

Physicochemical Analysis of *Apis dorsata* Honey from Terai Forests, Nepal*

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Abstract.- The multifloral honey produced by *Apis dorsata* from Shahabgunj, Dhakeri, Narayanpur and Perari forest, Nepal, were provided by International Center for Integrated Mountain Development (ICIMOD), Nepal. These ninety nine *Apis dorsata* honey samples were characterized physicochemical and were found to have values of various quality determining parameters well with in the permissible International standards. The honey samples had pH in the range of 3.8-4.68, free acidity 41-48 meq/kg, lactones 13-16 meq/kg, total acidity 55-65 meq/kg, moisture content 20.5-26%, electrical conductivity 0.22-0.63 mS/cm, proline content 76-160mg/kg, HMF content 30-56mg/kg, diastase number 5.1-29 DN, invertase number 390-499, apparent reducing sugars 73.78-77.78%, fructose 36.93-44.61%, glucose 19.61-27.51% and sucrose 12.07-20.38%.

Key words: *A. dorsata*, Nepal's honey, Shahabgunj forest, Dhakeri forest, Narayanpur forest, Perari forest, HMF content.

INTRODUCTION

In Nepal at least four species of honeybees are recognized as native. These include *Apis florea*, *Apis dorsata*, *Apis laboriosa*, and *Apis cerana*, while *Apis mellifera* has been introduced species. However, 50% honey in Nepal is harvested from *Apis dorsata*. Ten to twenty colonies of *Apis dorsata* may be found on the same tree, which is usually named as bee tree. Since up-to 50 colonies of giant honey bees (*Apis dorsata* and *Apis laboriosa*) may aggregate on the same nesting site and each colony can give 10 to 30 kg of honey in single harvest, honey hunting is an important apicultural feature in Nepal (Joshi *et al.*, 2000a). Most of the Nepalese beekeepers have several types of hives: log, house, straw and wood for beekeeping. All the Nepalese honey samples produced by *Apis dorsata*, were collected from four different forests which include Shahbgunj, Dhakeri, Narayanpur, Perari Forests. All these areas belong

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to Banke district of Terai, mid western development region Nepal. Narayanpur and Perari forests are located near Nepal-India boarder between 81-30'-8200'E and 2750-2800'N. The altitude approximately is 200m. Similarly Shahabgunj and Dhakeri areas are located near east-west highway of Nepal between 8130'-8200'E and 2800-2815'N. Sub-tropical climate prevails in this area.

MATERIALS AND METHODS

Nepalese honey samples were non-granulated, non-crystalline liquid and were finely packed in sealed glass bottles along with a piece of wax. No preservative or any heating was applied at any stage. For analysis Nepalese honey samples were stored under ordinary room temperature (25-29°C) in the laboratory until completely analyzed. Before performing any test, samples were homogenized by thorough stirring for at least 3 minutes. Honeys samples were diluted in distilled water at room temperature according to the requirements of the test. The physicochemical methods used for the characterization of honey are

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those as recommended by International Honey Commission (Bogdanov *et al.*, 1999). pH was determined in a 109/75ml solution of honey in deionized water. The free acidity, lactones and total acidity were quantified volumetrically, titrating a honey sample with a solution of 0.05 N NaOH, up to pH 8.3, and expressing the results in milliequivalent of acids at 1000g of honey. The diastase and invertase activity of honey samples was measured according to the procedure of Schade *et al.* (1958) and Siegenthaler (1977), respectively. Moisture content by refractometer (Chataway, 1932) and electrical conductivity was determined in a solution of 20% honey in deionized water (Vorwohl, 1964). proline and HMF content were estimated by Cough (1969) and Winkler (1955) methods, respectively. The sugars in honey samples were estimated by high performance liquid chromatography by using the column Aminex HPX-87H (Column dimensions, 300x7.8 mm) Chromatogram Index, OA3; Mobile Phase, 0.001 M H₂S₀; Gradient, isocratic elution; Flow rate, 0.6 ml/min; Temperature, 46°C; Detection, RI @ 32x. This procedure of sugar analysis by HPLC is based on Lopez and Gomes (1996).

Statistical analysis

The data was subjected to statistical analysis in which different physicochemical and biochemical parameters of honey were compared using analysis of variance (ANOVA) and Student's 't' test (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

Table I shows the results of physicochemical analysis of Nepalese honeys compared with that of International Honey Standards.

pH

The average pH of Nepalese honey from Shahabgunj was found to be 4.68 (4.3-5.1), from Dhakeri 4.58 (4.3-4.7), from Narayanpur 4.39 (3.7-4.6) and from Perari Forests 3.8 (3.7-4.3). The pH of all *A. dorsata* honeys from Nepal showed no significant difference and they fell within the prescribed range 3.42-6.1 (White *et al.*, 1962). These pH values were however, higher as compared to the ones obtained by Joshi *et al.* (2000a) in the

honeys of *A. dorsata* harvested from two different nesting sites (trees) in Chitiwan district Central Nepal which were 3.68 and 4.06, respectively.

Free acidity

Free acidity was determined in *A. dorsata* honey from Nepal. The mean free acidity of 44.45 meq/kg (26.5-51.5) was recorded for honey samples of Shahabgunj Forest, 43.16meq/kg (35-47.51) for honey samples of Dhakeri Forest, 43.14meq/kg (33.5-60) for honey samples of Narayanpur Forest and 48.9meq/kg (39.5-61) for honey samples of Perari Forest. The free acidity of all *A. dorsata* honey samples from Nepal was less than 50 meq/kg, a maximum limit for acidity prescribed by International Honey Standards and by the Directive 2001/110/EC from the Council of European Union. However, few of the samples had free acidity value above the permissible limit. Latif *et al.* (1956) from Pakistan, Mitra and Methaw (1968) from Calcutta, India and Phadke (1968) from India reported formic acid values in *A. dorsata* honeys. Joshi *et al.* (2000a) did not report free or total acidity in *A. dorsata* honey samples from Chitwan district, Central Nepal.

Lactone

Lactones were measured at an average of 13.2 meq/kg (0.0-20.5) from honey samples of Shahabgunj Forest, 18.79 meq/kg (11.5-18) from honey samples of Dhakeri Forest, 15.14meq/kg (12.5-18.5) from honey samples of Narayanpur Forest and 3.5meq/kg (7-21) from Perari Forest. Iglesias *et al.* (2004) noticed the lactone value in spanish honey at an average of 5.08 meq/kg.

Total acidity

The total acidity calculated for honey samples of Shahabgunj Forest was 61.74meq/kg (30-71.5), for Dhakeri Forest 67.98meq/kg (46.5-64) , for Narayanpur Forest 60.02meq/kg (46-77) and for Perari Forest 56.39meq/kg (53-79.5). Minh *et al.* (1971) from Philippines gave total acidity value of 40.2 meq/kg as average in *A. dorsata* honey. Iglesias *et al.* (2004) reported 33.23meq/kg mean total acidity in honey samples from central Spain. High acidity has been considered as indicator of purity of the honey (Suarez-Luque *et al.*, 2002). Another possible reason of elevated acidity values

in *A. dorsata* honey is that unlike *A. mellifera dorsata* have naturally high acidity because of its honey, the honey produced by giant honeybees (*A.*

Table I.- Physicochemical analysis of *A. dorsata* honey from four forests, Nepal.

Physiochemical parameters	Shahabgunj Forest (n=40)	Dhakeri Forest (n=29)	Narayanpur Forest (n=21)	Perari Forest (n=9)	Codex draft 1999	EU draft 1999	Directive 2001/EC **
pH	4.68±0.027	4.58±0.03	4.39±0.04	3.8±0.06			
Free acidity (meq/kg)	41.65±1.22	43.16±0.67	43.14±2.34	48.88±2.40	≤50	≤40	≤50
Lactone (meq/kg)	14.25±0.48	13.76±0.32	15.14±0.40	16.33±0.48			
Total acidity (meq/kg)	55.5±1.51	56.92±0.85	60.02±1.55	65.22±2.56			
Moisture content (%)	22.97±0.18	23.99±1.36	23.91±0.18	22.21±0.48	<21	<21	<21
Diastase activity (DN)	27.69±1.11	29.35±1.36	25.48±0.86	5.53±1.81	<8		<8
Proline content (mg/kg)	76.71±5.57	100.08±13.39	119.98±11.31	160.64±14.67	180*		
HMF content (mg/kg)	33.38±2.74	30.36±3.39	36.48±4.00	56.10±8.97	<60	<40	<60
Invertase Number (IN)	390.31±11.07	463.78±9.04	483.68±3.89	499.83±10.05	<10*		
Electrical conductivity (mS/cm)	0.60±0.00	0.61±0.01	0.48±0.74	0.22±0.03	0.80*		
Apparent reducing sugars (%)	74.99±0.64	77.76±0.48	76.02±0.74	73.76±0.90			
Fructose (%)	39.28±1.06	39.81±1.85	44.61±1.72	36.93±1.77			
Glucose (%)	19.61±0.55	20.17±0.89	25.52±1.37	27.51±1.50			
Sucrose (%)	17.88±0.88	15.95±0.63	20.38±0.63	12.07±1.17	≤5	≤5	≤5
Unidentified sugars (number)	3.43±0.31	2.97±0.28	2.38±0.21	2.22±0.32			
Unidentified – sugars (%)	1.13±0.08	0.92±0.13	1.14±0.03	1.01±0.20			

ANOVA, pH, $P < 9 \times 10^{-4}$; Free acidity, $P < 6.788 \times 10^{-4}$; lactone, $P < 6.079 \times 10^{-4}$; Total acidity, $P < 8.15 \times 10^{-4}$; Moisture, $P < 2.14 \times 10^{-4}$; Diastase activity, $P < 0.020$; Proline content, $P < 0.020$; HMF, $P < 0.012$; Invertase number, $P < 2.96 \times 10^{-3}$; Conductivity, $P < 0.013$; App. redu. sugars, $P < 3.59 \times 10^{-4}$; Fructose, $P < 1.49 \times 10^{-3}$; Glucose, $P < 5.88 \times 10^{-3}$; Sucrose, $P < 4.98 \times 10^{-3}$; Unidentified sugars no., $P < 7.67 \times 10^{-3}$; Unidentified sugars %, $P < 2.71 \times 10^{-3}$.

*The suggested values for invertase activity, Proline content and electrical conductivity for new honey standards (Bogdanov *et al.*, 1999)

**Council of the European Union. Council Directive 2001/ 110/EC of Dec 20, 2001, relating to honey. *Off. J. Eur. Commun.*, 2002, Jan 12, L10/47-L10/52.

multifloral foraging behaviour. Different types of nectars with various concentrations of acids may ultimately result into high level of acidity. These high acidity values of *A. dorsata* honey could be due to limited or short termed shelf life.

Moisture content

The study of moisture content showed an average of 23.5% (20.5-25.5) moisture in honey samples from Shahabgunj Forest, 23.99% (22.7-25) from honey samples of Dhakeri Forest, 23.91% (22-26) from honey samples of Narayanpur Forest and 22.2% (21.2-26) from honey samples of Perari Forest. This significantly high moisture content in honey of *A. dorsata* has also been recorded previously *viz.*, 21.5% by Joshi *et al.* (2000a), 20.9% (18.9-24.2) by Phadke (1968), 21% by Malakar (1997) from India, 23.1% and 27.8% by Laude *et al.* (1991) and Minh *et al.* (1971) from Philippines. The significantly elevated moisture contents are far above the limits of International

Honey Standards and of Directive, 2001/110/EC from Council of European Union, according to which water content of honey should not be more than 21%. These high water contents in *A. dorsata* honey could be because of the possible dilution of honey by rain water as this honeybee species forms hives in open air (Joshi *et al.*, 2000a). Therefore, uniformly high moisture content seems to be the peculiarity of *A. dorsata* honey and could be used to evaluate honey on the basis of honeybee species.

Electrical conductivity

An average electrical conductivity (EC) of *A. dorsata* honey from Nepal was 0.60mS/cm (0.44-0.70) from Shahabgunj Forest, 0.61mS/cm (0.51-0.74) from Dhakeri Forest, 0.48mS/cm (0.19-0.54) from Narayanpur Forest and 0.22mS/cm (0.18-0.42) from Perari Forest. These EC values for Nepalese honey samples were within the limits of International Honey Standards and of Directive 2001/110/EC from Council of European Union for

blossom honey (~0.8 mS/cm). Joshi *et al.* (2000a) recorded EC values of *A. dorsata* honey as 0.96mS/cm. The conductivity values found in the present study were, however, lower and found closer to the mean (0.558mS/cm) EC values reported by Iglesias *et al.* (2004). Moreover, EC values of 0.22mS/cm of Perari Forest honey samples seem to be closer to conductivity (0.19mS/cm) of citrus honey reported by Thrasyvoulou and Manikis (1995) in *A. mellifera* honey.

Diastase activity

Diastase is a starch hydrolyzing enzyme in the honey. Shahabgunj Forest honey samples showed 27.69 (16.66-43) DN, Dhakeri 29.35 (18-42.85) DN and Narayanpur Forest 25.49 (18.75-30) DN. The diastase activities in Nepalese *A. dorsata* honey samples were well within the DN range recommended as quality criteria by International Honey Standard and by Directive 2001/110/EC from Council of European Union, according to which DN should be ≥ 8 in healthy honey. However, naturally Diastase number 5.53 (3.44-20) of honey from Perari Forest depicts its citrus origin and falls within the limits prescribed for orange honeys by International Honey Standards, according to which the DN in citrus honey should be ≥ 3 .

Invertase enzyme

Invertase activity (IN) was also determined for all the *A. dorsata* honeys from four forests of Banke district of mid western Terai, Nepal. The average invertase activity (IN) of 390.11 (157-472.54) was recorded from Shahabgunj Forest honey samples, 464.11 (547.15-407.68) from Dhakeri, 487.77 (524.32-454.56) from Narayanpur and 493.27 (524.17-471.25) from Perari Forest samples. In the present analysis IN were found to be extraordinarily high in Nepalese honey samples. These values appear to be closer to the results obtained by Joshi *et al.* (2000a) for invertase number with an average of 875.8 in the honeys of same honeybee species, *A. dorsata*, from the same region, Chitwan district central Nepal. The explanation for comparatively low invertase number in present study as compared with that of Joshi *et al.* (2000b) is that invertase is more sensitive to heat and storage than diastase. Sometimes, during its

manipulation and packing and transport, honey is submitted to a temporary controlled heating in heat exchangers, for different purposes, such as diminishing the viscosity, dissolving the large crystallized particles, or destroying the yeasts (Detroy, 1979; Skowronek *et al.*, 1994; Crane, 1980). However, this kind of heating could destroy the enzymes. The higher quantities of invertase than diastase is because the honeybees have to add invertase to both honey and honeydew (Sancho *et al.*, 2001a) and *A. dorsata* (native honeybee of south east Asia) add more enzyme to the nectar keeping in view its multifloral foraging behaviour.

HMF

In order to check the freshness of Nepal's honey HMF contents were measured as 33.36mg/kg (7.68-61.28) in honey samples from Shahabgunj, 30.36mg/kg (3.84-57.6) from Dhakeri, 36.48mg/kg (7.68-59.52) from Narayanpur and 56.1mg/kg (21.12-76.16) in honey samples from Perari Forest. The reason of high HMF contents is that all the four forests from where these honey samples of *A. dorsata* were collected, were in Banke district of Terai region, Nepal where the climate is subtropical and according to LaGrange and Sanders (1988) the honey produced in subtropical climate has a high HMF value which exceeds 40mg/kg which is the maximum standard for HMF in the International Honey Standards.

Proline content

Proline content were estimated at an average of 98.23mg/kg (23.38-153.6) from Shahabgunj, 100.8 mg/kg (20.65-330) from Dhakeri, 119.98 mg/kg (42.15-193.18) from Narayanpur, and 160 mg/kg (101.97-198.81) from Perari Forest of Nepal. Joshi *et al.* (2000b) reported 875.82 ppm of proline in *A. dorsata* honey samples from Nepal. Sanchez *et al.* (2001b) reported 76.0mg/100g and 81.1mg/100g proline in Honey samples from two different geographical regions of Spain.

Honey sugars

The individual sugars spectrum was determined by HPLC which showed that in all *A. dorsata* honeys from four forests of Nepal, fructose contents were higher than glucose and sum of both

the monosaccharides fell within the limits of International Honey Commission according to which sum of fructose and glucose should not be less than 60%. The mean values for fructose in all honey samples were 43% (29-54) from Shahabgunj, 39.8% (29-73.5) from Dhakeri, 44.61% (33.3-60) from Narayanpur and 36.93% (31-48) from Perari Forest. Minh *et al.* (1971) from Philippines reported 30.7% (27.9-33.1) fructose in *A. dorsata* honey. Latif *et al.* (1956) from Pakistan reported 42.2%, Mitra and Mathew (1968) from Calcutta, India 35% (31.7-39.2), Phadke (1968) from India 37.4% (34.6-39.9) and Joshi *et al.* (2000a) from Nepal reported 48.01% (43.7-54.20) fructose in *A. dorsata* honeys. Results of fructose content in the present report are closer to the results obtained by Joshi *et al.* (2000a).

The glucose content was 18.45% (12-42.5) in honey samples from Shahabgunj, 20.17% (12.4-35.3) from Dhakeri, 25.52% (17.7-44) from Narayanpur, 27.51% (22-37) from Perari Forest in *A. dorsata* honeys collected from mid western teari, Nepal. Joshi *et al.* (2000a) from Nepal, however, reported a bit higher glucose contents 42% (33.54-49.85) in honeys of same honeybee species from Nepal. The sucrose content of honey was recorded as 23.6% (12-42.5) from Shahabgunj, 15.95% (9-25.7) from Dhakeri, 20.38% (16-26) from Narayanpur and 12.07% (4.14-16) from Perari Forest. The percentage of sucrose in Nepalese honey was extraordinarily high and far above the limits set for sucrose in blossom honeys by International Honey Commission, according to which it should not be more than 5%. In Narayanpur Forest every sample contained more than 15% sucrose. However, the possible reason for this high concentration may be because of the sugarcane cultivation in the area from where these honey samples were taken. Joshi *et al.* (2000a) reported an average of 0.33% (0.00-1.23) sucrose in Nepalese honeys from Chitwan district, Central Nepal.

The chromatogram of sugars also showed the peak areas of sugars other than fructose, glucose and sucrose. Shahabgunj Forest honey samples showed 1-7 unidentified sugars, Dhakeri Forest honey samples 1-5, Narayanpur Forest samples 1-4, and Perari Forest honey samples showed 3 unidentified sugars. Joshi *et al.* (2000a) also reported around nine, Sporns *et al.* (1992) five and Gomez *et al.*

(1993) determined eight sugars other than fructose, glucose and sucrose in the honey.

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