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Determination of trace elements (As, Ca, Cu, Fe, Pb, Zn) and identification of *Clostridium perfringens* in honeys originated from different region of Bangladesh

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Abstract

Trace elements and heavy metal residues in honey samples originating from different regions of Bangladesh were determined. High mineral contents were observed with Ca, and Fe being the most abundant. The concentrations of the trace elements were within the recommended limits, indicating that the honeys were of good quality. Pb was present in trace amount which is not harmful for consumption where Arsenic was absent. *Clostridium perfringens* spores were not found in the analyzed honey samples.

Key words: Trace elements, heavy metals, honey, Bangladesh.

INTRODUCTION

There is no available information on trace elements and *Clostridium perfringens* properties on Bangladeshi honey. As honey is an important food ingredient due to its nutritive and medicinal values. There have been reports that honey contains many microorganisms including bacteria and fungi.

As per Alimentarius (1982) honey is the natural sweetener produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants which honeybees collect, transform and combine with specific substances of their own, store and leave in the honeycomb to ripen and mature. Honey gets its sweetness from the monosaccharide's fructose and glucose, and has approximately the same relative sweetness as that of granulated sugar by Oregon (2012).

Honey in spite of its usefulness is known to contain certain microbes. It is in fact described as a reservoir for microbes. The presence of microorganisms in honey may influence quality and safety. Due to various reasons, most bacteria and other microbes cannot grow or reproduce in honey. Honey has antimicrobial properties that prevent the growth of many microorganisms (Cliver & Snowdon, 1996). In addition, honey has a low water activity, preventing the multiplication and the survival of bacteria. However, few pathogens have been found in honey (Cliver & Snowdon, 1996). Basically, microbes

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cannot multiply in honey and existence of high numbers of vegetative bacteria might be due to recent contamination. Study showed that various bacteria inoculated into aseptically collected honey held at 20°C loss viability within 8-24 days (Olaitan *et al*., 2007). However, spore forming microorganisms can survive in honey at low temperature. Honey contamination with spores of *Clostridium* has been documented in many countries. Many spores of *Clostridium botulinum* type F were detected in different containers of honey products (Nakano & Sakaguchi, 1991). The present study was undertaken to determine the content of trace elements and heavy metals residues in honeys originating from different regions of Bangladesh. We primarily investigate six different trace elements (As, Ca, Cu, Fe, Pb, Zn) and *Clostridium perfringens* presence in monofloral and different multifloral honey samples collected from different parts of Bangladesh.

MATERIALS AND METHODS

Monofloral and multifloral honey samples were collected from Dhaka, Chittagong, Rajshahi, Rangpur, Sylhet, Barisal and Khulna division.

Six fresh honey samples are collected having the weights of 0.4335 gm (S_1), 0.4394 gm(S_2), 0.4393 gm(S_3), 0.4234 gm(S_4), 0.4643 gm(S_5), and 0.4697 gm(S_6) respectively into different specially designed tubes of Microwave digestor (Ethos one) using electronic balance. Honey contains mostly organic compounds with minute metallic constituents. In atomic absorption spectrophotometer analysis, the metals are needed to be liberated from the organic milieu followed by extracting the metals in mineral acids. Viewing that, all the samples are firstly digested using Microwave digestor (Ethos one) where HNO_3 and H_2O_2 have been used in a ratio of 7:1. After digestion, the samples are transferred to six different 100 ml volumetric flasks and made up to the mark using de-ionized water followed by sonication and filtration. Samples are examined using AAS (Shimadzu AA-7000) at furnace mode for specific metals. Standard curve is prepared with the strengths of standard stock solution of that specific metal of 5ppb, 10ppb, 15 ppb and 20ppb. The wavelength (nm) of the emission peak for Arsenic is 193.7.

10 gm of honey sample was passed through 0.22 μm pore-size Millipore membrane filter and then the filter paper was placed onto modified *Clostridium perfringens* medium (mCP). This mCP medium was then be incubated in an anaerobic jar at 44°C for 24 hrs. The yellow colonies were counted as *Clostridium perfringens*. Then the colonies will be further tested by exposing to ammonium hydroxide. A highly specific reaction will occur due to phosphate producing *Clostridium perfringens* colonies to turn a distinctive dark pink color.

RESULTS AND DISCUSSION

The present study showed high mineral contents in the investigated honeys with Ca and Fe being the most abundant elements. The concentrations of the trace elements were within the recommended limits, indicating that the honeys were of good quality. Pb was

present in trace amount. This amount is not harmful for consumption. where as Arsenic was absent. Table I showed physical condition, color, Ca, Cu, Fe, Zn, Pd and As levels in different regional honey samples of Bangladesh.

Table 1. Physical condition, color, Ca, Cu, Fe, Zn, Pd and As levels in honey samples collected from different regions of Bangladesh

Name of regions	Physical Condition	Color	Ca (ppm)	Cu (ppm)	Fe (ppm)	Zn (ppm)	Pb (ppm)	As (ppm)
Dhaka	Liquid	Light Yellow	2.35	0.48	45.18	2.46	Trace	Not detected
Sylhet	Liquid	Light Yellow	1.29	0.48	42.78	3.51	Trace	Not detected
Chittagong	Liquid	Light Yellow	1.20	0.45	36.66	3.45	Trace	Not detected
Barisal	Liquid	Light Yellow	0.86	0.51	46.06	0.72	Trace	Not detected
Khulna	Liquid	Light Yellow	0.99	0.51	22.74	0.78	Trace	Not detected
Rangpur	Liquid	Light Yellow	2.31	0.48	19.95	1.47	Trace	Not detected
Rajshahi	Liquid	Light Yellow	2.48	0.48	18.91	1.35	Trace	Not detected

Clostridium perfringens spores were not found in the analyzed honey samples.

Honey can be a good source of major and trace elements needed by humans, it contains metals up to 0.17%. Metals such as Cr, Co, Cu, Fe, Mn and Zn are essential for humans, and they may play an important role in a number of biochemical processes (Garrett *et al.*, 1998). Some of them are present at the trace level, being toxic if they exceed safety levels (Przybyłowski & Wilczyńska, 2001). Honey is the result of a bioaccumulation process useful for the collection of information related to the environment where bees live. Honey has been recognized as a biological indicator of environmental pollution (Celli & Maccagnani, 2003; Rashed & Soltan, 2004; Muñoz & Palmero, 2006). As a food stuff used for healing purposes, honey must be free of any objectionable content and should contain only small amounts of pollutants, such as heavy metals (Jarić, 2011).

Since honey is a nutritional resource that depends on biotic and abiotic factors around the beehives, the presence of heavy metals could be related to its geographical and botanical origin. Several authors have indicated that bees and their products may be used as biological indicators of the environmental pollution present in the area where they fly. Honey is a natural product that is widely used for both nutritional and medicinal purposes, like other foods is prone to various types of contaminations and adulterations. Microbial and non microbial contaminants which include heavy metals, pesticides, herbicides, antibiotics have been reported in various honey samples all over the world. The present study was undertaken to determine the content of trace elements and heavy metals residues in honeys originating from different regions of Bangladesh. Calcium (Ca), Copper (Cu), Iron (Fe), Lead (Pb), Zinc (Zn) and Arsenic (As) were analyzed by atomic

absorption spectrophotometer. We find good amount of minerals (Fe, Cu, Ca, Zn) in the samples and Heavy metals like Pb and As was not found as well as these samples had no *Clostridium perfringens* spores.

Honey ingestion without knowing its source and safety might carry significant health hazards. Labeling of honey must be supported by analysis that confirms its provenance and safety. Further research projects should be launched to perform high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) etc. To detect the specific and particular identification of trace elements, more heavy metals and the factors by which some minerals and trace element contents are higher in some samples than others.

REFERENCES

Alimentarius, C. 1982. "Revised codex standard for honey." *Codex stan*, 12.

Celli, G. and Maccagnani, B. 2003. "Honey bees as bioindicators of environmental pollution." *Bulletin of Insectology,* **56**:1: 137-139.

Cliver, J. A. and Snowdon, D. O. 1996. "Microorganisms in honey." *International Journal of FoodMicrobiology,* **31**:1-3: 1-26.

Garrett, E.S., Jahncke, M.L. and Cole, E.A. 1998. "Effects of Codex and GATT." *Food Control,* **9**:2: 177-182.

Jarić, I. 2011. "Determination of differential heavy metal and trace element accumulation in liver, gills, intestine and muscle of sterlet (Acipenser ruthenus) from the Danube River in Serbia by ICP-OES." *Microchemical Journal,* **98**:1: 77-81.

Muñoz, E. and Palmero, S. 2006. "Determination of heavy metals in honey by potentiometric stripping analysis and using a continuous flow methodology." *Food Chemistry,* **94**:3: 478-483.

Nakano H. and Sakaguchi G. 1991. "An unusually heavy contamination of honey products by Clostridium botulinum type F and Bacillus alvei." *FEMS Microbiology Letters,* **79**:2-3: 171-177.

Olaitan P. B., Adeleke, O. E. and Ola, I. O. 2007. "Honey: a reservoir for microorganisms and an inhibitory agent for microbes." *African Health Sciences,* **7**:3: 159-165.

Oregon, S. 2012. "What is the relative sweetness of different sugars and sugar substitutes?"

Przybyłowski, P. and Wilczyńska, A. 2001. "Honey as an environmental marker." *Food Chemistry,* **74**:3: 289-291.

Rashed, M. and Soltan, M. 2004. "Major and trace elements in different types of Egyptian mono-floral and non-floral bee honeys." *Journal of food composition and analysis,* **17**:6: 725-735.