

Study on the Quality of Honey Collected from Three Floral Sources of Bangladesh

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Abstract- An investigation on quality parameters of honey collected from mustard, litchi and sundarban (multiflora) flora of Bangladesh was undertaken to compare with European Union (EU) quality standard viz., moisture content, electric conductivity, specific rotation, hydroxymethylfurfural (HMF) content, invertase content, antibiotic content and sulfonamides content. Sundarban honey (multiflora) (17.7g/100g) and mustard honey (19.5g/100g) were found in standard moisture condition and the litchi honey was found above the limit of EU standard (20g/100g). All the honey samples were found in the EU standard limit in terms of electrical conductivity and specific rotation. The HMF content was found higher (200.5mg/kg) in sundarban sample and no HMF was observed in the litchi honey. Highest invertase activity (99 IU/kg) was found in the litchi honey where as mustard and sundarban honey showed very low invertase activity with 2.0 IU/kg and 0.0 IU/kg respectively which was far below than EU standard (50 IU/kg). All the samples had shown negative reaction with tetrasensor and sulfasensor. So, it may be concluded that the honey from sundarban was highly overheated. On the other hand, the mustard and litchi honey were overheated and not heated respectively. Sundarban and mustard honey had failed to reach the quality standard to the EU. But litchi honey was found to met the all the EU quality requirements except the moisture content.

Index Terms- Hydroxymethylfurfural, Litchi honey, Mustard honey and Sundarban honey

I. INTRODUCTION

Honey has been called the “original medicine” and has been revered cross-culturally as a gift from the gods. It is a popular sweetener throughout the world. As the only available natural sweetener honey was an important food for *Homo sapiens* from his very beginnings. Indeed, the relation between bees and man started as early as Stone Age. The first written reference to honey, a Sumerian tablet writing, dating back to 2100-2000 BC, mentioned honey’s use as a drug and an ointment (Crane, 1975). In the long human tradition honey has been used not only as a nutrient but also as a medicine. An alternative medicine branch, called apitherapy, has developed in recent years, offering treatments based on honey and the other bee products against many diseases (Molan, 2001). From ancient times, honey was not only used as a natural sweetener but also as a healing agent. Many health-promoting and curative properties

attributed to it are the basis for some traditional folk medicine treatments throughout the world today. Of the consumers who use honey, 93 percent consider honey a healthful product, recognizing it as a pure, natural product. Seven percent think of it as a good home remedy (Krishna, 2005). At present, the annual world honey production is about 1.2 million tons, which is less than 1% of the total sugar production. The major honey exporting countries China and Argentina have small annual consumption rates of 0.1 to 0.2 kg per capita. Honey consumption is higher in developed countries, where the home production does not always cover the market demand. In the European Union, which is both a major honey importer and producer, the annual consumption per capita varies from medium (0.3-0.4 kg) in Italy, France, Great Britain, Denmark and Portugal to high (1-1.8 kg) in Germany, Austria, Switzerland, Portugal, Hungary and Greece, while in countries such as USA, Canada and Australia the average per capita consumption is 0.6 to 0.8 kg/year (Molan, 2001). However, in recent years, governments of many countries have increased control over food production, processing, and distribution to protect consumers against the biological, chemical, and environmental contamination of food. There have been a number of cases where contaminated honey has been found on sale, including India and China. Such cases have demonstrated that systems were inadequate to prevent contamination and led to demands for more stringent systems to prevent contamination in honey. Honey can be contaminated from the environment and from beekeeping practices. Environmental contaminants can reach the raw materials of bee products (nectar, honeydew, pollen, plant exudates) through the air, water, plants, and the soil. These contaminants include insecticides, herbicides, bactericides, fungicides, heavy metals and radio active substances. The main contaminants of honey from beekeeping practices are substances used to control the bee pest *Varroa* and the disease fowlbrood. Varroacides, antibiotics, residues of para-dichlorobenzene (PCDB) and other more toxic substances, such as naphthalene, which is used by beekeepers to control wax moths and storage vessels, are the main contaminants of from beekeeping practices. Honey can be contaminated with heavy metals from inappropriate storage containers as inorganic or organic components can diffuse from the inner surface of paraffinated, corrosive and painted vessels into honey. Increased iron concentration caused by storing honey in metal containers is a common problem. These chemicals are not only hazardous for health but also make a barrier for honey trade both national and international market. Bangladesh is exporting a little quantity of

honey in the Middle East especially in Saudi Arabia and in the United Arab Emirates. Beside these, due to low price in the domestic market beekeepers are trying to export their honey in the developed country mainly in the European countries. To get permission to export in the EU countries Bangladesh honey needs to qualify some parameters to meet the EU standard. Hence, the present study was undertaken to compare some important parameters of Bangladesh honey to meet the EU standard.

II. MATERIALS AND METHODS

Honey Sample:

Two samples were collected from migratory beekeepers who migrate their hives to mustard and litchi flora during its flowering season and were designated as mustard honey and litchi honey. Another honey sample was collected from Mowali who gathered honey from Sudarban (multifloral source) located in Khulna district of Bangladesh. This honey was named as Sundarban honey. Mustard and litchi honey were known to be processed honey and the processing method was also known. Mustard honey was processed through steaming and the litchi honey was processed through filtering. But processing method of the Sundarban honey samples was not known.

All the samples were tested in the laboratory of Instituut voor Landbouw- en Visserijonderzoek (ILVO), ministry of Agriculture, Belgium in June to August 2010 and 2011.

The parameters of honey quality were 1. Moisture content 2. Electric conductivity 3. Specific rotation 4. Hydroxymethylfurfural (HMF) content 5. Invertase content 6. Antibiotic content and 7. Sulfonamides content. All the quality parameters were tested as described by Reybroeck *et al.* 2007 and explained under the following subheadings:

Sample preparation for determining moisture content:

Honey samples were homogenized for 3 minutes. As the mustard honey sample is in solid form, a part of the honey was liquefied by heating in a water bath at 50°C ($\pm 2^\circ\text{C}$) for 20 minutes in a closed glass flask. The flask was in airtight condition. Honey sample were mixed firmly with a dry and clean glass rod. Honey samples were then passed through a 0.5 mm sieve to remove non soluble particles (bee particles, wax, egg), and any coarse material. Honey samples were kept for cooling at room temperature (25°C) and were stirred again and again.

Measurement of water:

Water has a refractive index of 1.3330 at 20°C (range 1.3327 – 1.3333). When the temperatures were above 20°C, a constant number of 0.00008 was added for each °C increased and when the temperatures decreased below 20°C, 0.00008 was subtracted for each °C.

Measurement of honey refractive index:

The refractive index was corrected for the temperature. It was done by adding 0.00023 per °C temperatures increase above 20°C and subtracting 0.00023 per °C the temperatures decrease below 20°C.

Calculation of the refractive index at 20°C

Measurements were repeated three times and mean measurements were taken. The difference among the measurements (corrected to 20.0 °C) were found between the range of 0.0004.

Calculation and expression of results

The mean refractive index (at 20°C) was converted to moisture content [in % (g/100 g)]: Moisture Content = $[-\log_{10}(\text{Corrected Refractive Index} - 1) - 0.2681] / 0.002243$.

The result was expressed as moisture content in % (g/100 g) with a 1 digit after the comma.

Norm

Council Directive 2001/110/EC of 20 December 2001 relating to honey:

In general : Not more than 20.0%

Heather (*Calluna*) an baker's honey in general: Not more than 23.0%

Baker's honey from heather (*Calluna*): Not more than 25.0%

Determination of electrical conductivity:

The electrical conductivity of honey is defined as that of a 20% weight in volume solution in water at 20°C, where the 20% refers to honey dry matter. Result is expressed in milliSiemens per centimeter ($\text{mS}\cdot\text{cm}^{-1}$).

Reagents required for the determination of electrical conductivity were freshly prepared distilled water, potassium chloride solution, 0.1M. Potassium chloride (KCl) weighing 7.4557 g was dissolved, dried at 130°C, in freshly distilled water in a 1000 ml flask and filled to volume with distilled water.

Equipment requirements were conductivity meter and cell. Model of conductivity cell was Consort SK10T, BB8. Water bath required at a temperature of 20°C \pm 0.5°C. Volumetric flasks of 100 ml and 1000 ml and Beakers, tall form were also required.

Sample preparation procedure for electric conductivity determination

A part of honey was dissolved, equivalent to 20.0 g anhydrous honey, in distilled water. The solutions were transferred quantitatively to a 100 ml volumetric flask and made up to volume with distilled water.

The beakers were placed with the sample solutions in the thermo stated water bath at 20°C \pm 0.5°C. The conductivity cell was dried with absorbing paper. The conductivity cell was immersed in the sample solutions. The conductances were read in mS after temperature equilibrium (20°C) has been reached. The conductance (G) and the temperature were read and noted.

As the determination was carried out at a different temperature, due to lack of thermostated cell, a correction factor were used for calculation of the value at 20°C:

For temperatures above 20°C: 3.2% value was subtracted for each °C increase and for temperature decrease below 20°C: 3.2% value was added for each °C decrease. The electrode was rinsed with distilled water.

Calculation and expression of results

The electrical conductivity of the honey solution was calculated, by using the following formula:

$$S_H = K.G$$

Where: S_H = electrical conductivity of the honey solution in mS.cm^{-1} , K = cell constant in cm^{-1} , G = conductance in mS. The results were expressed to the nearest 0.01 mS.cm^{-1} .

Determination of the cell constant

As the cell constant of the conductivity cell was not known, it was determined by transferring 40 ml of the 0.1M potassium chloride solution to a beaker. Then, the conductivity cell was connected to the conductivity meter. After that, the cell was rinsed thoroughly with the potassium chloride solution and immersed the cell in the solution, together with a thermometer. The electrical conductance of those solutions was read in mS after the temperature was equilibrated to exactly 20°C .

Calculation of the cell constant K by using the following formula: $K = 11.691 \times 1/G$. Where: K = the cell constant in cm^{-1} , G = the electrical conductance in mS, measured with the conductivity cell and 11.691 = the sum of the mean value of the electrical conductivity of freshly distilled water in mS.cm^{-1} and the electrical conductivity of 0.1M potassium chloride solution, at 20°C .

Norm

Council Directive 2001/110/EC of 20 December 2001 relating to honey.

- Honey not listed below, and blends of these honeys : $\leq 0.80 \text{ mS/cm}$

- Honeydew and chestnut honey and blends of these except with those listed below : $> 0.80 \text{ mS/cm}$

- Exceptions: strawberry tree (*Arbutus unedo*), bell heather (*Erica*), eucalyptus, lime (*Tilia* spp), Ling heather (*Calluna vulgaris*), manuka or jelly bush (*Leptospermum*), tea tree (*Melaleuca* spp.).

Determination of specific rotation:

The method can be applied to all honey samples. In particular, most of the honeydew samples have positive values of specific rotation whereas nectar honeys have negative values.

The specific rotation $[\alpha]_D^{20}$ is the angle of rotation of polarized light at the wavelength of the sodium D line at 20°C of an aqueous solution of 1 dm depth and containing 1g/ml of the substance.

Reagents required were distilled water, Carrez I solution (10.6 g potassium hexacyanoferrate(II), $(\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O})$ was dissolved in distilled water and diluted to 100 ml, Carrez II solution (24 g zinc acetate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$) was dissolved in distilled water, 3g (= 2.86 ml) of glacial acetic acid was added and diluted to 100 ml with distilled water.

Equipment required were Polarimeter+sodium lamp+2-dm polarimeter tube, volumetric flasks (100 ml), filtration funnels and filtration papers.

Sample preparation procedure

Twelve g honey samples were weighed (corresponding to about 10 g dry substance) and then the honey were dissolved in distilled water, 10 ml of Carrez I solution was added in each sample and mixed thoroughly for 30 seconds. Ten ml of Carrez II solution was added in each sample and mixed again for 30 seconds and was made up to volume in a 100 ml volumetric flask with distilled water. The samples were allowed to stand overnight at room temperature. The next day, the solutions were filtered.

Test procedure

Zero was set with distilled water. A clean 2-dm polarimeter tube was rinsed and filled with the filtered honey solution. The tube was placed in the polarimeter and the angular rotation (α) was read. The measurements were taken at the temperature of 20°C .

Calculation and expression of results

Specific angular rotation $[\alpha]_D^{20} = \alpha \times 100 / l \times p$ (When using 10 g of honey (dry matter) and a 2 dm polarimeter tube $[\alpha]_D^{20} = \alpha \times 5$).

Where, α = angular rotation found, l = length in decimeters of the polarimeter tube and p = grams of dry matter taken.

(If the wavelength of the light used is 589 nanometer (the sodium D line), the symbol 'D' is used. The sign of the rotation (+ or -) is always given. The formal unit for specific rotation is $\text{deg cm}^2 \text{ g}^{-1}$ but scientific literature uses just degrees). Results were at one decimal place. Most of the honeydew samples have positive values of specific rotation whereas nectar honeys have negative values.

Determination of Hydroxymethylfurfural (HMF) (as described by White, 1979):

The method determines the concentration of 5-(hydroxymethyl-) furan-2-carbaldehyde. The result is usually expressed in milligrams per kilogram. The HMF content was used as a heat-treatment indicator. The determination of the hydroxymethylfurfural (HMF) content was based on the determination of UV absorbance of HMF at 284 nm (White, 1979). In order to avoid the interference of other components at this wavelength the difference between the absorbances of a clear aqueous honey solution and the same solution after addition of bisulphate was determined. The HMF content is calculated after subtraction of the background absorbance at 336 nm. This method is based on the original work of White.

Reagents required were Carrez solution I: [15 g of potassium hexacyanoferrate (II), $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$ was dissolved in water and was made up to 100 ml], Carrez solution II: [30 g zinc acetate, $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ was diluted and made up to 100 ml], sodium bisulphate solution 0.20 g/100g [0.20 g of solid sodium hydrogen sulphite NaHSO_3 , (sodium metabisulphite, $\text{Na}_2\text{S}_2\text{O}_5$) was dissolved in water and diluted to 100 ml. Freshly prepared 5-Hydroxymethyl-2-furaldehyde, Sigma H40807.

Equipment required, UV-Visible Spectrometer operating in a wavelength range including 284 and 336 nm. 1 cm quartz cells or disposable UV-cuvettes (Brand 7591 50 or equivalent), vortex mixer, filter paper.

Preparation of sample and reference solutions

Five g of honey was accurately weighed into a 50 ml beaker (filtration tube). The samples were dissolved in approximately 25 ml of water and transferred quantitatively into a 50 ml volumetric flask. Now 0.5 ml of Carrez solution I was added and mixed. Then 0.5 ml of Carrez solution II was added and mixed and made up to the mark with water (a drop of ethanol was added to

suppress foam). Filtration was done through paper; the first 10 ml of the filtrate was rejected. Five ml in each of two 2 test tubes (18 x 150 mm) were pipetted.

Dilution of sample and reference solutions were carried out as follows:

Additions to test-tube	Sample Solution	Reference solution
Initial honey solution	5.0 ml	5.0 ml
Water	5.0 ml	-
0.2 % sodium bisulphate solution	-	5.0 ml

Test procedure

The absorbance of the sample solutions were determined against the reference solution at 284 and 336 nm in 10 mm quartz cells within one hour.

(If the absorbance at 284 nm exceeds a value of about 0.6, the sample solution was diluted with water and the reference solution with sodium bisulphate solution to the same extent in order to obtain a sample absorbance low enough for accuracy)

If dilution was necessary,

$$\text{The Dilution factor 'D', where } D = \frac{\text{Final volume of sample solution}}{10}$$

If no dilution was necessary, the dilution factor = 1.

Calculation and expression of results

$$\text{HMF in mg/kg} = (A_{284} - A_{336}) \times 149.7 \times 5 \times D/W$$

Where:

A₂₈₄ = absorbance at 284 nm, A₃₃₆ = absorbance at 336 nm,
 $\frac{126 \times 1000 \times 1000}{16830 \times 10 \times 5}$

149.7 = $\frac{16830 \times 10 \times 5}{126}$ = Constant, 126 = molecular weight of HMF, 16830 = molar absorptivity of HMF at $\lambda = 284$ nm, 1000 = conversion g into mg, 10 = conversion 5 into 50 ml, 1000 = conversion g of honey into kg, 5 = theoretical nominal sample weight, D = dilution factor, in case dilution is necessary, W = Weight in g of the honey sample. The results were expressed in mg/kg to 1 decimal place.

Note

Some honeys, such as lime, may show strong absorbance at 284nm due to interfering substances. If these have an absorbance at 336 nm different to that at 284 nm, the result will be in error. This difficulty is normally overcome by using a double-beam spectrophotometer. In the absence of such instrument, sample dilution may be tried. In this case the dilution factor must be taken into account when calculating the result. If dilution is too great to give adequate accuracy, an alternative method should be used.

Council Directive 2001/110/EC of 20 December 2001 relating to honey.

Hydroxymethylfurfural content (HMF) determined after processing and blending

In general, except baker's honey: Not more than 40 mg/kg
Honeys of declared origin from regions with tropical climate and blends of these honeys: not more than 80 mg/kg

Determination of invertase as described by White (1994)

The invertase activity, a freshness parameter is expressed in units, where one unit is defined as the number of micromoles of substrate destroyed per minute and expressed per kilogram of honey. p-Nitrophenyl- α -D-glucopyranoside (pNPG) is used as a substrate for the determination of the sucrase number in honey. p-Nitrophenyl- α -D-glucopyranoside (pNPG) is split into glucose and p-nitrophenol by α -glucosidase (invertase, sucrase). By adjusting the pH to 9.5 the reaction was stopped and at the same time nitrophenol was transformed into the nitrophenolate anion, which corresponds to the amount of converted substrate and is determined photometrically at 400 nm.

Reagents required for the determination of invertase

Buffer solution (0.1 M, pH=6.0) [11.66 g of potassium hydrogen phosphate $\text{KH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ was dissolved in water and diluted to 1L], Substrate p-nitrophenyl- α -D-glucopyranoside (pNPG)solution, 0.02M [6.0252g pf pNPG was dissolved in buffer solution and made up to 1L (dissolved by heating (never above 60°C)]. Up to 1 month it was stored in a dark bottle in the refrigerator. STOP solution (3M, pH = 9.5) [363.42g of tris-(hydroxymethyl) aminomethane was dissolved in water and diluted to 1L. pH was adjusted to 9.5 with 3M hydrochloric acid. Equipment required: Photometer at 400 nm, 1 cm disposable UV-cuvettes (Brand 7591 50 or equivalent), Thermostated water bath (40 ± 0.5 °C) and vortex mixer.

Preparation of sample: Honey solution: 5g of honey + buffer solution in 25 ml flask and made up to the mark with buffer solution (was kept in the refrigerator for 1 day).

Procedure

Five ml of substrate solution was placed in test tube at 40°C. 0.50 ml of honey solution was added (starting time). The samples were then mixed and incubated at 40°C. After exactly 20 minutes

0.50 ml of STOP solution was added and mixed (sample solution).

For the blank, 5.0 ml of substrate solution was incubate at 40°C at the same time. 0.50 ml of STOP solution was added and mixed and 0.50 ml of honey solution was added. The solutions were cooled to room temperature and within one hour the absorbances were measured for the samples and the blank solutions in 1 cm cells at 400 nm.

Calculation and expression of results:

The amount of p-nitrophenol (μM) produced corresponds to the amount of substrate (μM) utilised.

Invertase (1 U/kg) = $1 \mu\text{mol pNPG} / \text{minutes} \times \text{kg honey}$

$\text{Invertase in U/kg} = 6 \times 0.05 \times 0.05298 \times 104 \times \Delta\text{A}400 = 158.94 \times \Delta\text{A}400$

Where, U = 1 international unit with a defined utilisation of $1 \mu\text{M}$ per minute, 6 = factor for the ml of sample solution used (total volume), 0.05 = converts reaction time from 20 minutes to 1 minute, 104 = converts the amount of honey taken (0.1 g in 0.5 ml) to 1 kg, 0.05298 = $7.37 / 139.11$; conversion factor for μg into μM per ml, where 7.37 = factor for p-nitrophenol from the corresponding graph, 139.11 = molecular weight of p-nitrophenol. Reported to one decimal place.

Invertase number (IN) = $21.64 \times \Delta\text{A}400$ where 21.64 = slope of linear regression of IN (y-axis) on $\Delta\text{A}400$ (x-axis).

Norm

No norm in Council Directive 2001/110/EC of 20 December 2001 relating to honey.

International Honey Commission proposal:

In general, except baker's honey: $\geq 50 \text{ (IU)}$

Honey with a low natural enzyme content: $\geq 20 \text{ (IU)}$

Arbutus, Robinia, Erica : $\geq 10 \text{ (IU)}$

IU: Siegenthaler units

Determination of tetracyclines in honey (as described by Reybroeck, 2007):

This method describes a procedure to determine residues of tetracyclines in honey by means of TetraSensor Honey (Unisensor s.a., Wandre, BE). The method can be applied to all types of honey.

Principle of the Tetrasensor Honey

The Tetrasensor Honey is a receptor assay for the fast screening of residues of tetracyclines in honey. During this first incubation period tetracyclines possibly present in the honey bind with the specific receptor. During the second incubation the sample is migrating over an immunochromatographic dipstick with two capture lines. The first line (TEST) captures the remaining active receptor and the second line (CTRL) serves as a control line. The colour intensity at both capture lines is interpreted visually.

Reagents required

Tetrasensor Honey testkit (the kit was stored in a refrigerator ($2-8 \text{ }^\circ\text{C}$) until the expiration date stated on the kit label), vials with lyophilised receptor and the immunochromatographic dipsticks (kept 1 day at room temperature), the testkit TH00616 was for 25 assays, testkit TH00624 was for 100 assays,

chlortetracycline hydrochloride (Sigma C4881, stored at -18°C), distilled water.

Standards

Blank honey (free from tetracyclines) and Honey with $10 \mu\text{g/kg}$ (ppb) chlortetracycline.

Equipment required: Small spatula or glass rod and waterbath at $37 \pm 2 \text{ }^\circ\text{C}$.

Sample preparation

For liquid and semisolid honey sample preparation was not required. Solid honey was liquified by heating in a glass test tube in a water bath at $37 \pm 2 \text{ }^\circ\text{C}$.

Test procedure

The lid of the honey dilution tube was filled with honey. Then, the samples were vigorously mixed to dissolve all honey. $200 \mu\text{l}$ of the dilution was added to the lyophilised receptor into the reagent vial. The sample was mixed by swirling the vial. After that, the samples were incubated at room temperature ($20 \pm 5^\circ\text{C}$) for 15 minutes. A dipstick (with the arrows downwards) was dipped into the vial. The dipstick was making contact with the liquid. The incubation was continued for 15 minutes. The coloured lines were interpreted as shown in the figure.

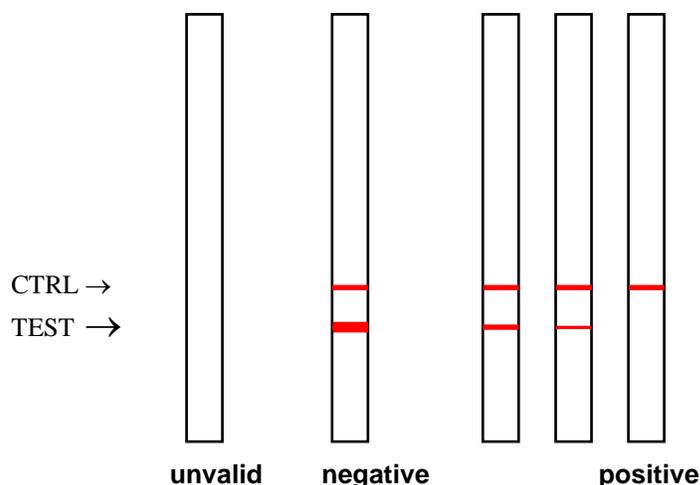


Figure 1. Tetrasensor strip reaction

The visual interpretations were as follows: when the colour of the test line is more intensive than the colour of the control line, the honey sample is negative ('vis neg'). In all other cases the honey is contaminated with tetracyclines ('vis pos') (Figure 1).

Quality control and control criteria

Besides the samples to be tested, always include a blank control honey and a honey doped with $10 \mu\text{g/kg}$ chlortetracycline the same day. The blank honey must give a negative result. The honey doped with $10 \mu\text{g/kg}$ chlortetracycline must give a positive result. The second capture line on the dipstick serves as a control line and always has to become visible; otherwise the test is invalid. This is shown in the figure 1.

Expression of results: A positive result was expressed as 'Result: +, tetracyclines present); a negative result was expressed as 'Result: -, tetracyclines not detectable'.

Norm

Regarding the European legislation (EEC Regulation 2377/90 and modifications) the use of antibiotics is not allowed in apiculture: no MRLs are fixed for tetracyclines in honey (zero tolerance).

Determination of sulfonamides in honey (as described by Reybroeck, 2007):

This method describes a procedure to determine residues of sulfonamides in honey by means of SulfaSensor Honey (Unisensor s.a., Wandre, BE). The method can be applied to all types of honey.

Principle of the SulfaSensor Honey

The SulfaSensor Honey is a receptor assay for the fast screening of residues of sulfonamides in honey. Sample pretreatment = hydrolysis to release the sulfa's possibly bound on the sugars. During the first incubation step of the assay, sulfonamides possibly present in the honey bind with the specific receptor. During the second incubation the sample is migrating over an immunochromatographic dipstick with two capture lines. The first line (TEST) captures the remaining active receptor and the second line (CTRL) serves as a control line. The colour intensity at both capture lines was interpreted visually.

Reagents required

SulfaSensor Honey testkit (the kit was stored in a refrigerator (2–8°C) until the expiration date stated on the kit label), vials with lyophilised receptor and the immunochromatographic dipsticks (kept 1 day at room temperature), Sulfamethazine (Sigma S6256, stored between 2-6°C) and distilled water.

Standards

Blank honey (free from sulfonamides) and honey with 20 µg/kg (ppb) sulfamethazine.

Equipment required: Small spatula or glass rod, gas burner, beaker or pot in heat-resistant glass or in metal and dry-block Heatsensor incubator at 40°C (Waterbath at 37±2 °C).

Sample preparation

Solid honey was liquified by heating in a glass test tube in a water bath at 37±2 °C. The neutralizing buffer was completely dissolved before use. Boiling water was prepared. The cap of the tube was filled with honey (650 mg). 600 µl (3 micropipettes) of acid buffer was added to the tube and vigorously mixed to dissolve all honey. When water was boiling the water was removed from the fire and directly incubated the honey tube sample for 5 minutes using the floating tube support. The tube from the warm water was removed and was open carefully. 600 µl (3 micropipettes) of neutralizing buffer was added, the cap was closed and vortex was done. 1800 µl (9 micropipettes) of honey buffer (total volume = 3.5 ml) was added, the cap was closed and vortex was done.

Test procedure

The corresponding number of freeze-dried reagent microwells was opened and placed them in the Heatsensor at 40°C. Directly 200 µl of the honey sample tube solution was added to the lyophilised receptor, the samples were mixed 5-10 times by pushing up and down the plunger of the micropipette. Incubation was done for 5 minutes at 40°C. A dipstick (with the arrows downwards) was dipped into the vial. The dipstick was making contact with the liquid. The incubation was continued for 15 minutes at 40°C. The dipstick and the filter were removed directly. The samples were interpret within 10 minutes and the coloured lines as shown in the figure or perform an instrumental reading.

The visual interpretations were as follows: when the colour of the test line is more intensive than the colour of the control line, the honey sample is negative ('vis neg'). In all other cases the honey is contaminated with sulfonamides ('vis pos') (Figure 2).

Quality control and control criteria

Besides the tested samples, a blank control honey and a honey doped with 20 µg/kg sulfamethazine was included on the same day. The blank honey gave a negative result. The honey doped with 20 µg/kg sulfamethazine gave a positive result. The second capture line on the dipstick served as a control line and always became visible.

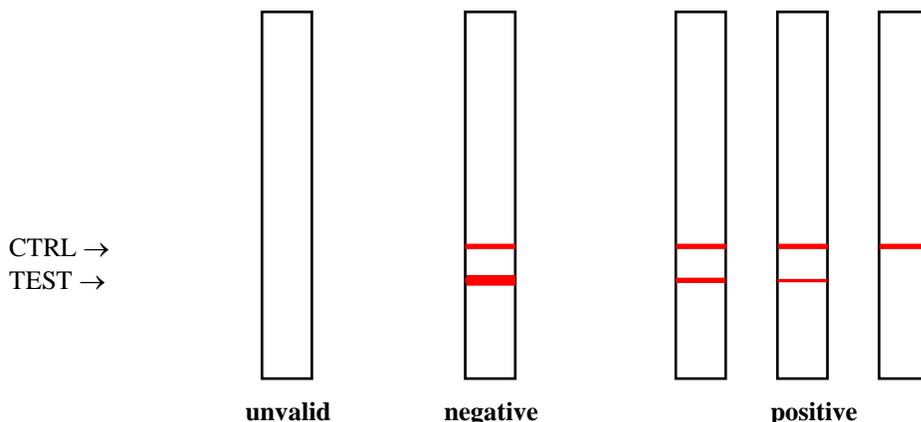


Figure 2. Sulfasensor strip reaction

Expression of results

A positive result is expressed as ‘Result: +, sulfonamides present); a negative result is expressed as ‘Result: -, sulfonamides not detectable’.

Norm

Regarding the European legislation (EEC Regulation 2377/90 and modifications) the use of antibiotics is not allowed in apiculture: no MRLs are fixed for sulfonamides in honey (zero tolerance). In some countries an action limit is applied (e.g.in Belgium, set at 20 µg per kg).

III. RESULTS AND DISCUSSION

Moisture content

Highest moisture content (21.0g/100gm) was obtained in litchi honey which is followed by mustard (19.5g/100gm) and sudarban honey (17.7g/100gm) respectively (Table 1). Among the three honey samples sudarban honey and mustard honey met the EU standard (20g/100gm) but moisture content in litchi honey was higher and failed to reach export quality.

Table 1. Comparative moisture content of Bangladesh honey to EU standard

Type of honey	Mean moisture content (g/100 g)	EU standard* (g/100 g)
Mustard honey	19.5	not more than 20
Litchi Honey	21.0	not more than 20
Sudarban honey	17.7	not more than 20

* Council Directive 2001/110/EC of 20 December 2001 relating to honey

In general, high moisture content occurred if honey is harvested in unripe condition and in that case moisture content is crossed over EU limit (20g/100gm). To maintain the acceptable limit honey is processed either in processing plant or manually through steaming method. As the mustard honey samples were steamed its’ moisture content was found low. Sundarban honey generally marketed after processing manually or through processing plant and its moisture content remained below 20%. The litchi honey was collected as fresh condition and no processing was done (only filtered), and therefore, its moisture content was high. This finding is supported by the work of Reybroeck *et al.* (2007).

Electrical conductivity

Highest electrical conductivity (0.213 mS/cm) was recorded in sudarban honey (Table 2). The lowest electrical conductivity (0.158 mS/cm) was found in litchi honey. All the honey samples were in the acceptable limit of EU standard (≤ 0.80 mS/cm). So, each type of honey has the quality to enter in the EU market in terms of electrical conductivity.

Table 2. Comparison of electrical conductivity of Bangladesh honey to EU standard

Type of honey	Average electrical conductivity (mS/cm)	EU standard* (mS/cm)
Mustard honey	0.175	≤ 0.80
Litchi Honey	0.158	≤ 0.80
Sudarban honey	0.213	≤ 0.80

* Council Directive 2001/110/EC of 20 December 2001 relating to honey

The results indicated that the honey samples were from the floral source only. If honey is mixed with sugar syrup its electrical conductivity crossed EU standard (0.80 mS/cm).

Specific rotation

The highest specific rotation (-11.0 deg cm² g⁻¹) was recorded in mustard honey and the lowest specific rotation (-13.0 deg cm² g⁻¹) was in Sudarban honey (Table 3). All the honey samples showed negative specific rotation. So, all the honey samples were obtained from floral origin (nectar honey) and all the samples were in the acceptance status of EU standard.

Table 3. Comparison of specific rotation of Bangladesh honey to EU standard.

Type of honey	Average specific rotation (deg cm ² g ⁻¹)	EU standard* (deg cm ² g ⁻¹)
Mustard honey	-11.0	(+) indicate extra floral honey (-) indicate nectar honey
Litchi Honey	-12.0	(+) indicate extra floral honey (-) indicate nectar honey
Sudarban honey	-13.0	(+) indicate extra floral honey (-) indicate nectar honey

* Council Directive 2001/110/EC of 20 December 2001 relating to honey

It is known that mustard nectar and litchi nectar found in the flower which is a good natural source for foraging by bees. As sudarban honey was obtained from different floral source of sudarban (multifloral source), it also showed negative specific rotation. The results also suggested that sudarban honey was also from the nectar of flowers not from the extra floral nectar.

Hydroxymethylfurfural (HMF) content

The highest HMF was obtained from the sudarban honey (200.5 mg/kg) and the lowest HMF (0.0 mg/kg) was obtained from litchi honey which was 8.2 mg/kg in mustard honey (Table 4). From the tested sample litchi honey and the mustard honey showed the acceptable limit of EU standard (80.0 mg/kg). On the other hand, sundarban honey showed more HMF content in comparison to mustard and litchi honey and was not in the acceptable limit of EU standard (80.0 mg/kg).

Table 4. Comparison of Hydroxymethylfurfural (HMF) content of Bangladesh honey to EU standard.

Type of honey	Average hydroxymethylfurfural (HMF) (mg/kg)	EU standard* (mg/kg)
Mustard honey	8.2	not more than 80.0
Litchi Honey	0.0	not more than 80.0
Sundarban honey	200.5	not more than 80.0

* Council Directive 2001/110/EC of 20 December 2001 relating to honey

Hydroxymethylfurfural content increases with the increase of temperature when the honey is processed. Fructose of honey is damaged when honey is heated. As the litchi honey samples were freshly harvested, HMF was not found. Mustard honey was processed through steaming, and therefore the mustard samples showed slightly higher HMF. In case of Sundarban honey, the honey samples were not processed in the processing plant and the honey might be overheated. The results are also supported by the work of Reybroeck *et al.* (2007).

Invertase activity

Invertase activity was the highest in number (99.0 IU/kg) in the litchi sample and lowest (0.0 IU/kg) in the sundarban honey (Table 5). Mustard honey showed higher number (2.0) of invertase activity in comparison to sundarban honey but lower than the samples of litchi honey. Both the mustard and sundarban honey had lower invertase than the acceptable limit of EU standard (≥ 50 IU/kg).

Table 5. Comparison of invertase activity of Bangladesh honey to EU standard.

Type of honey	Average invertase activity (IU/kg)	EU standard* (IU/kg)
Mustard honey	2.0	≥ 50
Litchi Honey	99.0	≥ 50
Sundarban honey	0.0	≥ 50

* Council Directive 2001/110/EC of 20 December 2001 relating to honey

Enzyme is denatured in high temperature. If the honey is processed in overheated condition invertase activity will be dropped to lower level. As the litchi honey was in fresh condition i.e. not processed its invertase activity was found in higher. The

mustard honey was processed through steaming and it might be overheated and hence resulted lower invertase activity. In case of sundarban honey, the enzyme was damaged fully and it was might be through overheating, and therefore no enzyme activity was found in this sample.

Tetrasensor reaction

All the samples were found having no antibiotics (tetracycline) (Table 6). So, all the honey samples were in the acceptable limit of EU standard.

Table 6. Comparison of tetrasensor reaction of Bangladesh honey to EU standard.

Type of honey	Reaction to tetrasensor	EU standard*
Mustard honey	(-) negative	(-) not detected/ zero tolerance
Litchi Honey	(-) negative	(-) not detected/ zero tolerance
Sundarban honey	(-) negative	(-) not detected/ zero tolerance

*Council Directive 2001/110/EC of 20 December 2001 relating to honey

In Bangladesh, most of the beekeepers do not use antibiotics to control foulbrood or nosema disease. Even the mustard and litchi sample were collected from the hive where use of antibiotics was not observed. Marketed Sundarban honey was also may be from the beekeepers/ mowalis who did not apply any antibiotics in the hives. In addition there was no record of foulbrood and or nosema disease in the hives.

Sulfasensor reaction

All the samples were found having no sulfur containing compound or sulfonamide drugs (Table 7). So, all the honey samples were found in the acceptable limit of EU standard in respect of sulfasensor reaction.

Table 7. Comparison of sulfasensor reaction of Bangladesh honey to EU standard.

Type of honey	Reaction to sulfasensor	EU standard*
Mustard honey	(-) negative	(-) not detected
Litchi Honey	(-) negative	(-) not detected
Sundarban honey	(-) negative	(-) not detected

*Council Directive 2001/110/EC of 20 December 2001 relating to honey

Most of the beekeepers of Bangladesh do not use sulfonamide drugs to control parasitic mite. Even the mustard and litchi sample were collected from the hive where the use of sulfur containing chemical or drugs was not observed. Marketed Sundarban honey was also may be from the beekeepers who did not apply sulfonamide drugs in the hives because of the absence of parasitic mites in the hives.

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