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Physico-chemical characteristics of *Apis dorsata*, *A. cerana* and *A. mellifera* honey from Chitwan district, central Nepal

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Abstract – Honey samples from *Apis dorsata*, *A. cerana* and *A. mellifera* were collected by cutting a piece of honey comb directly from the colonies on the same day and from the same floristic region of Chitwan district, central Nepal. Physico-chemical analysis of the honey samples was performed. The values obtained for moisture content, electrical conductivity (EC), invertase, and proline content were significantly different between all the honey groups. The analytical values for *Apis dorsata* (n = 28), *A. cerana* (n = 26), and *A. mellifera* (n = 27) respectively were: 21.5, 20.1 and 17.1 moisture content (g/100 g honey); 0.96, 0.65 and 0.31 EC (mS/cm), 373.4, 218.2 and 110.9 invertase (Siegenthaler U/kg), and 875.8, 323.0 and 610.2 proline (mg/kg). There were no significant differences between the honey types in pH, glucose oxidase, and the amount of glucose. However, the amount of fructose was significantly higher in *A. dorsata* and *A. cerana* than in *A. mellifera* honeys. Similarly, the amount of oligosaccharide L_2 was significantly higher and sucrose was significantly lower in *A. dorsata* honeys than in *A. cerana* and *A. mellifera* honeys.

honey / *Apis dorsata* / *Apis cerana* / *Apis mellifera* / physico-chemical characteristics / Nepal

1. INTRODUCTION

Honey is generally evaluated by a physico-chemical analysis of its constituents. Several of these constituents are of great importance to the honey industry as they influence the storage quality, granulation, texture, flavour and the nutritional and medicinal quality of the honey. The International Honey Commission (IHC) has

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therefore proposed certain constituents as quality criteria for honey. These include: moisture content, electrical conductivity, reducing sugars, amount of fructose and glucose, sucrose content, individual sugars, minerals, free acidity, diastase, HMF, invertase, proline, and specific rotation [2].

In Nepal, there is no effective legislation or policy for the quality control of honeys. Honey is generally harvested by cutting the combs and squeezing the honey, and it is then packed in an odd assortment of glass bottles, mostly pre-used, e.g., in bottles which have contained alcoholic drinks; or in plastic containers. Honey has been used more for medicinal and religious purposes than as a nutritional food. For most Nepalese, it is still a luxury item which is scarcely available and very expensive (US $4/kg); i.e., 1 kg honey is equivalent to 8 kg rice, or 3 kg chicken, or 3 days' wage. The price varies from place to place. At present, honey from commercial beekeepers produced by exotic *A. mellifera* is sold at a higher price than the squeezed honey harvested from native *A. cerana* colonies. However, honey from native bee colonies harvested in a 'correct way', or sealed comb honey is of excellent quality (i.e., without any pollutant or residues).

The amount of scientific information on the chemistry of Nepalese honeys is very limited. Phadke [13] in India and Latif et al. [6] in Pakistan made an extensive study of *A. cerana* honeys. Phadke [14] also studied *A. dorsata, A. florea* and *A. trigona* honeys from India. However, these authors did not measure the invertase and proline content which have recently been proposed as quality criteria for honey [2]. Laude et al. [7] compared *A. dorsata, A. cerana* and *A. mellifera* honeys from the Philippines. They collected honey samples from the market and from beekeepers, but the influence of different methods of honey harvesting and processing could not be quantified. Therefore, we collected honey samples from different bee species at the same time and from the same floristic area of Nepal to determine the differences, if any, in the physico-chemical characteristic of these honeys.

2. MATERIALS AND METHODS

Eighty-one honey samples, i.e., 28 *A. dorsata*, 26 *A. cerana* (kept in Newton B hives and traditional log hives), and 27 *A. mellifera* (kept in Langstroth hives) were collected by cutting a piece of honey comb directly from the colonies and storing in a deep freezer within a week. Samples were collected on the same day and from the same floristic region in the Chitwan district, central Nepal.

The moisture content, pH and electrical conductivity (EC) were measured according to the standardised methods of the European Honey Commission [1]. Invertase, proline and glucose oxidase were measured according to the methods of Siegenthaler [17], Ough [12] and Shepartz and Subers [15], respectively. Sugar spectra were analysed by high-performance liquid chromatography (HPLC) based on DIN 10758 at the Beekeeping Institute, Celle, Germany. The limits of detection (LOD) and limits of quantification (LOQ) are given in Table I.

The statistical analysis was carried out with the SAS (Statistical Analysis System) for Windows 6.12. Significance tests were carried out using the Bonferroni-Holm method.

3. RESULTS

Mean results and basic statistics obtained for moisture content, pH, EC, invertase, proline and glucose oxidase are summarised in Table II. The carbohydrate composition of honey (the amount of specific sugars, fructose-glucose ratio and the total of all identified sugars) is given in Table III. The correlations calculated between EC and invertase, proline content and the oligosaccharide L_2 are given in Table IV.

Table I. Sugar spectra were analysed by high performance liquid chromatography (HPLC) based on DIN 10758 at the Beekeeping Institute, Celle, Germany. The limits of detection (LOD) and limits of quantification (LOQ) are shown below.

Sugars (g/100 g honey)	X	SD (%)	*r*	SD (%)	LOD	LOQ
Fructose	34.36	0.67	1.91	1.96	0.04	0.15
Glucose	22.0	0.48	1.37	2.20	0.05	0.18
Sucrose	5.55	0.17	0.49	3.12	0.03	0.10
Turanose	2.25	0.15	0.42	6.52	0.03	0.11
Maltose	2.18	0.14	0.38	6.22	0.04	0.15
Trehalose	2.11	0.08	0.23	3.91	0.04	0.14
Isomaltose	1.94	0.08	0.22	4.07	0.07	0.25
Melibiose	2.01	0.13	0.36	6.35	0.12	0.44
Erlose	2.18	0.09	0.27	4.35	0.10	0.34
Melezitose	2.20	0.08	0.23	3.69	0.09	0.33
Raffinose	2.21	0.09	0.26	4.14	0.13	0.45
Panose	2.94	0.23	0.66	7.91	0.21	0.75
Maltotetraose	3.24	0.21	0.60	6.48	0.40	1.41
Oligosaccharide L_1					0.24	0.85
Oligosaccharide L_2					0.27	0.96

X: mean; SD: standard deviation; *r*: repeatability. The HPLC configurations used were: pump: Gynkotek M480 (Gynkotek, Germering, Germany); injector: Rheodyne 7125 (Rheodyne, Cotati, USA); oven: STH 585 (Gynkotek, Germering, Germany); detector: Shodex RI, SE-71 (Showa Deko K.K., Tokyo, Japan); integration: Chromeleon 4.12 (Softron, Germering, Germany/Chromatographie-Datensystem; column: 250 × 4 mm + 4 × 4 mm, 5 µm Lichrosher 100 NH_2 (Merck); eluent: acetonitrile 80%, water 20% (Merck); flow: 1.3 mL/min, pressure ca. 90 bar; temperature: 30 °C; injection volume (sloop): 20 µL; analysis: external standard.

Table II. Analytical data for Nepalese honeys.

Bee species Parameters	*Apis dorsata* (*n* = 28)		*A. cerana* (*n* = 26)		*A. mellifera* (*n* = 27)	
	Mean	SD (±)	Mean	SD (±)	Mean	SD (±)
Moisture (%)	21.51^a	2.38	20.12^b	2.66	17.14^c	2.56
pH	3.68^a	0.36	3.62^a	0.4	3.52^a	0.32
EC (mS/cm)	0.96^a	0.75	0.65^b	0.45	0.31^c	0.14
Invertase (U/kg)	373.37^a	269.64	218.23^b	135.34	110.93^c	58.27
Proline (ppm)	875.82^a	497.07	323.0^b	169.52	610.19^c	220.11
Glucose Oxidase (µg $H_2O_2 \cdot g^{-1} \cdot min^{-1}$)	8.51^a	10.96	5.51^a	4.78	6.92^a	3.58

Bonferroni-Holm test (the different superscripts $^{a, b, c}$ in a row with no common superscript denote a statistical significance between the values of three honey groups (multiple $\alpha < 0.05$).

4. DISCUSSION

4.1. Moisture content, pH and EC

Moisture content was found to be significantly higher in *A. dorsata* than in *A. cerana* and *A. mellifera* honeys. The majority (57.15%) of *A. dorsata* honey samples was found to be higher in moisture content (> 21%) than the maximum allowable content for honey determined by the International Honey Commission [2]. Of *A. cerana* honey samples, 34.62% exceeded the

Table III. Carbohydrate composition of honeys (sugars are in g/100 g carbohydrate; minimum and maximum values are given in brackets).

Bee species Parameters	*Apis dorsata*		*A. cerana*		*A. mellifera*	
	Mean	SD (±)	Mean	SD (±)	Mean	SD (±)
Fructose	48.01[a] (43.7–54.20)	2.35	48.25[a] (44.52–51.29)	1.62	45.93[b] (42.32–50.45)	1.8
Glucose	42.23[a] (33.54–49.85)	4.94	44.02[a] (33.05–52.52)	4.54	41.95[a] (36.33–46.25)	2.53
Sucrose	0.33[a] (0.00–1.23)	0.29	1.39[b] (0.00–6.83)	1.71	1.96[b] (0.00–7.8)	1.93
Turanose	1.42[a] (0.30–2.06)	0.49	0.97[b] (0.00–2.59)	0.7	1.66[a] (0.53–2.89)	0.5
Maltose	2.22[a] (0.91–3.51)	0.73	2.09[a] (0.00–3.66)	0.86	3.26[b] (1.61–4.13)	0.61
Oligosacchaide L_2	2.16 (0.0–9.19)	3.29	0.45 (0.0–3.21)	0.89	0.31 (0.0–2.24)	0.75
* Others	3.63		2.83		4.93	
Sum of all sugars per g/100 g honey	73.46[a] (65.59–80.07)	3.87	75.42[a] (61.2–83.02)	6.58	82.0[b] (74.58–86.84)	4.22
Fructose: glucose ratio	1.15[a] (0.97–1.35)	0.13	1.11[a] (0.85–1.49)	0.13	1.1[a] (1.0–1.2)	0.05

Bonferroni–Holm test (the letters [a, b, c] in superscript after the means denote a significant difference between the analytical values of three honey groups (multiple $\alpha < 0.05$).
* 'Others' includes the sum of kojibiose, trehalose, isomaltose, melibiose, erlose, melezitose, maltotriose, raffinose, and maltotetraose; statistical analysis was not carried out for these sugars.

maximum permissible level of moisture content (> 21%), whereas in *A. mellifera*, only 7.4% of the samples had more than 21% moisture content. Phadke [13, 14] in India recorded 20 ± 2% water content in *A. indica* (*cerana*) honeys and 20.9% in *A. dorsata* honeys. Mitra and Mathew [10] also found 20.5% for *A. cerana* honeys and 23.5% for *A. dorsata* in Calcutta, India. Laude et al. [7] recorded similar average values for moisture content in *A. dorsata* (23.1 ± 2.3%), *A. cerana* (22.0 ± 3.7%), and *A. mellifera* (19.5 ± 1.6%) honeys from the Philippines. However, lower values (17–21%) were obtained by Shrestha [16] in Nepalese honeys, and (16.2%) by Latif et al. [6] in Pakistan honeys.

The pH values were not significantly different between *A. dorsata*, *A. cerana* and *A. mellifera* honeys (Tab. I). The values obtained for Chitwan honeys agree with the results obtained by Laude et al. [7] for Philippine honeys, and with those of Malakar [8] for the same type of honeys in West Bengal, but are slightly lower than the results obtained by Olek et al. [11] and Shrestha [16] for *A. cerana* honeys.

The EC values were significantly different between all three groups of honeys (Tab. I).

Table IV. Pearson's correlation coefficients between EC and invertase, proline and oligosaccharide L_2 (total No. of samples, $n = 81$; $n = 28$ for *A. dorsata*; $n = 26$ for *A. cerana*; and $n = 27$ for *A. mellifera*).

Variables	Invertase (Siegenthaler U/kg)			Proline (ppm)			Oligosaccharide L_2 (g /100 g carbohydrate)		
Bee species	*A. dor.*	*A. cer.*	*A. mel.*	*A. dor.*	*A. cer.*	*A. mel.*	*A. dor.*	*A. cer.*	*A. mel.*
EC (mS/cm)	0.80	0.50	0.36	0.85	0.34	0.32	0.94	0.61	0.61
	$P = 0.0001$	$P = 0.0212$	$P = 0.0660$	$P = 0.0001$	$P = 0.144$	$P = 0.1026$	$P = 0.0001$	$P = 0.004$	$P = 0.001$
Invertase (U/kg)				0.64	0.24	0.22			
				$P = 0.0004$	$P = 0.3115$	$P = 0.2683$			
Proline (ppm)									

A. dor.: Apis dorsata; A. cer.: Apis cerana; A. mel.: Apis mellifera.

The highest EC was recorded in *A. dorsata* honeys followed by *A. cerana* and *A. mellifera* honeys, respectively. The percentage of samples with more than 0.7 mS/cm EC (the limit proposed for honeydew honey compared to < 0.7 mS/cm for nectar or blossom honey [1]) was 39.3% in *A. dorsata*, 30.8% in *A. cerana*, and 3.7% in *A. mellifera* honeys. However, the latest IHC publication has proposed the minimum EC value for honeydew honey of 0.8 mS/cm [2]. The taste, odour and colour of honeydew honey were different from that of nectar honey, but the honeydew honeys from *A. dorsata* and *A. cerana* were quite similar to European honeydew honey. Shrestha [16] recorded 360–1 060 μS/cm EC in Nepalese honeys (the source of the honey was not mentioned). The data obtained for EC and the pollen spectrum [5] suggest that *A. dorsata* and *A. cerana* bees collect more honeydew than *A. mellifera* bees.

4.2. Invertase, proline and glucose oxidase

Invertase and proline were significantly higher in *A. dorsata* honeys than in *A. cerana* and *A. mellifera* honeys (Tab. I). But interestingly, in *A. cerana* honeys the proline content was significantly lower and the invertase activity was significantly higher than that of *A. mellifera* honeys. Glucose oxidase, however, did not show a significant difference between the honey types.

Five honey samples from *A. cerana* were found to contain 140–200 ppm proline, the indicator level for honey adulteration [4, 18, 20, 22]. Since samples were collected directly from the combs and the sugar spectrum did not show any adulteration or sugar feeding (i.e., the sucrose content was < 5%), the indicator level of proline content for *A. cerana* honey should be lower than that for *A. mellifera* honey. The proline content of all *A. mellifera* honey samples was above the indicator level, ranging between 343–1 118 (average 610) ppm. White and Rudyj [22] reported the mean for 740 samples of *A. mellifera* honey as 503 ppm. Thrasyvoulou and Manikis [19] also reported the proline content of 80 Greek honey samples as 526 ppm, ranging between 264–1 205 ppm.

Contrary to our results, Laude et al. [7] found very low amounts of invertase in *A. cerana* honeys. In their *A. cerana* honey samples, HMF (51.5 ± 48.9 ppm) and sucrose content (9.5 ± 4.1%) were very high, and EC was very low compared to that of *A. mellifera* honeys. They suggested that the EC, invertase activity and other honey-related factors were influenced by different methods of beekeeping or treatment of honey by beekeepers, and not due to the different use of honey sources by bee species. In the present study, samples were collected directly from the combs and from the same floristic region. The operational factors or methods of treating honey were also the same. Therefore, the differences in their physico-chemical properties found in the present study were mainly due to the bees' different foraging preferences [5]. It has already been reported that honeydew honey contributes various enzymes, mainly invertase, from the gut and saliva of the insect that has produced it [9].

As reported by earlier investigators, honeydew honey contains higher amounts of invertase, proline and oligosaccharides [3]. The positive correlations between EC, invertase, proline content and oligosaccharide L_2 found in *A. dorsata* honey support this theory (Tab. IV).

4.3. Sugar spectrum

Sugar analyses gave results that were similar in glucose and fructose content to those obtained by Phadke [13, 14] for *A. dorsata* and *A. cerana* honeys in India. The proportion of fructose and glucose and the total amount of reducing sugars were also found to be similar to those recorded

by Crane [3] for *A. dorsata, A. cerana* and *A. mellifera* honeys. Sugar feeding is not a common practice in the present study area; therefore, the sucrose content in all the honey types was within the limits of the quality criteria established by the IHC [2]. However, it was higher in *A. cerana* and *A. mellifera* honeys than in *A. dorsata* honey. Oligosaccharide L_2 (which is a honeydew-specific sugar) was higher in *A. dorsata* than in other honeys, and was recorded only in the samples which had more than 0.7 mS/cm EC. Therefore, in combination with EC, the measurement of this sugar could be useful in determining the botanical origin of the honey, whether honeydew or nectar [21].

From the present study we can conclude that in the same floristic area, the native bees collected more honeydew honey which contained more EC and enzyme invertase than honeys from the exotic bee *A. mellifera.* Our continuing studies will detail the foraging preferences of different honeybee species in the same floristic area. A further study with year-round honey samples is necessary to fully determine whether the differences in the physico-chemical properties of honeys were mainly due to the different botanical origin (honeydew or nectar) of honey, or to other factors.

Résumé – Caractéristiques physico-chimiques des miels d'*Apis dorsata*, d'*Apis cerana* et d'*Apis mellifera* du district de Chitwan, Népal. Quatre-vingt-un échantillons de miels provenant de 28 colonies d'*Apis dorsata*, 26 d'*Apis cerana* et 27 d'*Apis mellifera* ont été prélevés en découpant un petit morceau de rayon de miel directement dans chaque colonie, le même jour et dans la même région floristique du district de Chitwan dans le centre du Népal. Les échantillons ont été conservés dans les mêmes conditions et leur analyse physico-chimique effectuée (Tabs. II et III). La teneur en eau, le pH et la conductibilité électrique ont été déterminés selon les méthodes harmonisées de la Commission Européenne pour le Miel [1]. L'invertase, la proline et la glucose oxydase ont été respectivement mesurées selon les méthodes originales de Siegenthaler [18], Ough [12] et Shepartz et Subers [15]. Les spectres de sucres ont été analysés en chromatographie liquide haute pression d'après la norme DIB 10758 à l'Institut d'apiculture de Celle, Allemagne. Les résultats montrent que la teneur en eau, la conductibilité électrique, l'activité de l'invertase et la teneur en proline sont significativement plus élevées dans les miels provenant d'*A. dorsata* que dans ceux provenant d'*A. cerana* ou d'*A. mellifera*. Dans les miels d'*A. cerana*, la teneur en eau, la conductibilité électrique et l'activité de l'invertase sont significativement plus élevées que dans les miels d'*A. mellifera*, mais la teneur en proline est significativement plus faible. Le pH et la glucose oxydase ne présentent pas de différence significative entre les trois types de miel. Le fait que la conductibilité électrique et que l'activité de l'invertase soient plus élevées dans les miels d'*A. dorsata* et d'*A. cerana* pourrait être dû à la plus grande teneur en miellat de ces miels. Dans les miels d'*A. dorsata* il existe une corrélation positive entre la conductibilité électrique, l'invertase et la proline. Il existe aussi une très bonne corrélation entre la conductibilité électrique et l'oligosaccharide L_2. Ce sucre a été trouvé dans les échantillons de miel qui avaient une conductibilité électrique > 0,7 mS/cm. Il pourrait donc être possible de déterminer si le miel est un miel de fleurs ou un miel de miellat en mesurant la conductibilité électrique et le sucre L_2. Le nourrissement au sucre n'est pas une pratique répandue dans le région étudiée ; la teneur en saccharose reste donc dans les limites des critères de qualité définis par la Commission Internationale du Miel (IHC) [2].

miel / *Apis dorsata* / *Apis cerana* / *Apis mellifera* / caractéristique physico-chimique / Népal

Zusammenfassung – Chemisch-physikalische Eigenschaften der Honige von *Apis dorsata, A. cerana* und *A. mellifera* aus Chitwan Distrikt, Nepal. Vom gleichen Trachtgebiet im Chitwan Distrikt, Nepal und am gleichen Tag wurden 81 Honigproben als Wabenstücke direkt von den Völkern entnommen. 28 Proben stammten von der Bienenart *Apis dorsata*, 26 von der Art *A. cerana* und 27 von *A. mellifera*. Alle Proben wurden unmittelbar nach der Entnahme aus den Völkern gemeinsam bis zur chemisch-physikalischen Analyse tiefgefroren. Der Wassergehalt, der pH-Wert und die elektrische Leitfähigkeit wurden nach den standardisierten Methoden der Europäischen Honig Kommission (IHC) [1] bestimmt. Invertase-Aktivität, Prolingehalt und Glucose-oxydase-Aktivität wurden nach den Originalmethoden von Siegenthaler [17], Ough [12] beziehungsweise Shepartz und Subers [15] gemessen. Basierend auf der DIN 10785 wurde mittels HPLC (NH_2 –Säule, Laufmittel 80 % Acetonitril und 20 % Wasser, RI Detektor) das Zuckerspektrum am Bieneninstitut Celle, Deutschland analysiert. Die Ergebnisse zeigen, dass Wassergehalt, elektrische Leitfähigkeit, Invertase-Aktivität und Prolingehalt bei den Honigen von *A. dorsata* signifikant höher waren als bei den Honigen der anderen zwei Bienenarten. Bei Honigen von *A. cerana* ist der Wassergehalt, die elektrische Leitfähigkeit und die Invertase-Aktivität signifikant höher, der Prolingehalt aber signifikant niedriger als bei Honigen von *A. mellifera*. Glucose-oxydase-Aktivität und pH-Wert zeigen in den Honigen der drei Bienenarten keine signifikanten Unterschiede. Die höhere elektrische Leitfähigkeit und Invertase-Aktivität in den Honigen von *A. dorsata* und *A. cerana* können vom höheren Honigtauanteil dieser Honige herrühren. In den Honigen von *A. dorsata* wurde eine positive Korrelation zwischen elektrischer Leitfähigkeit, Invertase-Aktivität und Prolingehalt festgestellt. Ebenso zeigte sich eine starke positive Korrelation zwischen der elektrischen Leitfähigkeit und dem Gehalt an dem Oligosaccharid L_2. Dieser L_2-Zucker wurde in allen Honigproben mit einer elektrischen Leitfähigkeit höher als 0,7 mS/cm gefunden. Daher ist es anhand von Messungen der elektrischen Leitfähigkeit und/oder dieses Zuckers möglich, zu unterscheiden, ob der Honig vorwiegend aus einer Honigtau- oder Nektartracht stammt. Zuckerfütterung ist im Gebiet der Probenahme nicht üblich, daher ist der Saccharosegehalt innerhalb der Limits der IHC [2].

Honig / *Apis dorsata* / *Apis cerana* / *Apis mellifera* / Chemisch-physikalische Eigenschaften / Nepal

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