ABSTRACT

Pure natural honeys are very expensive and difficult to identify by consumers. These have given unscrupulous people the opportunity of counterfeiting the genuine honey. Bacterial and fungal load counts in samples were found very high. Microorganisms of faecal origin were isolated. The nature of the micro flora suggested that most of the honeys in our market are adulterated or rather artificial. These findings may have some health implication and may be of value in food protection and control. It was recommended therefore that tools for mycobacterium detection and isolation or at least microscopy need to be put in place to assess and monitor the widespread of this organism through honey in our environment and in the market places.

Key words: natural honey, adulterated honey, fungi, bacteria and food borne diseases.

INTRODUCTION

Honey is a saccharine product gathered by bees from the nectar of flowers. It is available in myriad colours, varying from clear rich to dark rich amber. Its unique flavor is attributable to the floral source from which honey bees obtain nectar.

Adulteration of food is an act of debasing a commercial product with object of limiting or counterfeiting a pure genuine commodity for a superior one in order to gain an illegitimate profit (karma, 1974). Honey adulteration may potentially lead to the production of foods that are either harmful or indeed fatal. Perhaps the most publicized case has been the surveillance conducted by the federal ministry of agriculture (1991) that showed: 17 out of 21 honey samples bought from local marketers were adulterated with various sugar syrups.

Pure natural honeys are very expensive and are very difficult to identify by consumers. These have given the unscrupulous people the opportunity of counterfeiting a genuine honey. The addition of inexpensive sweeteners to honey increase and lower production costs. These are unwanted practices that harm the business of bee keepers. For the past twenty years cases of morbidity and mortality due to food poisoning and food borne illnesses has been on the rise. It must of course be remembered that cases of food borne illnesses that are notified represent the tip of the iceberg.

These studies would help consumers in identifying pure natural honey. It would draw the attention of indiscriminate users of honey on the dangers relating to the pathogenic microorganisms found in honey. The study would therefore serve as an aspect of food security/food control.

METHODS AND PROCEDURES.

Samples were purchased from the following areas: Wunti (WT), Central market (CM), Railway (RW), Bayara (BY) and Muda lawal (MD). All in Bauchi metropolis, Pure/ natural honey (PH) was sourced direct from bee keepers from Dumi village.

Bacterial and fungal (mould and yeasts) counts

Serial dilutions of 10^{-3} and 10^{-4} of the samples were prepared and 1ml was pour plated on nutrient agar (NA). The plates were incubated at 37^\circ C for 24 hours, (Jay, 2000). The bacteria count was carried out on daily basis using 0.1ml of 10^{-3} dilution Plates were inoculated in duplicate and incubated at 37^\circ C for 2-5
days as described by Odepidan and IL0 (1996). The result was calculated and expressed as colony forming unit per ml (cfu/ml) of the sample using the method of Odepidan, (1996). This was determined by multiplying the number of colonies on each plate by the specific dilution factor. The procedure was based on the assumption that each viable cell developed into a colony, hence the number of colonies on the plate revealed the approximate number of organism contained in the sample (Odepidan and IL0, 1996). The samples were plated out on potato dextrose agar for fungal count as described by Warmp (1950). The plates incubated at 25°C for 5 days and then examined for fungal growth. The plates were re-examined after one week for development of additional fungal species. Representative fungal colonies that develop were then stored on PDA slants in the refrigerator and later used for characterization and identification of the species.

**Isolation and Identification**

The bacterial isolate were subjected to Gram and Spore staining as described by Sneath et al (1986): smear was prepared from 18-25 hour culture, it was stained with crystal violet solution for at least 1– 2 minutes. Gram-negative bacteria are decolorized, losing the purple colour of crystal violet; Gram-positive bacteria were not decolorized and remain purple. Vegetative spores of the bacteria stains pink-red. While the spores stains green.

**Biochemical characteristics.**

Coagulase test, indole test, hydrogen sulphide, catalase test, Methyl red test, Starch hydrolysis and sugar utilization were conducted according to sneath et, al (1986).

The Fungal isolate were identified by making reference to Mycology atlas by Bernard and Gabriel (1980).

The yeast isolates were stained as outlined by FAO (1979) and then subjected to sugar fermentation test using glucose, fructose sucrose and maltose. The honey samples showed presence of sucrose, glucose, and fructose sugar in varying proportion. The mean sucrose content values range from 1.4±0.0 in PH, the lowest, to 3.8± 2.4 in CM, the highest.

**RESULTS**

Table : Bacteria and fungal mean counts of honey samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Bacteria (cfu/ml)</th>
<th>mould (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM</td>
<td>5.95x10⁵ ± 2.48</td>
<td>5.25x10⁵± 6.7</td>
</tr>
<tr>
<td>MD</td>
<td>7.52x10⁵ ± 2.82</td>
<td>4.37x10⁵ ± 2.5</td>
</tr>
<tr>
<td>WT</td>
<td>4.18x10⁵ ± 2.29</td>
<td>3.89x10⁵ ± 4.11</td>
</tr>
<tr>
<td>PH</td>
<td>3.45x10⁵ ± 4.2</td>
<td>2.72x10⁵ ± 3.2</td>
</tr>
<tr>
<td>BY</td>
<td>4.40x10⁵ ± 3.83</td>
<td>3.40x10⁵ ± 5.7</td>
</tr>
<tr>
<td>RW</td>
<td>9.06 x 10⁵ ± 3.32</td>
<td>4.44x10⁵ ± 3.42</td>
</tr>
</tbody>
</table>

Key: Values are mean ± standard deviation of triplicate determination.

The bacterial population is higher than fungal population. RW has higher bacterial count of 9.06x 10⁵ ± 3.32. Mould and yeast counts were higher in CM; 5.25 x 10⁵ ± 6.65. While PH had the lowest mould count; (2.72x10⁵ ± 3.2) than CM (5.95x10⁵ ± 2.48), WT (4.18X10⁵ ± 2.92), while PH had the lowest count of 2.72x10³±3.2.

**Species of Micro organisms Associated with the Collected Honey Samples**

The species of microorganisms associated with all the honey samples are Staphylococcus aureus, Bacillus sp and Clostridium Botulinum were the most common Bacteria isolated. While Mucor sp and Saccharomyces cerevisiae are the most common fungal isolate.

**DISCUSSION**

The bacterial load is found to be variable in the table. The mean load of RY had the highest count while the PH sample registered the lowest count. The bacterial population is high generally, which contradicted the low count expected in honey. Bacterial counts are often close to zero in honey, but may occasionally reach 10,000 cfu/gram. An average count might be 4.00cfu/g. (NHB, 2002). The high load of bacteria may be attributed to adulterations as this provide bacteria the opportunity to multiply and spoil the product (Doner, 1977).
Mould and yeast count
Fungal count is also found to be high with CM having the highest count, and PH recording the lowest count as shown in Table. The low fungal count in PH sample may be attributed to the freshness of the sample and genuineness of the samples. There are naturally occurring bacteria and fungi in honey; bacteria naturally present in honey is responsible for the antimicrobial property of honey (Marcia, 2002).

Distribution of Microbial Isolates.
The most common Bacteria isolate in all the samples was Clostridium Botulinum. This agrees with the survey made by National Honey Board (NHB, 2002), which stated that viable spores of C. Botulinum was examined in 10% of Honey samples. Consequently it is inadvisable to feed honey to children less than a year old (NHB, 2002). Other most common bacteria isolated from the samples were Staphylococcus aureus, Bacillus cereus, and Escherichia coli. The presence of Staphylococcus cereus, E. coli is an indication of faecal contamination. Although isolates S. aureus and E. coli were found in few samples from different areas of collection, but the microorganisms are pathogenic to man. Bacillus cereus cause toxic food poisoning and opportunistic infection in immunocompromised persons. (Cheesbrough, 2000). Some samples indicate honey adulteration some pure honey was found to inhibit effectively (NHB, 2002).

Mould and yeast isolates
Mucor sp. had the highest occurrence. Penicillium sp ranked second. Other fungi isolated were Aspergillus sp. These microbes form aflatoxin during storage of foods. The etiology of diseases such as liver carcinoma in rainbow front and hepatitis X in dogs which had been described nearly a decade but had remained a mystery (Adams, 1995). The moulds isolate (Penicillium sp.) causes spoilage of foods especially at high water activity as a result of adulteration. The yeast isolated were Toruplosis, Candida, Saccharomyces cerevisiae, Candida albicans and candida kruesei. The yeast are responsible for fermentation of honey, which occurs naturally in honey, in that they can germinate and grow at much higher sugar concentrations than other yeast, and, therefore are called “Osmophilic”. Honey ferments at higher moisture level, which may be due to adulteration (addition of water to honey) or poor storage that allows contamination. Saccharomyces cerevisiae is the most common isolates from the entire sample. It is the most frequently encountered yeast in fermented beverages and foods based on fruits and vegetables an observation, which is reflected in the existence of more than eighty synonyms and varieties for the species (Adams, et al.) When honey absorbs moisture, yeast grow aerobically (using oxygen) at the surface and multiply rapidly where as below the surface the growth is slower (White, 1980).

Analysis of variance
The one-way analysis of variance on Bacterial count (cfu/ml) showed that there was a significant different (P<0.05) between groups of sample. Also a significant difference P<0.05 was observed between the mould/ yeast count. The variability may be due to quality of the honey samples.

REFERENCES
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