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

1KhaliqurRahman, 1Imdadullah muhammadzai, 2Arshad Hussain, 3HalimurRahman, 2Javid Ali

1Institute of Chemical Science University of Peshawar Pakistan
2Pakistan Council of Scientific and Industrial Research Peshawar
3Sarhad University of Science and Information Technology Peshawar Pakistan

Corresponding Author: khaliqjan244@yahoo.com

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 B1 and 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.


Key words: Aflatoxins, heavy metals, contamination, honey

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 (B1, B2, G1 & G2) 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 (Huttunen 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 B₁ aflatoxin B₂, aflatoxin G₁ and aflatoxin G₂
were purchased from company Biopure (Austria).
Standard stock solutions of AfB₁, AfB₂, AfG₁ and
AfG₂ (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) twice. 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
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 Absorption 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, lead, mercury, nickel, cobalt and cupper) in branded and unbranded honey samples.

Table 1 presents heavy metals concentration in branded and unbranded honey samples.

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>(0.13±0.02)</td>
</tr>
<tr>
<td>Mercury</td>
<td>(0.23±0.03)</td>
</tr>
<tr>
<td>Cadmium</td>
<td>(0.47±0.02)</td>
</tr>
<tr>
<td>Nickel</td>
<td>(0.65±0.02)</td>
</tr>
<tr>
<td>Cobalt</td>
<td>(0.17±0.01)</td>
</tr>
<tr>
<td>Manganese</td>
<td>(0.19±0.03)</td>
</tr>
</tbody>
</table>

In our samples Lead concentration (0.94±0.03) found maximum and copper (0.46±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 copper (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 been 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), cupper (0.46±0.02), nicle (0.41±0.03) and lead (0.39±0.03) were in moderate concentration.

Mercury 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 mercury (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 deflection, 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).

**Conclusion**

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)

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

* mean ± standard deviation

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

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

* mean ± standard deviation

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

<table>
<thead>
<tr>
<th>Honey Samples</th>
<th>B1</th>
<th>B2</th>
<th>G1</th>
<th>G2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marhaba</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Qarshi</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Versatile</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Al-hayat</td>
<td>1.25</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.25</td>
</tr>
<tr>
<td>Young’s honey</td>
<td>ND</td>
<td>2.14</td>
<td>ND</td>
<td>ND</td>
<td>2.14</td>
</tr>
<tr>
<td>Pak-salman</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Langness</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND= Not detected

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

<table>
<thead>
<tr>
<th>Honey Samples</th>
<th>B1</th>
<th>B2</th>
<th>G1</th>
<th>G2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Big bees honey</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Small bees honey</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Beera</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Palosa</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sperkay</td>
<td>ND</td>
<td>2.15</td>
<td>ND</td>
<td>ND</td>
<td>2.15</td>
</tr>
<tr>
<td>Bekerr</td>
<td>2.33</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>2.33</td>
</tr>
<tr>
<td>Granda</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND= Not detected
References

3/5/2014