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Evaluating the Physico-chemical Properties of Forest Honey

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ABSTRACT

Honey is a widely consumed functional food which has a valued place in traditional medicine. The purpose of this study was to compare the physicochemical properties of honey which was collected from the forest regions of Bastar division. Physico-chemical parameters include moisture, ash, pH, free acidity, electrical conductivity and sugar content which were determined by following the Food Safety Standard Authority of India and International Honey Commission methods. Each honey is unique on the basis of chemistry, quantity and combination of the various components that attributes towards its quality. The control and characterization of quality are of great importance and interest in apiculture. The present study analyzed the quality parameters of honeys from different tribal farmers and market under Bastar division and compared the results with the quality criteria laid by Food Safety Standard Authority of India and Codex Standard. Processing was noted to have detrimental effect on several quality parameters of honeys; in spite of which it was remarkably within the critical limits. The moisture values of all honey samples analyzed were above 20%. The free acidity was above 0.15% in 17 out of 18 samples (94.44%) collected tribal farmers, while 1 out of 18 collected honey samples (5.56%) showed lower values than 0.15%. In this study, 2 out of 18 honey samples (11.11%) from Bastar forest region had soluble solids content below 80 Brix. In terms of HMF, only 1 out of 18 honey samples (5.55%) exceeded 80 mg/kg, which is the maximum content of HMF set in standards. The values of some physicochemical parameters (free acidity, HMF, and soluble solids) of local and imported honey samples are not within the quality limits set in legislation. Further studies are needed to evaluate the properties of fresh honey produced in Bastar forest region and the stability of honey during prolonged storage.

Key word: *Honey testing, Physiochemical property, Forest honey, Honey analysis, Quality test*

Introduction

Honey is known and recognized as a wholesome food consumed due to its extraordinary composition, in terms of nutritional and therapeutic features (Solayman *et al.*, 2016 and Pita- Calvo and Vazquez, 2017). Honey was considered to have healing properties by the ancient civilizations going back in time from the Chinese Empire to the Egyptian Empire. Nowadays, it is quite a trend to investigate alternative natural foods and molecules, such as bee products, which have been supposed to trigger active pharmacologic and metabolic pathways and to generate beneficial effects on consumers' health (Pasupuleti *et al*., 2017). Different researchers have investigated the physicochemical properties of honey from many countries worldwide (Escuredo *et al.*, 2013 and Won *et al.*, 2008). The moisture content is important as it influences many other parameters in honey, such as the sugar content,

hydroxymethylfurfural (HMF), viscosity, crystallization, as well as sensory and microbial properties. Codex Standard for Honey of 2001 (CAC, 2001) stipulates that the moisture content in honey should not exceed 20 g 100 g/1. Authors have also mentioned that honey, being hygroscopic, can absorb moisture, thus the moisture in honey can increase depending on the processing operations, as well as due to inadequate storage conditions (Escuredo *et al.*, 2013 and Karabagias *et al.*, 2014).

India has an ancient tradition of beekeeping and now is one of the most important honey producers in Asia, due to the variety of landforms as well as the diverse and very rich flora. In the Indian flora, there is a series of species of honey plants that stand out through a high honey production. This country has potential to offer sufficient and variate botanical resources to the indigenous bees (*Apis cerena indica, A. dorsata, A. florea* and exotic bee*; Apis mellifera*) in order to obtain a wide panel of unifloral and multifloral honeys, such as the *Brasicca* type, the acacia sort, the sunflower variety (*Helianthus anuus*) and, of course, the multifloral type (usually from spring–summer meadows and grasslands). Central East India, specifically the dense forest Bastar, region of Chhattisgarh, is known as the hug tribal honey production (Anonymous, 2021). From the mountains to the plains, the southern region of Chhattisgarh is rich in cultivated and spontaneous polliniferous and nectariferous plants.

Obtaining physical-chemical parameters of honey is important not only for its characterization (CAC, 2001), but it is also essential to ensure product quality on the market. Because of the diversity of *Apis*

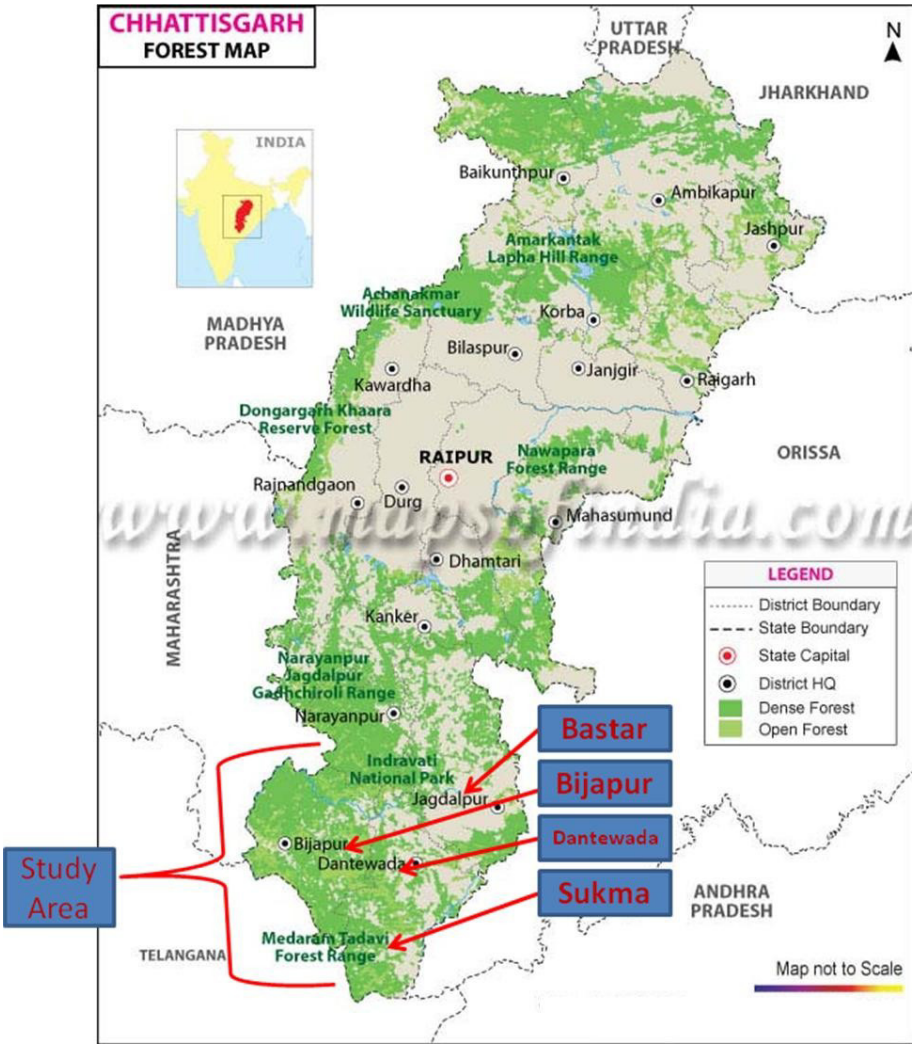

Fig. 1. Map of Chhattisgarh with study area; Bastar division

genus and little information regarding the characteristics of the honey collected by tribals of Bastar division, determining the physical-chemical parameters of the honey is crucial for the process of creating specific legislation for collection and marketing with quality in forest area of Bastar division. Therefore, efforts to characterize the physiochemical parameters of wild honey are important to generate information on the quality standard of this product. In this study, we analyzed samples of collected honey from Bastar division of Chhattisgarh region.

Materials and Methods

Bastar, Dantewada, Bijapur and Sukma districts are located approximately from 19°12' to 19°83' N latitude and from 81°56' to 9°96' E longitude, 18°51' to 49°29' N latitude and from 81°20' to 50°64' E longitude, 16°49' to 43°68' N latitude and from 75°43' to 04°00' E and 18°21' to 50°16' N latitude and from 81°40' to 5°86' E longitude longitude, respectively in Bastar division of Chhattisgarh state.

The forests play an important role in the lives of the people, providing food security and livelihood through the collection of minor forest produce, and employment (as casual labour) in the Forest Department of Chhattisgarh. The forests provide for people's consumption needs - fuel and firewood, medicines, food and drink, implements and housing materials. The forty percent of livelihoods are forest based, 30 percent are agriculture based and 15 percent of livelihoods are dependent on animal husbandry. Another 15 percent of the income of people comes from wage labour. Recorded Forest Area in the Chhattisgarh State is 59,772 sq km of which 25,786 sq km is Reserved Forest, 24,034 sq km is Protected Forest and 9,952 sq km is Unclassed Forest. In the state, during the period 1st January 2015 to 5th February 2019, a total of 3,793.05 hectares of forest land was diverted for various non-forestry purposes under the Forest Conservation Act, 1980 (MoARD, 2010). The Bastar division two main tree species are Sal (*Shorea robusta*) and Teak (*Tectona grandis*). Other major species are Bija (*Pterocarpus marsupium*), Saja (*Terminalia tomentosa*), Dhavdha (*Anogeissus latifolia*), Mahua (*Madhuca indica*), Tendu (*Diospyros melanoxylon*) and bamboo (*Dendrocalamus strictus*) etc. Mostly Arjuna (*Terminalia arjuna*), Jamun (*Syzigium cumini*), Kosam (*Schleichera oleosa*), Mahua (*Madhuca indica*), Baheda (*Terminalia bellerica*), Bhelua (*Semecarpus anacardium*), Koliyari (*Bauhinia purpurea*), Karra (*Cleistanthus collinus*), Bamboo (*Bambusa vulgaris*), Tutlani (*Dolichandrone falcata*), Kudi (*Holarrhena antidysenterica*), Kummi (*Careya arborea*), Pengu (*Celastrus paniculatus*), Saaj (*Terminalia tomentosa*), Chironji (*Buchanania lanzan*), Semal (*Bombax ceiba*), Siadi (*Bauhinia vahlii*), Kadasali (*Nyctanthes arbor*), Dumar (*Ficus racemosa*), Jam (*Psidium guajava*), Peepal (*Ficus religiosa*), and Sevana (*Gmelina arborea*) are present in forest areas of Bastar division. The livelihood of the people in the bastar division is very diverse and the main economic activities are mixed farming of crop and livestock production and beekeeping.

Sampling Techniques and Sample size

In this study for honey quality analysis, a total of 18 samples: four samples from each of the four district of Bastar division; Three samples per traditional beekeeper and the other one from commercial beekeeper) were collected. The collected honey samples were put in clean food grade plastic containers and placed at room temperature until analysis. The honey quality analysis was carried out at Interated Beekeeping Development Centre (IBDC), Ramnagar, District Kurukshretra, Haryana. The parameters viz., moisture, reducing sugars, sucrose, ash, water insoluble solid, acidity and pH were analyzed.

Honey quality data, namely moisture, reducing sugars, sucrose, ash, water insoluble solid, acidity and pH, was analysed by one way ANOVA using OPSTAT (2020). The mean values of honey samples, hive types (traditional and commercial beekeepers) and locations, were compared by using least significant difference (LSD), whenever ANOVA showed statistically significant difference among means. The following statistical Model was used to compare both beekeeper types and locations independently:

$$yij = \mu + Ti + eij$$

Where, yij = Honey quality parameters (response variable), μ = overall mean, Ti = the effect of ith hive type or location and eij = random error.

Results and Discussion

Physico-chemical properties of honey produced in the study area

The minimum, maximum and mean values for each physico-chemical parameter of the analyzed honey samples (hive types, locations and over all) are summarized in Tables 1 and 2. The physicochemical

properties of the different honey samples (hive types and locations) were compared in relation to Food Safety and Standards Authority of India (FSSAI) and Codex Alimentarius Commission (CAC). The overall physicochemical properties of the study area's honey samples are described below.

Moisture: The minimum, maximum and mean moisture contents of the study area's honey are given in Table 1. Majority of the honey samples collected from the study area (89%) had a moisture content of more than 23%. Moisture content of all samples were higher the maximum limit (21%) established by FSSAI and other international agencies (QSAE, CAC and EU). The mean moisture contents of honey samples collected from different locations and hive types are reported in Tables 1 and 2, respectively. There were no significant differences ($P > 0.05$) in moisture between honey samples obtained from the two hive types and among locations. Honey moisture content depends on the environmental conditions such as temperature relative humidity of the area and the manipulation of honey during the harvest period by beekeepers, and it can vary from season to season (Acquarone *et al*., 2007). Moisture variability depends on climatic factors, season of production and maturity of honey (Cantarelli *et al*., 2008). The low moisture content of the study area's honey might be attributed to low relative humidity of the area when the honey samples were harvested (November) and collected

Table 1. Comparison of physicochemical properties of honey in different districts of Bastar division

Parameters	District				Mean (n=4)
	Bastar district	Sukma district	Dantewada district	Bijapur district	
Total reducing sugar (%)	80.04	81.70	81.55	82.22	81.38
Sucrose (%)	2.22	1.32	2.17	1.54	1.81
Moisture (%)	23.15	22.70	22.65	23.85	23.09
Fiech's test	-	-	-	-	
HMF (mg/kg)	69.61	49.49	69.66	74.20	65.74
F:G ratio	1.38	1.34	1.39	1.39	1.38
Specific gravity (at 27 ^{0}C)	1.37	1.39	1.40	1.39	1.39
Water Insoluble matter (%)	0.09	0.08	0.08	0.08	0.08
Acidity (%)	0.18	0.18	0.15	0.19	0.18
Fructose (%)	46.34	46.85	47.37	47.68	47.06
Glucose (%)	33.70	34.86	34.19	34.40	34.29
Proline (mg/kg)	79.52	-	29.96	-	54.74

ND= not detected

Table 2. Comparison of physicochemical properties of honey sample collected from Bastar Market

Parameters	Location		Mean (n=2)
	Gumiyapaal (Bastar district)	Borla (Bijapur district)	
Total reducing sugar (%)	80.47	80.28	80.375
Sucrose (%)	2.25	1.80	2.025
Moisture (%)	27.0	21.2	24.1
Fiech's test	Negative	Negative	-
HMF (mg/kg)	48.14	31.13	39.64
F:G ratio	1.49	1.58	1.54
Specific gravity (at 27 ^{0}C)	1.37	1.37	1.37
Water Insoluble matter (%)	0.07	0.08	0.08
Acidity (%)	0.15	0.16	0.16
Fructose (%)	48.18	50.05	49.12
Glucose (%)	32.29	31.78	32.04
Proline (mg/kg)	199.7	ND	199.7

% by mass; ND= not detected

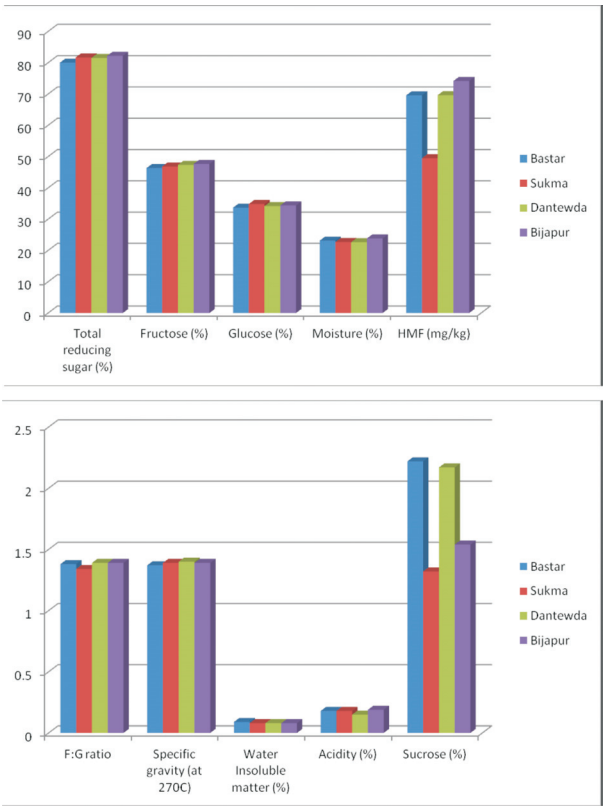

Fig. 1. Comparison of physicochemical properties of honey samples collected from four districts of Bastar division

(months of peak honey flow season, October-November, has relatively low humidity as compared to the minor season, May- June), experience of the beekeepers on harvesting ripened honey, the predominance of moisture stress areas in the study district which dictates the natural vegetation type and climate of the area. The mean moisture content (23.59%) of the present study is higher than the moisture content of the country's average (20.6%), Burie district (18.80 %) and Sekota (15.98%) district, Ethiopia, those reported by Nuru (1999), Tessega (2009) and Tewodros (2010), respectively. The maximum limit of moisture content of Ethiopian honey so far analyzed is 32% (Nuru, 1999). The low moisture content of the honey samples analysed indicates good storage ability of the study area honey, since high moisture content could lead to fermentation during storage.

Reducing sugars: The minimum, maximum and mean reducing sugar contents of the study area's honey are given in Table 2. The overall mean reducing sugar content of the analysed honey samples was 80.87% (Table 2). Total reducing sugar contents in all honey samples are with in quality requirement limits (≥65%) (QSEA; CAC; EU). All honey samples had a total reducing sugar content above the minimum limits of local and international honey quality standards. There were no significant differences ($P > 0.05$) in reducing sugars content between honey samples obtained from the two hive types and among locations. Ninety nine percent of the composition of honey constitutes sugars and water (Bradbear, 2009). Similarly, the average reducing sugars content of honey obtained from market location (80.38%) was significantly higher ($p<0.05$) than the average reducing sugar content of honey obtainedfrom the two agro ecologies (collected directely from beekeepers). The reducing sugars content observed in this study (80.87%) is higher than the finding of Tessega (2009) and Tewodros (2010) who reported 65.73% and 67.33% for honey samples collected from Burie and Sekota, respectively. Similarly, the mean reducing sugars content of the study area honey is higher than the Ethiopian

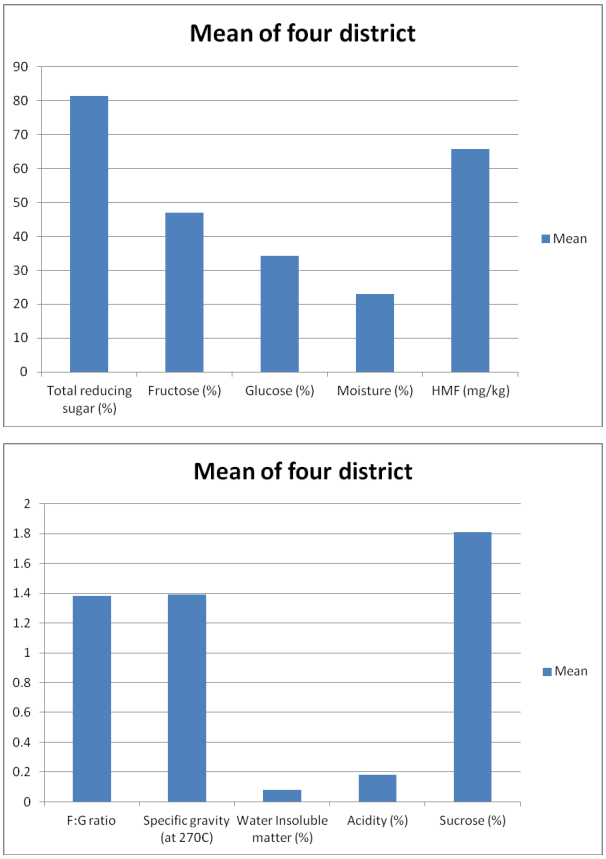

Fig. 2. Comparison of physico-chemical properties of honey (Mean of four districts)

national average (65.5%) reported by Nuru (1999). Thus, the analysis result of the mean reducing sugars content (69.04%) shows that the study area honey meet the quality requirements for reducing sugars established by local and international legislation.

Sucrose: The minimum, maximum and mean sucrose contents of the honey samples analyzed are indicated in Table 1. The average sucrose content of honey samples collected from traditional hives (1.81%) and honey obtained from modern hives (2.02%) was not statistically significant ($p>0.05$). Similarly, no significant difference in sucrose content was observed between honey samples collected from different locations ($p>0.05$). The overall mean sucrose content of honey samples analysed was 1.92% (Table 1). Of all the samples there is no samples were recorded for above the maximum limit (≤ 5g/100g) set by Indian and international standards, whereas all (100%) honey samples were within the maximum limit for sucrose content according to Indian quality standards (<5%). About 38.88% sample collected from beekeepers honey had optimum percentage of sucrose limit and 50% of market honey samples had higher percentage among the sample locations. Higher sucrose contents could be the result of an early harvest of honey, i.e., the sucrose hasnot been converted to fructose and glucose (Azeredo *et al.*, 2003). The amount of sucrose in honey differs according to the degree maturity and nectar compound of the honey. Unripened honeys that were very early harvested contain too much sucrose (White, 1960; Belitz and Grosch, 1999). As the degree of ripeness increase, the amount of sucrose found in honey decreases, these indicate the level of sucrose decrease with the maturity of honey. The Sucrose content of honey lower than 0.20% can be attributed to the enzymatic activity of invertase which causes a decrease in the amount of this non-reducing disaccharide during the storage (Anklam, 1998). Both physical and chemical actions are involved in transformation of nectar into honey, with the activity of enzymes be-

Table 3. Comparison of physico-chemical properties of honey samples collected from different hive types

Parameters	Traditional (n=16)	Movable (n=2)
Total reducing sugar (%)	81.37	80.37
Sucrose (%)	1.8125	2.025
Moisture (%)	23.0875	24.1
Fiech's test	Positive	Negative
HMF (mg/kg)	65.74	39.64
F:G ratio	1.3775	1.54
Specific gravity (at 27^{0}C)	1.305	1.37
Water Insoluble matter (%)	0.09	0.08
Acidity (%)	0.18	0.16
Fructose (%)	46.275	49.12
Glucose (%)	34.2875	32.04
Proline (mg/kg)	ND	199.7

Table 4. Physico-chemical properties of honey produced in the study area in relation to national and international standards (n=18)

S. No	Parameters	Unit	Current study (Range)	FSSAI	CAC	EU	QSAE
1	Total reducing sugar (%)	% by mass	80.37-81.37	65(mm)	≥ 65	≥ 65	Minimum 65
2	Sucrose (%)	% bymass	1.81-2.02	5(max)	≤ 5	≤ 5	Maximum 10
3	Moisture (%)	% by mass	23.08-24.10	20(max)	≤21	≤21	Grade A 17.5-19 Grade B 19.1-20 Grade C 20.1-21
4	Fiech's test	-		Negative			
5	HMF (mg/kg)	mg/kg	39.64-65.74	80 (max)			
6	F:G ratio	% bymass	1.37-1.54	0.95-1.50			
7	Specific gravity (at 27 ^{0}C)	% by mass	1.30-1.37	1.35 (min)			
8	Water Insoluble matter (%)	% by mass	0.08-0.09	0.10(max)	≤ 0.1	≤ 0.1	Maximum 0.1
9	Acidity (%)	% bymass	0.16-3.08	0.20(max)			
10	Fructose (%)	% bymass	46.27-49.12	-			
11	Glucose (%)	% bymass	32.04-34.28/	-			
12	Proline (mg/kg)	mg/kg	29.96-199.60	180 (min)			

FSSAI= Food safety & standard authority of India, CAC = Codex Alimentarius Commission; EU = European Union; QSEA = Quality and Standards Authority of Ethiopia.

ing most prominent. Since these enzymes remain in the honey, their action may continue at a declining rate. The long- noted decrease in the sucrose content of honey after extraction has been ascribed to a continuing action of the invertase added by the bee (White, 1960). Sucrose content does not reach zero after several years of storage, however, even though a honey may still contain an active invertase (white, 1960). The determination of sucrose and fructose: glucose ratio is valuable for assessing adulteration by sucrose and to predict honey crystallization tendency (Ruoff, 2006).

Water insoluble solids: The minimum, maximum and mean water-insoluble contents of the honey samples analyzed are indicated in Table 1. About 5.55% of (only one) sample honeys from traditional hives exceeded the established water insoluble solids content limit (0.10%) set by FSSAI and other international standard has a maximum limit of 0.1 g/100 g for water-soluble solids of honey for extracted honey (CAC, 2001; QSAE, 2005). The water-insoluble solids content is directly dependant up on honey handling and high concentrations are a sign of improper handling during harvest. The water-insoluble solids of honey include wax, pollen, honeycomb debris, bees and filth particles. This indicates that honey's water insoluble matter is used as a criterion of honey cleanliness. The honeys from traditional hives had the higher percentage of impurities (up to 1.53%). The higher water-insoluble matter content observed in traditional hives might be due to inappropriate extraction and handling methods employed by producers and traders, as there were lack or shortage of harvesting, storage and processing equipment like honey extractor, strainers, honey containers and skill gaps of producers and traders in the study area. On the other hand, there was no significant difference ($p>0.05$) in the amounts of water-insoluble solids among honey samples collected from the different localities (Table 1). A mean value of 0.09% was obtained for water-insoluble solids of honey in the present study, this may be attributed to suspended wax particles and/or bees and vegetable debris in honeys, as indicated by Mendes *et al*, (1998). The mean (0.26%) water-insoluble solids observed in this study has lower than the water-insoluble content of Tewodros (2010) who reported a mean value of 0.62% for water-insoluble solids for honey samples collected from Sekota district, Ethiopia. This result shows that the average amount of water insoluble matter of the study area's honey is within the acceptable limit, except for one (5.55%) honey samples from traditional hives that exceeded the established water-insoluble solids content limits (0.10%) set by FSSAI and others. Giving practical training to local beekeepers about the way how to harvest, handle, process, package and sales honey and provision of quality apicultural equipment can improve the problem of high water-insoluble solids in honey samples collected from traditional hives.

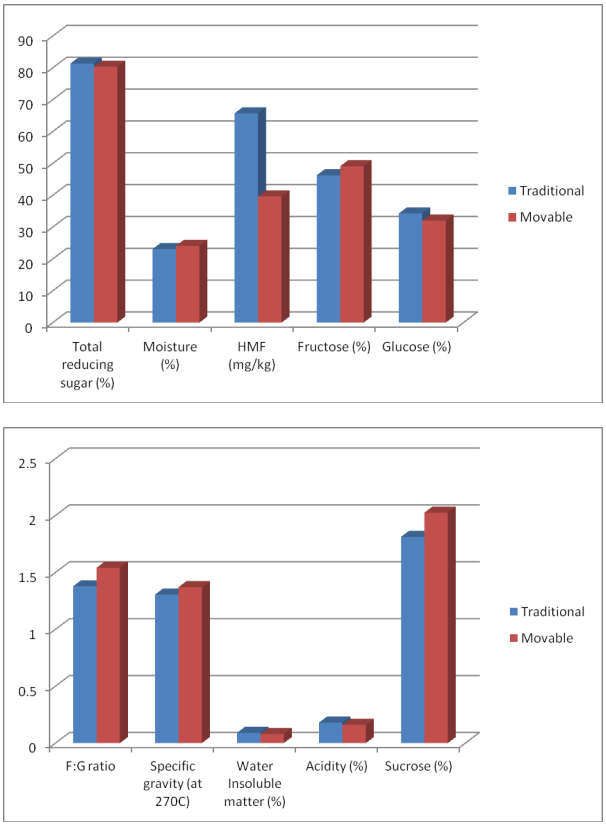

Fig. 3. Comparison of physico-chemical properties of honey samples collected from different hive types

Free acidity: The minimum, maximum and mean free acidity values of the honey samples analyzed are indicated in Table 1. The overall mean free acidity of honey samples analysed was 0.18% (Table 1). Free acidity values of all honey samples were within the acceptable limits (≤0.20%) set by FSSAI. None of the samples exceeded the limit set, which may be taken as indicative of freshness of all the honey samples of the study area. The average acidity content of honey samples collected from frame hives (0.16%) and honey obtained from traditional hives (0.18%) was not statistically significant ($p>0.05$) (Table 1). Free acidity may be explained by taking into account the presence of organic acids, which are

proportional to the corresponding lactones, or internal esters, and some inorganic ions such as phosphates or sulphates (Finola *et al*., 2007).

Honey Collected from traditional beekeepers and the market

The mean and range results of honey from market and farm gate are presented in Appendix Table 2. Comparison of the two sources, showed that marketed honey samples had significantly ($p<0.05$) higher reducing sugar content than beekeepers honey, but no significant difference ($p> 0.05$) was observed with the remaining parameters. The result also showed that the honey samples collected from both sources fulfill all quality parameters set by local and international legislations, except for few samples from traditional hives those exceeding water insoluble content limits. However, this analysis result did not show any typical adulteration problem on marketed samples as compared to honey samples collected directly from beekeepers.

Conclusion

Laboratory evaluation showed that the Total reducing sugar, Sucrose, Moisture, Fiche's test HMF, F:G ratio, Specific gravity, Water Insoluble matter, Acidity, Fructose, Glucose and Proline contents of the honey samples collected from the study area were 8 2.22%, 1.54%, 23.85%, 74.20%, 1.39%, 1.39%, 0.08%, 0.19%, 47.68% and 34.40%, respectively. The results revealed that all the physicochemical parameters lie with-in limits of local and international standards set by Food Safety and Standards Authority of India, Codex Alimentarius Commission and EU Council. There were significant differences for acidity ($p<0.01$) of honey among locations and water insoluble solids ($p<0.05$) between hive types. But, there were no significant differences ($p<0.05$) between hive types and among locations for moisture, reducing sugar, sucrose, and specific gravity contents of honey samples tested. The Water insoluble solids content of the analysed honey samples collected from traditional hives is beyond the standards set for the same parameter by Food Safety and Standard Authority of India. This implies contamination of honey during harvesting, extraction, processing and storage by the traditional beekeepers. Major reasons identified during the survey for increased water- insoluble solid content of honey were lack or shortage of harvesting, storage and processing equipments like honey extractor, strainers, honey containers and skill gaps of producers and traders.

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