bacterial colonies resulting from the first culture after incubation at 37°C for 48 h were transferred to fresh media, streaked and incubated again. After successive transfers, the resulting pure isolates were Gram stained and identified, based on the color, size, shape, and differential biochemical tests. For yeasts, incubation was at room temperature (22°C) and 37°C for four days. The resulting colonies were examined, streaked and grown successively until pure cultures were obtained. Identification was based on the color and shape (13).

TAFs Analysis

Reagents and apparatus: All reagents and solvents used were of high performance liquid chromatography (HPLC) grade. AF standards were purchased from Sigma Chemical Company, USA. Aflatestimmunoaffinity columns (IAC) were purchased from VICAM Company, Watertown, MA, USA. Apparatus characteristics were WATERS 1525 binary HPLC pump, and 2475 Multy λ fluorescence detector. HPLC column (C18, 250 x 4.6 mm: 4 µm) was purchased from Waters, USA.

Extraction and clean up: Samples were analyzed using a HPLC following AOAC (1995) with some modifications. Samples were extracted with methanol: water: n-hexane (240:60:100, v/v/v). The mixture was shaken for 30 min on a mechanical shaker. The solution was left to sediment and filtered through a Whatman Filter No.1. The samples were diluted with water and after filtered. Aflatest was used for samples to clean up. First, 10 ml phosphate buffer saline (PBS) was passed through the IAC. Then, 75 ml of the filtrate was passed through the IAC at a flow rate of 1 ml/min. The column was washed with water and dried using vacuum. Finally, AF was eluted with methanol using the following procedure. First, 0.5 ml methanol was applied to the column which passed through by gravity.

After 1 min, the second portion of 0.75 ml methanol was applied and collected. The Aflatest was diluted with water and analyzed using HPLC (14).

AFT standard: After preparation of standard solutions for AFT, its concentration was determined using a UV spectrophotometer. This standard was used to prepare mixed working standard for HPLC analysis (9,15).

Analysis of AFT using HPLC: AFT was quantified by reverse-phase HPLC and 2475 Multy λ fluorescence detector with post column derivatization (PCD) involving bromination. The waters HPLC system was applied with a Kobra cell and the addition of bromide to the mobile phase. After dilution of AF eluate with water, 100 µl was injected into the HPLC. Mobile phase was water: methanol: acetonitrile (600:300: 200, v/v/v) and 350 µl of nitric acid 4 M and 120 mg of potassium bromide with a flow rate of 1 ml/min. The fluorescence detector was operated at an excitation wavelength of 365 nm and emission wavelength of 435 nm.

Statistical methods: The data obtained from this study were analyzed by T test using SPSS version 22 and the differences between the data in the case of $P<0.05$, was considered significant.

Results

The results of microbial analysis showed that the aerobic mesophil bacteria count ranged from 0-5. 102 cfu/g (mean= 50 cfu/g) and the mean of fugal counts was 1.5. 102 cfu/g. 29.4% of samples tested positive for Bacillus cereus, 14.7% for B. mycoides, 8.8% for B. megaterium, 50% for Aspergillus flavus, 32.3 % for A. niger, 11.76% for A. fumigatus, 5.8% for A. candidus, 5.8% for A. Vesicolor, 8.8% for Penicillium sp., 14.7% Candida sp. and 20.58% for other fungi (Table1 and figure1). Coli form bacteria were not detected in any of the honey sample.

All samples of honey analyzed were found to contain AFs. The concentrations of AFs ranged between 0.74-8 ppb with a mean value of 3.67 ppb (Table2). Also the AF levels in 35% honey samples were higher than the maximum allowable amount of Europe Union (4 µg/kg).

The mean concentrations of AFs in samples of warm and cold season were compared in Figure2; they did not contain significantly different AFs concentrations.

Table1) Microbiological contamination of Qazvin honey samples (n=34).

Microbes	Frequency (%) (n=34)
Bacteria	
Bacillus cereus	29.41
Bacillus mycoides	14.7
Bacillus megaterium	8.8
Fungi	
Aspergillus flavus	50
Aspergillus niger	32.3
Aspergillus fumigatus	11.76
Aspergillus candidus	5.8
Aspergillus vesicolor	5.8
Penicillium sp.	8.8
Candida sp.	14.7
Other yeasts	20.58

Table2) TAF contamination (ppb) in honey samples (n=34).

seasons	Sample size (N)	Positive sample (N)	Mean ± SE	Min-Max	maximum permitted level (EU regulations)	Higher than the maximum Permissible level
warm	17	17	4.15± 2.28	0.96-8	4 µg.kg^{-1}	47% (n=8)
Cold	17	17	3.2 ± 1.82	0.74-7.57	4 µg.kg^{-1}	23.5% (n=4)

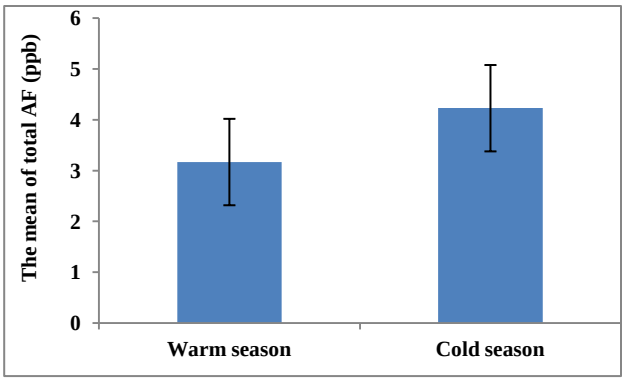

Figure2) The mean of TAF concentration in warm and cold seasons.

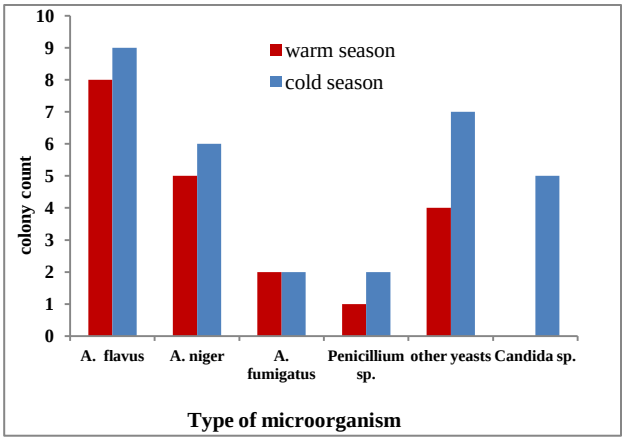

Figure1) Microorganisms isolated from honey samples of warm and cold seasons.

Discussion

AFs could pose a signifcant threat to human health because they are toxigenic, carcinogenic, mutagenic and teratogenic, besides the economic loss due to food contamination.

a great attention has been about the acute toxicity and carcinogenicity of the AFs as well as the pathways leading to their biosynthesis by some strains of Asp. flavus, Asp. nomius and Asp. parasiticus (16). All samples of honey analyzed were found to contain AFs. The concentrations of AFs ranged between 0.74-8 ppb with a mean value of 3.67 ppb (Table1). The mean concentration of TAF in samples of warm and cold season was compared in Figure1. They did not contain significantly different AF concentrations. All samples of honey analyzed by Swaileh and Abdulkhaliq in Palestine, were found to contain AFs. The concentrations of AFs ranged between 0.49-22 µg.kg-1 with a mean value of 12.10 µg.kg-1 (10). The study of KhaliqurRahman et al. (2014) showed that aflatoxins were detected only in 4 honey samples out of 24 different branded and unbranded honey samples of Khyber pukhtounkhwa, Pakistan (17). In the study in Lisbon, Portugal none of examined samples revealed to be contaminated with AFs (16). Similar results as their study were detected in Jeddah, Saudi Arabia (18).

According to EU regulations, the maximum permitted level of TAF in dried fruit intended for direct human consumption is 4 µg/kg-1 (10). In the present study, 12 honey samples out of 34 (35.3%) contained TAF levels of higher than 4 µg kg-1. These results were different from those obtained by Swaileh and Abdulkhaliq (10): only 3 honey samples out of 21 (14%) contained TAF levels of less than 4 µg kg-1. The production of AFs by fungi depends not only on the genetic competence of the strains, but is also influenced by a quite wide range of factors (substrate composition, very low aW, and acidity of honey) and ecological conditions (16).

The isolation of different microorganisms from honey samples, confirms other findings that fungi and spore forming bacteria may be present in honey for a limited period of time (13). Our result on mesophilic bacteria count is lesser than that reported by some others researchers (16,19,20), but higher than that obtained by Mahmoudi et al. (13). This variation in bacterial counts may be due to the type of sample, freshness of the honey, the time of harvest and the analytical techniques used (21).

Similar to our result, Omafubve and Akanbi (2009) isolated B. cereus, B. megaterium, B.

polymyxa, B. licheniformis, B. firmus and B. pumilus from honey samples (20). The isolation of different Bacillus species from honey has also been reported from other countries (16,19, 22). Bacillus species are among the main spoilage organisms in food due to their versatile metabolism and heat-resistance spores. B. cereus, B. subtilis and B. licheniformis have been associated with food poisoning (20).

Similar as our study Kacaniova et al. (2007) and Adenekan et al. (2010) did not detect coli forms in any of the honey sample. This may be explained by the evidence that honey is well preserved against bacteria so that these microorganisms would not survive unfavorable conditions (23,24).

Malika et al. (2005((16) reported fungal counts of less than 10 cfu/g-1 in Moroccan honey while some French honeys (22) and some Nigerian honey (20) had zero counts of moulds and yeasts.

Among the isolated fungi, the highest prevalence was assigned to the genus Aspergillus. From the genus, 5 species were identified of which A. flavus and A. niger were the most prevalent species. These results were nearly similar to those obtained by other researcher (25). They noticed that species of Aspergillus were the most prevalent fungi in honey samples tested with the most predominant species being A. flavus. A. flavus and A. fumigatus cause stonebrood infections in larvae and adult honeybees. The agents may contaminate humans via honey and cause dysentery-like infections and hepatic cancer (26).

Candida sp. occurred in 14.7% of samples and other yeasts were isolated in 20% of samples. In this respect, the other researcher reported that the yeast species identified (Candida humicola and Saccharomyces sp.) were detected in a very high frequency and at high levels of contamination (16).

These osmophilic yeasts are probably good indicators for microbiological quality of honey (26). Penicillium sp. in rare frequency of occurrence, emerging collectively about 8.8% of the honey samples. These yeasts were also, isolated from honey bees (26).

Conclusion

According to the results of this paper (AF contamination of honey samples and fungal contamination), should have more control on the hygienic quality of honey over the production, storage and supply periods in this area.

Footnotes

Conflict of Interest:

The authors declared no conflict of interest.

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