Contaminants Analysis of Different Branded and Unbranded Honey of khyber pukhtounkhwa Pakistan

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Abstract: This study presents evaluation of aflatoxins (B1, B2, G1, G2) and heavy metals (cadmium, manganese, lead, mercury, nickel and cobalt) contamination in branded and unbranded honey. Higher concentration (µg/kg) of heavy metals was found in branded honey as compared to unbranded honey. As in Marhaba, Ni concentration (0.49±0.03) found maximum while Co (0.15±0.02) was lowest. Pb concentration (0.85±0.03) was maximum whereas Cd (0.16±0.03) found lowest in Qarshi. Versatile contains maximum Pb (1.34±0.02) while lowest Cd (0.12±0.02). In Al-hayat Cu concentration (1.23±0.03) was maximum while Pb (0.11±0.03) was lowest. Young's honey contains maximum Ni (2.41±0.01) while lowest Mercury (0.16±0.03). Ni (1.25±0.02) was found maximum and Mn (0.14±0.03) lowest in Pak-salman, whereas in Langness Hg concentration (0.71±0.03) found maximum while Cd (0.13±0.02) was lowest. The contamination level of aflatoxins (B1, B2, G1 and G2) was also evaluated in both types of honey. Minimum level of aflatoxins were detected in branded and unbranded honey sample are B1and B2 such as (2.14, 1.25) and maximum concentration are (2.33, 2.15) respectively. It is concluded that contaminants are less as compare to the reported values so mostly the honey produces in Khyber pakhtunkhwa are good for use and export can be enhanced.

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Introduction

Honey can be utilized as a final product or ingredient in food. It contains a mixture of carbohydrates, such as glucose (25 - 37 %), fructose (25 - 45 %), sucrose (0.5 - 3 % and maltose (2 - 12 %) having some trace amount of other sugars depending on water (15 - 18 %) and the floral source. Honey also contains range of nutritiously important elements and is high-viscous liquid (Matthews, W., 2005). Storage may produce various changes in honey, as osmophilic yeast cause spontaneous fermentation is one of the most significant changes in honey (Jiménez *et al.,* 1994).

Mould, yeasts and spore forming bacteria are primarily the microbes of great concern in honey. These microorganisms may take part in various activities such as production of enzymes, spoilage of provisions, antibiotics, metabolic conversion of provisions, mycotoxins, growth factors (amino acids and vitamins) and inhibition of competing microorganisms. Commercial distribution of honey can be presented in bulk quantity and also packaged for retail sale. Microbiological characteristics of honey are inherent to quality and safety (Goerzen *et al.,* 1991).

Certain fungi that can grow on food such as dried fruits, nuts, cereals, legumes and spices produces naturally-occurring toxins called Mycotoxins. The most commonly observed mycotoxins are found aflatoxin (B_1, B_2, G_1 & G_2) and ochratoxin-A. Aflatoxins directly damages DNA and in many developing countries have been shown to cause cancer of the liver in laboratory (Matthews, W., 2005). Besides the economic loss due to food contamination, among mycotoxins, aflatoxins could be more hazardous to human health there are carcinogenic, toxigenic, teratogenic and mutagenic (Hsieh 1986).

In a preliminary study on honey in Portugal, reported less contamination with fungi such as Mucor species, yeasts, Penicillium species and many species of genus *Aspergillus*, particularly *A. Candidus*, *A. flavus*, *A. niger* and *A. fumigates*. Predisposed patients can get harm from these potentially pathogenic species. In clinical form of botulism, spores of bacteria apparently germinate and produce toxin in the intestinal tract of affected infants less than one year of age (Hutttanen *et al.,* 1981). Honey also contains about 0.17 % mineral contents, although it differs within a wide range. Honey has been considered as a biological indicator of environmental pollution because honey bees create bio-accumulation process. Therefore, the concentration of heavy metals in honey represents their amount in the whole region as the forage area of the hive is very large and the bees come in contact not only with soil and air but also with water (Przybylowski *et al.,* 2001).

Cadmium (Cd) and Lead (Pb) are the most toxic heavy metals. These originate mainly from vehicle traffic, metal industry, incinerators is transported from the soil to plants and also through air can contaminate directly nectar and honeydew (Byrne D., 2005). Nutritional aspects and quality control is based on heavy metals in honey. Metals known or supposed toxicity are undesirable, so that, in some countries a limit of 1 mg/kg for lead is set (Buldini *et al.,* 2001).

Metals in very small quantities are vital for all life forms, they enter into the cells like cations, but their inclusion is strictly regulated, because actually, in large quantities, all metals are toxic. The human being, like the other vertebrates, need metal's cations, because they assure the development of many processes of vital importance, The division of the metals in required, neutral and toxic may be inaccurate and often mislead, because all the required elements in small doses become toxic and very toxic in large doses (Popescu Gheorghe,2005).

Honey shows therapeutic values and is a dietary supplement due to its important levels of trace minerals that are essential to health (Alissandrakis *et al.,* 2007). Metals in trace amount are important in daily diets due to their essential nutritious value. Copper, iron, manganese and zinc are essential trace minerals as they are important part of biological systems (Tuzen *et al.,* 2007). Food is one of the main sources of heavy metals for human and diet is the main route of exposure to trace metals. So, to assess risks to human health for these elements, analysis of food samples and collecting information regarding dietary intake is also important (Soylak *et al.,* 2008).

During the foraging activities in the areas surrounding the apiary, the Honeybees may continuously expose to contaminants (Conti *et al.,* 2001). Bees and their products can serve as bioindicators for contamination as they fly intensively in area of about 3 kilometer (Bogdanov *et al.,* 2003). The aim of this study to analyze aflatoxins and toxic heavy metals (Mn, Cd, Co, Hg, Cu, Pb, Ni) in different branded and unbranded honey.

Material And Method

Collection Of Samples

Different varieties of branded and unbranded honey (n = 14) samples were collected from bee keepers and local market of Khyber Pakhtunkhwa Pakistan. Samples were kept in plastic containers or glass vessels with tight plastic covers. These were brought to the Food Technology Centre, PCSIR Laboratories Complex Peshawar, stored in cool and dark place till analysis for aflatoxins and heavy metals.

Aflatoxin Analysis

Chemicals

Analytical grade chemicals were procured from Merck (Darmstadt, Germany), BDH (England) and Sigma Chemicals (USA). Aflatoxins standards such as aflatoxin B_1 aflatoxin B_2, aflatoxin G1 and aflatoxin G_2 were purchased from company Biopure (Austria). Standard stock solutions of AfB_1, AfB_2, AfG_1 and AfG_2 (1 µg / ml) each were prepared by diluting in benzene / acetonitrile (98: 2; v / v). These solutions then stored at 4°C in refrigerator, covered in aluminum foil to prevent aflatoxins degradation in UV light.

Determination Of Total Aflatoxin

Determination of total aflatoxins (B1, B2, G1 and G2) was carried out by standard method of AOAC, using thin layer chromatography technique (AOAC 200). Briefly, 50g sample was blended for 3 minutes with 250 ml solution of acetone / water (85:15 v / v), then filtered through Whatman filter paper. A 150 ml of filtrate was collected in 400 ml beaker. Then 170 ml of 0.02M sodium hydroxide and 30 ml ferric chloride along with about 3 gm basic copper carbonate added to the filtrate in 400 ml beaker, mixed well and added to the mixture in 600 ml beaker. This solution mixture was filtered and transferred 150 ml to 500 ml separating funnel. To this 150 ml of 0.03 % sulphuric acid was added and then extracted with chloroform (10 ml) twicely. Lower layer of chloroform was transferred to another separating funnel. Added 0.02 M potassium hydroxide swirled gently for 30 seconds and left it for layer separation. Chloroform extract layer was collected in a vial. 8 ml extract was evaporated to dryness on heating bath at 45 °C in the presence of gentle flow of nitrogen gas. The dried residue obtained was redissolved in 200 µl solution of benzene / acetonitrile in ratio 98:2 (v/v). Known concentration was spotted on TLC plates and subjected to development for 45 minutes. Plates were developed in a glass chamber with solution of chloroform / xylene / acetone in ration 60:30:10 (v/v/v). After the spots on plate were observed under long wave ultraviolet light (λ = 365 nm). Intensity was observed by visual comparison with aflatoxins standard spots. The identity of aflatoxins was confirmed by spraying of 50 % sulphuric acid solution and Trifluoroacetic acid reaction (Scott 1984).

Heavy Metals Analysis

Sample Preparation

One gram of honey samples was taken and transfer to digestion flask then added about 20ml of per chloric acid and nitric acid. This mixture was heated on 250 in the digestion tube. After digestion 1ml digested solution were diluted up to 100 ml with distilled water. The concentration of heavy metals was analyzed on atomic absorption spectrophotometer (Erdtman 1952).

Atomic Absorbtion Spectro Photometer

The estimation of heavy and toxic metals such as Mn, Cd, Co, Hg, Cu, Pb and Ni was carried out by atomic absorption spectrophotometer model (Hitachi zee man Z-8000 Japan). Different working standards were used for calibration and standardization of the instrument. The concentration of different elements in each sample was determined.

Statistical Analysis

Triplicate determination were carried out and standard deviation was calculated (Steel *et al.,* 1997). Calibration curve of the standard elements was obtained for concentration vs. absorbance / division. Data for each sample was subjected to one way analysis of variance (ANOVA)and the mean comparison was performed according to the turkey multiple comparison test (post hock test) significance value of alpha=0.01 was used to distinguish significance difference of mean with the verities (Angus *et al.,* 2005).

Result And Discussion

This study presents the quantitative evaluation of aflatoxins (B1, B2, G1 & G2) and heavy metals (cadmium, manganese, lead, mercury, nickel, cobalt and cupper) in branded and unbranded honey samples. Table 1 presents heavy metals concentration in branded honey samples, which showed that in Marhaba, Ni concentration (0.49±0.03) found maximum and cobalt (0.15±0.02) was lowest, while manganese (0.23±0.03), mercury (0.21±0.01) cadmium (0.17±0.02), cupper (0.16±0.03) and lead (0.16±0.01) were in moderate concentration. Its reported that, lead and cadmium contents in honeys were (8.4-105) ppb and (0.9 - 17.9) respectively (Tuzen *et al.,* 2007). In our samples Lead concentration (0.85±0.03) found maximum and cadmium (0.16±0.03) found lowest in Qarshi, while nicle (0.52±0.02), cupper (0.42±0.01), manganese (0.34±0.02) cobalt (0.27±0.03) and mercury (0.25±0.02) were in moderate concentration. Versatile contains maximum lead (1.34±0.02) while lowest cadmium (0.12±0.02), while nicle (1.13±0.03), cupper (0.35±0.02), mercury (0.24±0.01), manganese (0.17±0.02) and cobalt (0.13±0.01) were in moderate concentration. On the other hand, according to World Health Organization the average recommended daily intake of Cd and Pb are 60 µg/d and 210 µg/d, respectively. Therefore, consuming 20 g per day of honey from Sanlıurfa provides: 0.06 µg Cd day and 6.98 µg Pb in a day (WHO 1982).

In Al-hayat cupper concentration (1.23±0.03) found maximum and lead (0.11±0.03) was lowest, while nicle (0.44±0.02), mercury (0.28±0.02), Cobalt (0.19±0.01), cadmium (0.18±0.02) and manganese (0.12±0.02) were in moderate concentration. Young's honey contains maximum nicle (2.41±0.01) and lowest mercury (0.16±0.03), while lead (1.03±0.03), cupper (0.77±0.03), manganese (0.24±0.03), cadmium (0.20±0.03) and cobalt (0.18±0.02) were in moderate concentration. It has been reported that manganese level was 1.0 ppm in honey obtained from different areas of southeastern Anatolia (Yılmaz *et al.,* 1999). But in our samples the nicle concentration (1.25±0.02) found maximum and manganese (0.14±0.03) lowest in Pak-salman, while cupper (1.19±0.01), mercury (0.44±0.02), lead (0.27±0.02), cobalt (0.22±0.02) and cadmium (0.22±0.01) were in moderate concentration. Mercury level in various bee's products were reported as (0.00001 – 0.006 mg / Kg) (Madras *et al.,* 2002)

In Langness honey samples mercury concentration (0.71±0.03) found maximum and cadmium (0.13±0.02) was lowest, while cupper (0.47±0.03), manganese (0.18±0.03), lead (0.17±0.03) cobalt (0.14±0.03) and nicle (0.13±0.01) were in moderate concentration. It has also been reported that around the world, cadmium contents of some honey samples were (0.020-0.490 mg/kg) (Conti *et al.,* 2001).

Table 2 represents heavy metals concentration in unbranded honey samples, which showed that in big bees honey, lead concentration (0.73±0.03) found maximum and cadmium (0.15±0.03) was lowest, while manganese (0.63±0.02), cupper (0.36±0.01) , nicle (0.33±0.01), mercury (0.18±0.03) and cobalt (0.16±0.02) were in moderate concentration. Lead concentration (1.25±0.03) found maximum and cadmium (0.13±0.03) lowest in small bee's honey, while cupper (0.32±0.01), mercury (0.27±0.03), cobalt (0.17±0.01), nicle (0.15±0.02) and manganese (0.14±0.02) were in moderate concentration. It has benn reported that the manganese concentrations of Turkish honey were 0.31 ppm respectively (Uren *et al.,* 1998).

Beera contains maximum cupper (1.29±0.02) and lowest manganese (0.19±0.03), while mercury (0.61±0.01) nicle (0.61±0.03), lead (0.43±0.02), cadmium (0.23±0.02) and cobalt (0.21±0.03) were in moderate concentration. In Palosa, lead concentration (1.27±0.02) found maximum and cadmium (0.11±0.02) was lowest, while cobalt (0.65±0.01), mercury (0.55±0.02), manganese (0.26±0.01), nicle (0.19±0.03, cupper (0.13±0.01) were in moderate concentration. It has been reported that manganese level of honey was (0.49) ppm (Yarsan *et al.,* 2007). Sperkay honey contains maximum manganese (1.15±0.02) and cadmium (0.14±0.02) was lowest, while nicle (0.94±0.03), mercury (0.69±0.01), cobalt (0.46±0.02), cupper (0.41±0.03) and lead (0.39±0.03) were in moderate concentration.

Mercurry concentration (0.46±0.03) found maximum and cupper (0.46±0.03) was lowest in Bekerr, while nicle (0.46±0.02), manganese (0.32±0.03), cobalt (0.17±0.02), cadmium (0.17±0.01) and lead (0.16±0.03) were in moderate concentration. In Granda honey, nicle concentration (2.25±0.01) found maximum and mercurry (0.12±0.02) was lowest, while lead (0.52±0.02), cobalt (0.25±0.03), cadmium (0.24±0.03), cupper (0.15±0.02) and manganese (0.15±0.03) were in moderate concentration. Ni levels

(0.004 – 3.23 mg/kg) have been reported in honey under the Swiss MRL study (Porrini *et al*., 2002). Lead is another heavy metal; their higher concentration leads to brain defection, hypertension, hearing difficulty, anemia, kidney disease and loses of intelligence (Darrell 1991). The average recommended daily intake of Cd and Pb are 60µg/d and 210µg/d, respectively (Frias *et al.,* 2008).

Detection of aflatoxins level in branded and unbranded honey has been reported in Table 3 and 4. It showed that aflatoxins were not detected mostly in honey samples. Higher concentration of aflatoxin B2 was found in Young's honey (2.14 ppb), and lowest in Al hayat honey (1.25 ppb). Higher concentration of aflatoxin B1 (2.33 ppb) in Bekerr honey, while the lower concentration of aflatoxin B2 were detected in Sperkay (2.15ppb).

Conclosion

It is concluded that, some branded and unbranded honey samples from local markets of khyber pukhtunkhwa are contaminated with aflatoxins, while most of the samples contains toxic heavy metals contamination in branded and unbranded honey. The contaminated samples have lower concentration than the permissible limits set by European commission and WHO. The aflatoxin analysis revealed mainly the presence of aflatoxin B1 and B2, which shows the possibility of fungal contamination during their production, marketing and storage. While the toxic metals contamination in honey may be from environment. Thus is concluded that branded and unbranded honey are not contaminated as to the permissible levels represents good quality honey. So, utilization and export of honey could be enhanced. It is also essential to investigate further the presence of other contaminants in these commodities to monitor their quality for food safety.

Table 1. Heavy metals concentration in branded honey (µg/kg)

Honey Samples	Cd	Cu	Pb	Ni	Mn	Co	Hg
Marhaba	0.17±0.02*	0.16±0.03	0.16±0.01	0.49±0.03	0.23±0.03	0.15±0.02	0.21±0.01
Qarshi	0.16±0.03	0.42±0.01	0.85±0.03	0.52±0.02	0.34±0.02	0.27±0.03	0.25±0.02
Versatile	0.12±0.02	0.35±0.02	1.34±0.02	1.13±0.03	0.17±0.02	0.13±0.01	0.24±0.01
Al-hayat	0.18±0.02	1.23±0.03	0.11±0.03	0.44±0.02	0.12±0.02	0.19±0.01	0.28±0.02
Young's honey	0.20±0.03	0.77±0.03	1.03±0.03	2.41±0.01	0.24±0.03	0.18±0.02	0.16±0.03
Pak-salman	0.22±0.01	1.19±0.01	0.27±0.02	1.25±0.02	0.14±0.03	0.22±0.02	0.44±0.02
Langness	0.13±0.02	0.47±0.03	0.17±0.03	0.13±0.01	0.18±0.03	0.14±0.03	0.71±0.03

* mean ± standard deviation

Table 2. Heavy metals concentration in unbranded honey (µg/kg)

Honey Samples	Cd	Cu	Pb	Ni	Mn	Co	Hg
Big bees honey	0.15±0.03	0.36±0.01	0.73±0.03	0.33±0.01	0.63±0.02	0.16±0.02	0.18±0.03
Small bees honey	0.13±0.03	0.32±0.01	1.25±0.03	0.15±0.02	0.14±0.02	0.17±0.01	0.27±0.03
Beera	0.23±0.02	1.29±0.02	0.43±0.02	0.61±0.03	0.19±0.03	0.21±0.03	0.61±0.01
Palosa	0.11±0.02	0.13±0.01	1.27±0.02	0.19±0.03	0.26±0.01	0.65±0.01	0.55±0.02
Sperkay	0.14±0.02	0.41±0.03	0.39±0.03	0.94±0.03	1.15±0.02	0.46±0.02	0.69±0.01
Bekerr	0.17±0.01	0.12±0.03	0.16±0.03	0.46±0.02	0.32±0.03	0.17±0.02	0.13±0.03
Granda	0.24±0.03	0.15±0.02	0.52±0.02	2.25±0.01	0.15±0.03	0.25±0.03	0.12±0.02

* mean ± standard deviation

Table 3. Aflatoxins concentration in branded honey (µg/kg)

Honey Samples	B1	B2	G1	G2	Total
Marhaba	ND	ND	ND	ND	ND
Qarshi	ND	ND	ND	ND	ND
Versatile	ND	ND	ND	ND	ND
Al-hayat	1.25	ND	ND	ND	1.25
Young's honey	ND	2.14	ND	ND	2.14
Pak-salman	ND	ND	ND	ND	ND
Langness	ND	ND	ND	ND	ND

ND= Not detecte

Table 4. Aflatoxins concentration in unbranded honey (µg/kg)

Honey Samples	B1	B2	G1	G2	Total
Big bees honey	ND	ND	ND	ND	ND
Small bees honey	ND	ND	ND	ND	ND
Beera	ND	ND	ND	ND	ND
Palosa	ND	ND	ND	ND	ND
Sperkay	ND	2.15	ND	ND	2.15
Bekerr	2.33	ND	ND	ND	2.33
Granda	ND	ND	ND	ND	ND

ND= Not detected

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