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Effect of Storage on Various Honey Quality Parameters of *Apis dorsata* Honey from Nepal*

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Abstract.- Nepalese honey samples stored for 8 months showed pH in the range of 3.9-4.6, free acidity 48.5-53meq/kg, lactone 15.5-17.1meq/kg, total acidity 61-70meq/kg, electrical conductivity 0.24-0.64mS/cm, Proline content 148-241mg/kg, HMF content 53.4-122mg/kg, Diastase Number 1.02-13.25DN and Invertase Number 0.58-10.5IN. After 16 months of storage the various parameters recorded were: pH 3.7-5.08, free acidity 46.1-57.07meq/kg, lactone 17-19meq/kg, total acidity 64-74meq/kg, electrical conductivity 0.29-0.71mS/cm, Proline content 66.43-120mg/kg. HMF content were beyond international maximum limit even after 8 months storage. Similarly diastase Number (0.22-0.86DDN) and Invertase Number (00-0.71IN) were much below than the minimum standard. Although honey produced by *A. dorsata* from Nepalese forest showed various quality parameters close to International Honey Quality Standards, yet its shelf life was shorter due to high moisture content.

Key words: Shelf life, *A.dorsata* honey, physicochemical characteristics of honey, proline content, HMF content, Diastase number, Invertase number

INTRODUCTION

In Nepal at least four species of honey bees are recognized as native. These include *Apis florea, A, dorsata, A, laboriosa,* and *A, cerana,* while *A, mellifera* is an 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 (*A. dorsata* and *A. laboriosa*) may aggregate on the same nesting site and each colony can give 10 to 30 kg of honey in a single harvest, honey hunting is an important apicultural practice in Nepal (Joshi *et al*., 2000b). Most of the Nepalese beekeepers have several types of hives: log, house, straw and wood for beekeeping. Improper storage of honey under ordinary conditions causes the deterioration of some of the useful compounds as well as the accumulation of certain unwanted compounds which lessen its quality, thus making it a less profitable commodity from a commercial point of view. The purpose of present study is to find out the extent of biochemical changes which take place during storage in the honey leading to the undesirable variations in its composition.

MATERIALS AND METHODS

All the Nepalese honey samples produced by *Apis dorsata,* were collected from four different forests which include Shahagunj, Dhakeri, Narayanpur, Perari Forests. All these areas belong to Banke district of Terai, mid western development region. The honey samples were stored under room temperature (25-29°C) in the laboratory until analyzed for the total time period of 16 months. There was no sign of granulation in honey samples. No preservative or any heating was applied at any stage.

All physicochemical determinations were carried out according to the European Honey Commission methods (Bogdanov *et al*., 1999). pH and electrical conductivity were determined in a 10g/75ml solution of honey in deionized water. Free, lactonic and total acidities were titrated in the same solution used for pH measurement (AOAC, 1975). Water content was determined by refractive index and correlation with Chataway Charts (Chataway, 1932). Proline content of honey was

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determined according to Cough (1969). Winkler's method was used for the determination of HMF content (Winkler, 1955). The diastase and invertase activity of honey samples was determined according to the procedure of Schade *et al*. (1958) and Siegenthaler (1977), respectively. ANOVA (Steel and Torri, 1981) was performed for statistical analysis of data.

RESULTS AND DISCUSSION

pH

Shahabgunj Forest showed significant increase in the pH, from 4.68-5.01 (8.54%) in the last eight months of the storage while pH remained the same during the initial eight months. After eight months of the storage, honey samples of the same nesting sites experienced decreased in the pH except the sample no.15, 23, 24, 28, 29, 32, 33 and 36, whereas each sample of the honey from the same nest showed increase in pH after sixteen months of storage. On the whole, Dhakeri Forest honey samples showed continuous increase in the pH during storage. The pH increased 2.4% during the initial eight months and 2.1% during the subsequent eight month of storage (Fig. 1). The pH increase during the initial eight months of storage which was 4.73% (4.42-4.68) in Narayanpur sample of the honey and the maximum pH of 5.0 was shown by sample #89. Instead of further increase, pH of Narayanpur Forest honey samples showed a decrease, becoming lower than the one initially recorded. The pH increased 5.88% (pH 3.8-4) in Perari Forest's honey during the initial eight months. Instead of further increase, pH of the honey samples decreased 3.7 (12.17%) at the end of sixteen months of storage. 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 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

On the whole, at the end of sixteen months of storage, the free acidity value in all Shahabgunj, Dhakeri, Narayanpur Forest honey samples showed 16.56% (41.4-8.55meq/kg), 15.06% (43.16-49.66meq/kg), 8.12% (44.9-48.55meq/kg), increase in the free acidity after eight months of storage and 11.74% increase (48.55-54.25meq/kg), 14.92% (49.66-57.07meq/kg), 10.50% (48.55-53.65meq/kg), respectively, after sixteen months of storage. When stored, Perari honey samples had high free acidity values, which according to the international standards was close to the upper permissible limit (60meq/kg). These samples showed 8.24% (48.9-52.92meq/kg) increase in the free acidity during initial eight months of storage and in the later eight months free acidity of the honey decreased by 12.86% (52.92-46.11meq/kg) (Fig. 2). Ageing increased the free acidity value of every independent sample during sixteen months. The free acidity of all *A. dorsata* honey samples from Nepal was less than 50 meq/kg, a maximum limit (50meq/kg) for acidity prescribed by International Honey Standards and by the Directive 2001/110/EC from the Council of European Union [but you said 60 was close to the upper limit](this limit is for HMF). However, a 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.

Lactones

Impact of aging on lactones is uniform as its amount showed a steady increase during sixteen months of storage. Shahabgunj, Dhakeri and Narayanpur (Fig. 3) samples had 8.42% (14.25-15.45meq/kg), 19.98 % (13.76-16.71meq/kg), 9.01% (14.98-16.33meq/kg) more lactones at the end of first 8 months of storage and 9.06% (15.45-16.85meq/kg), 13.8% (16.71-18.79meq/kg), 4.65% (16.33-17.09meq/kg), more lactones during the subsequent eight months, respectively. In Perari Forest honey samples the lactone increased 5.08% (16.33-17.16meq/kg) and 7.98% (17.16-18.52meq/kg) after eight and sixteen month's storage, respectively. Sporns *et al.* (1992) reported

5.9 meq/kg with maximum of 15.4 lactone values for Alberta honeys from Canada. Gomez *et al.* (1993) noticed the lactone value in Spanish eucalptus honey at an average of 2.9 meq/kg. Iglesias *et al.* (2004) noticed the lactone value in spanish honey at an average of 5.08 meq/kg.

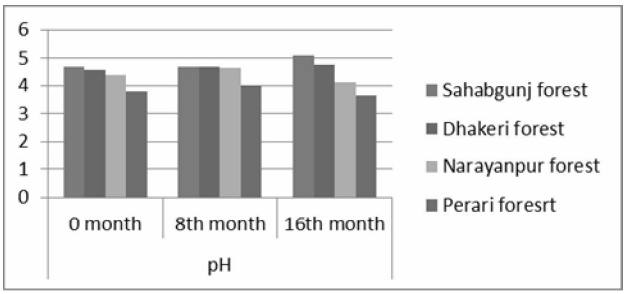

Fig. 1. Effect of 16 month storage on pH of *A.dorsata* honey collected from four forests of Teri district, Nepal.

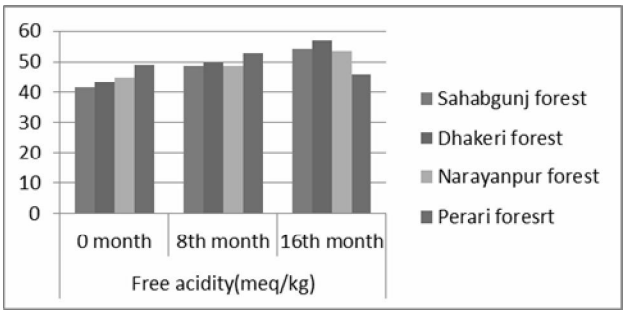

Fig. 2. Effect of 16 month storage on free acidity (meq/kg) of *A.dorsata* honey collected from four forests of Teri district, Nepal.

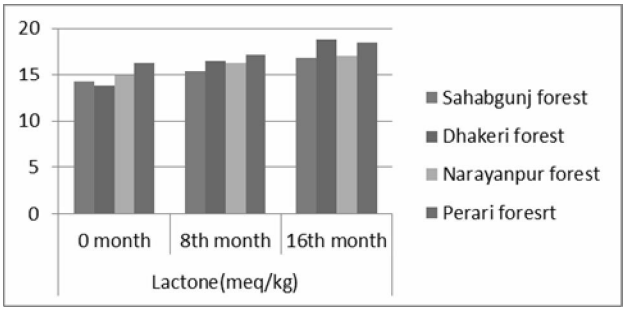

Fig. 3. Effect of 16 month storage on lactone content (meq/kg) of *A. dorsata* honey collected from four forests of Teri district, Nepal.

Total acidity

The total acidity of honey samples increased due to aging 11.24% (55.5-61.7meq/kg) and 15.16% (61.7-71.1meq/kg), respectively, after eight and sixteen months of aging. Aging resulted in 19.43% increases in total acidity (56.92-67.98meq/kg), 8.6% (59.73-64.88meq/kg) after eight months and 10.46% increase (67.98-74.89meq/kg), 9% (64.88-70.74 meq/kg) after sixteen months of storage in Dhakeri and Narayanpur Forest honey samples. On the whole, due to aging of eight months storage, the total acidity of Perari Forest honey samples increased 7.4% (65.22-70.05meq/kg), whereas it decreased 7.73% (70-64.63meq/kg) after sixteen months (Fig.4). A similar trend of increase in total acidity has also been observed by Cervantes *et al.* (2000) who first heated the honey samples at 55ºC and then kept those samples at 26ºC for three and half months. One explanation to this significantly high total acidity is aging of Nepalese honey. 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* honey, the honey produced by giant honeybees (*A. dorsata*) have naturally high acidity because of its multifloral origin. 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 lead to lessen its shelf life.

Electrical conductivity

An overall trend towards increase in the electrical conductivity of Nepalese honey was observed due to aging. Nevertheless after sixteen months of storage the conductivity values did not exceed the codex honey quality standards in any sample of the honey of any forest. Shahabgunj Forest showed striking increase in the electrical conductivity of the honey samples from initial EC value. This increase was 6.66% (0.6-0.64mS/cm) and 18.33% (0.64-0.71mS/cm) after eight and sixteen months of storage, respectively. Honey samples from Dhakeri, Narayanpur (Fig. 3), Perari Forest showed an overall increase of 13.11%, 24%, 31.8% due to aging in electrical conductivity (EC) after sixteen months storage, respectively (Fig.5). These EC values for Nepalease 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

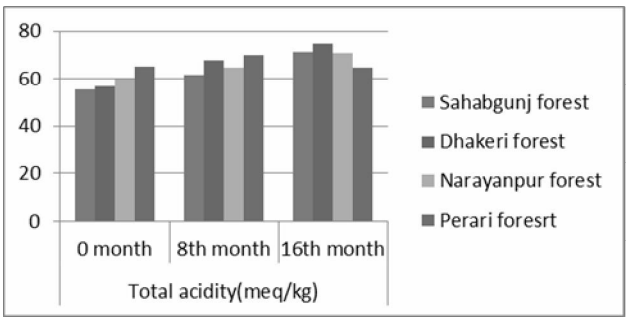

Fig. 4. Effect of 16 month storage on total acidity (meq/kg) of *A. dorsata* honey collected from four forests of Teri district, Nepal.

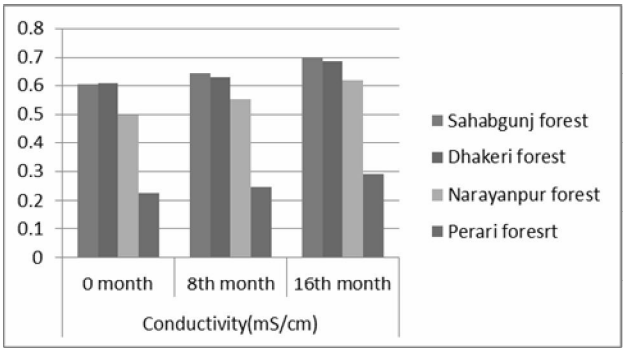

Fig. 5. Effect of 16 month storage on electrical conductivity (mS/cm) of *A. dorsata* honey collected from four forests of Teri district, Nepal.

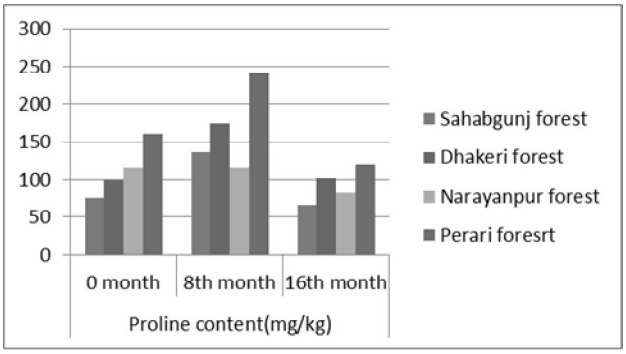

Fig. 6. Effect of 16 month storage on proline content of *A. dorsata* honey collected from four forests of Teri district, Nepal.

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 produced by A. mellifera, as reported by Thrasyvoulou and Manikis (1995). Qamer *et al.* (2009) recorded similar increasing tendancy in Sidder honey produced by *A.mellifera* collected from Bannu (0.50-0.61mS/cm), Karak (0.50-0.56mS/cm) and Chunian (0.22-0.44mS/cm). An overall trend towards increase in the EC of Nepalese was observed due to aging. Nevertheless after sixteen months of storage the EC values did not exceed the Codex Honey Quality Standards in any of the Nepalese honey

Proline content

Despite increase in Proline content during the initial eight months, there was a decline of 13.4% in the Proline content of honey stored for more than a year of the Shahabgunj Forest of Nepal. The increase in proline content was 92.93% (76.71-136mg/kg) during the first eight months followed by 55.1% (136-66.43mg/kg) decrease during the next eight month (Fig. 6). In Dhakeri forest honey samples Proline content increased 74.47% (100-174.61mg/kg) during first eight months but decreased 41.24% (174.61-102.58mg/kg) during the subsequent eight months of storage. The proline content decreased 28.5% due to aging at the end of sixteen months 6in Narayanpur honey samples. Perari honey samples showed 80.39% increase after aging during first of eight months whereas proline content decreased 50.2% during the subsequent eight months.

Diastase

The sixteen months storage deprived all honey samples of Shahabgunj and Dhakeri Forest (96%, 92.54%) of their diastase activity. Honey samples of both forests showed 58.03% (27.69-11.62DN), 71.58 % (29.35-8.34DN) reduction in diastase number during initial eight months and 92.59% (11.62-0.86DN), 92.54% (8.34-0.64DN) loss in diastase during the subsequent eight months of storage, respectively. The diastase number was within permissible limit even after eight months of

storage, although the diastase activity decreased 48.2% (25.6-13.25DN) in Narayanpur samples after eight months of storage (Fig. 7). This decline was more pronounced (96.22%) during the next eight months. The diastase activity of honey samples from Perari Forest was decreased 81.55% during the initial eight months and 78.43% by the end of sixteen months. The same behaviour of loss of diastase activity was also noticed by White and Subers (1963), Iman (1990) and Qamer *et al* (2009). Sancho *et al.* (1992) studied the effect of storage for two years on diastase activity by keeping the honey samples at 20ºC. Cervantes *et al.* (2000) first heated the honey at 55ºC and then stored for three and half months at 26ºC. Both scientists observed depletion in diastase activity of honey under different storage conditions.

Invertase number

Under all conditions aging reduced the enzyme activity. Tremendous loss of invertase activity occurred due to aging during sixteen months storage was reduced to an extent the enzyme number fall below the level of Invertase required by codex honey regulations. The loss of invertase enzyme in the samples of the honey from Shahabgunj (100% (59-0.71) Siegenthaler U/kg), Dhakeri (99.67%), Narayanpur (99.58%) and Perari (90.8% (4.60-0.42IN) Forest reached its maximum after sixteen months storage whereas it was 84.8% (390-59 Siegenthaler U/kg), 82.12%, 87.32%, 99.07% (499.83-4.6IN) during initial eight months, respectively (Fig.8). The decrease in invertase activity has also been studied by Takenaka and Echigo (1974) and Ivanov (1981) after six months, White *et al*. (1964) after eighteen months, by Krauze and Krauze (1991), Qamer *et al*.(2009) after sixteen months and Sanchez *et al.* (2001a) after twenty-four months.

HMF content

The HMF content in the samples of honey from Shahabgunj Forest increased continuously during storage. After eight months of storage HMF content was less than permissible limit of 40mg/kg in the samples no.6, 7, 8, 14, 15, 18, 19, 23 and 39, whereas the HMF in other honey samples raised up to the concentration which made the honey unfit for consumption according to codex honey regulations. Shahabgunj honey samples showed significant increase in HMF content, 60.04% (33.38-

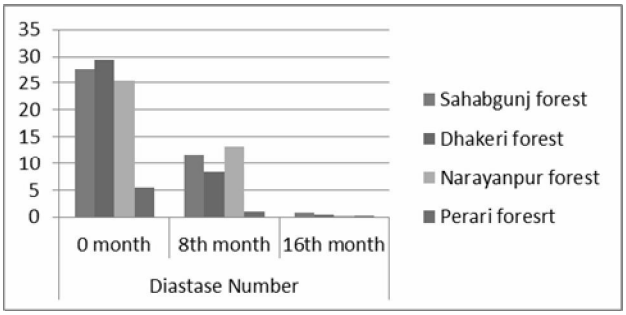

Fig. 7. Effect of 16 month storage on diastase number of *A. dorsata* honey collected from four forests of Teri district, Nepal.

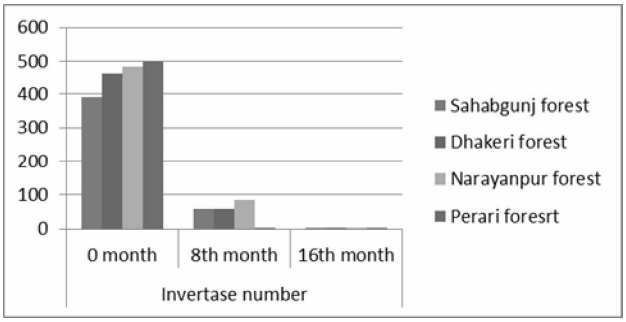

Fig. 8. Effect of 16 month storage on invertase number of *A. dorsata* honey collected from four forests of Teri district, Nepal.

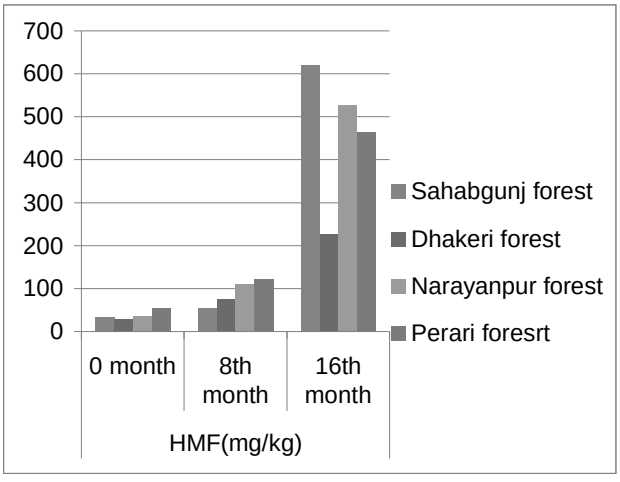

Fig. 9. showing 16 month storage effect on HMF of *A.dorsata*,*s* honey collected from four forests of Teri district, Nepal.

53.42mg/kg) during the first 8 months and 1063% (53.42-621.28%) by the end of 16 months (Fig. 9). The maximum increase in HMF content after sixteen months was 976mg/kg shown by sample #3. The same significant increase in HMF content of honey is also observed in other studies (Iman, 1990; Sancho *et al*., 1992, Cervantes *et al.*, 2000; Sanz *et al.*, 2003; Qamer *et al*., 2009).

CONCLUSIONS

Although honey produced by *A. dorsata* collected from Nepalese forest showed various quality parameters close to the International Honey Quality Standards, yet it, showed shorter shelf life due to natural high moisture contents. Two quality parameters *i.e.* Invertase number and moisture content are quite distinctive and can be used as parameters to identified honey on the basis of bee species type. It appears that International Honey Standards are based on honey produced by *A. mellifera*. There is an urgent need that fresh standards be delimited keeping in view the honey composition of Asian species.

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