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Quality Analysis of Bee Honeys

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ABSTRACT

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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. Henceforth the present study analyzed the quality parameters of honeys from different bee origin and compared the results with the quality criteria laid by European Union Directive for honeys. Processing was noted to have detrimental effect on several quality parameters of honeys; in spite of which it was remarkably within the critical limits. Moisture and electrical conductivity were within the limits as specified by EU, while slight elevation was noticed in honeys viz., *Apis mellifera* and *Apis florea* in their ash content, sucrose content and acidity. Hydroxymethyl furfural content and diastase activity of the honeys were within the levels of EU directive. The microbial analysis indicated that none of the honeys analyzed were infested with pathogenic coliforms.

Introduction

In India, honey is used as nutritional supplement, in therapeutic purposes and also in cosmetic products. Many of these substances give its specific aroma, flavor and some of its biological activity. But these substances are unstable over time and thermolabile. Hence processing methods and period of storage have an impact on the ultimate quality of honey which needs to be analyzed to know the efficacy of raw honey even after processing. Honey has a high commercial significance, although knowledge of the product quality is not adequate. Hence it has become an indispensable part of honey

researches to study the quality of honey with specific to the physicochemical characters.

When the food scandals rule the market consumers slowly got linked with the quality of honey based on origin. For example, honey produced in non-EU countries was discovered within European Community, adulterated with high fructose corn syrup; there were identified other incidents where excessive antibiotics residues have been found in honey derived from third-world countries (Ochi, 2005). Therefore the quality standards stress the importance of the quality of honey that is

linked to the origin and which is used to promote sales through specific labeling.

Unifloral or multifloral, organic protected designation of origin are a few of designations used for honey, that provide scientists with a challenge in terms of confirming labeling that will satisfy consumers, honey industry and control bodies. Moreover, scientists are requested to provide methods and markers that establish the compliance of honey with regulatory requirements. Consequently, finding suitable chemical markers indicating the specific quality of honey linked to origin has been the focus of many studies in the last decade. This includes the studies dealing with the volatile compounds (Cuevas-Glory *et al.*, 2007), phenolic content (Pyrzynska *et al.*, 2009), physico chemical parameters (Gobessa *et al.*, 2012), in honey of different floral and geographic origins.

The latest and the most comprehensive one is the study pertaining to the honey of different bee origin that provides the state-of-the-art results attempting to define typical honey compounds most closely associated with bees from which they are obtained. In this context, the present study aims at a systematic approach to understand the quality aspects of different bee honeys.

Materials and Methods

Sample collection

A total of five multifloral honey samples (in quadruples each) of, the Indian hive bee, *Apis cerana indica* F. (Apidae) (Ac), the European or Italian bee, *Apis mellifera* L. (Apidae) (Am), the rock bee, *Apis dorsata* F. (Apidae) (Ad), the little bee, *Apis florea* F. (Apidae) (Af) and *Trigona irridipennis* S. (Stingless bee) (Ti) were collected from the local beekeepers of different areas of southern zone of Kerala in raw as well as processed form. The processed form of *Apis florea* bee honey

was not available due to the paucity of inventory. The samples collected in January – February of 2015 were stored in half liter pet containers duly labeled with name codes and date of collection. All chemicals used in this study were of analytical grade.

Determination of physicochemical characteristics

The major physicochemical characteristics viz., moisture, electrical conductivity, ash content, specific gravity, water activity, free acidity, pH and total soluble solids were determined according to AOAC (2005).

Determination of hydroxymethyl furfural content

The method used in this study determines the concentration of 5(hydroxyl methyl-) furan – 2 – carbaldehyde, defined as the constituents of honey which are capable of combining with barbituric acid and p – toluidine under the conditions of the test. This method describes the determination of hydroxyl methyl furfural in honey and is based on the original method of Winkler (1995).

To aliquot parts of a honey solution, solutions of p- toluidine and barbituric acid are added and the resultant colour is measured against a blank in 1-cm cuvettes at 550 nm.

Determination of diastase activity

A standard solution of starch, capable of developing with iodine a colour in a defined range of intensity, is acted upon by the enzyme in the honey under standard conditions. The diminution in the blue colour is measured at intervals. A plot of absorbance against time is used to determine the time t_x required to reach absorbance 0.235. The diastase number is calculated as 300 divided by t_x .

Determination of sugars

The major analysis done in this category includes total sugars, reducing sugars and non-reducing sugars according to AOAC.

Microbiological analysis

The honey samples were assessed for the presence of various microorganisms viz., bacteria, coliforms, actinomycetes, fungi and yeasts. Nutrient Agar (NA), Eosin Methylene Blue (EMB), Ken Knights reagent (KE), Rose Bengal (RB) and Yeast Malt Agar (YMA) medium were used for culturing of bacteria, coliforms, actinomycetes, fungi and yeast respectively. The serial dilution of honeys followed by pour plating was employed to estimate the population of viable microorganisms (Johnson *et al.*, 1973).

Statistical analysis

A complete randomized design and Pearson's correlation were used to calculate the significant differences and correlations in the treatment means respectively. The mean values of the replicate determinations were reported for microbiological analysis.

Results and Discussion

The results of the major physicochemical characteristics, sugar composition and microbial contamination of the raw and processed honeys analyzed are as follows.

Physicochemical characteristics of bee honeys

Moisture

The raw *Trigona iridipennis* bee honey had the highest (19.79%) moisture followed by *Apis cerana* honey (16.6%), while raw *Apis dorsata* honey had lowest (10.03%) moisture

content followed by *Apis florea* honey (11.7%) and *Apis mellifera* honey (14.38%). The moisture content of the processed honeys analyzed viz., Ac, Am, Ad and Ti, the highest moisture percentage was obtained in *Trigona iridipennis* (Ti) honey of 18.34% followed by Ad honey with 15.01% of moisture. In the case of processed honeys the lowest moisture content was for *Apis mellifera* honey (13.3%) followed by *Apis cerana* honey (14.7%). Significant difference in the moisture content was recorded in raw and processed honeys at $p < 0.025$ and processing found to reduce the moisture content among all the honeys except in processed *Apis dorsata* honey, this might be due to the unscientific way of processing given to Ad honey. All the honeys had moisture content within the limits of EU (European Union) directive of not $> 20\%$. The moisture content of the analyzed samples was consistent with the previously reported values of some Indian honey for which the values ranged from 17.2% to 21.6%. Overall, the low moisture content in the investigated honey samples suggests that they are of good quality and storage capability. The maximum moisture content was found to be in raw honey species due to the less amount of processing they obtain.

Electrical Conductivity

The EC values in the investigated honey samples varied in the range of 0.13-0.56mS/cm and were within the recommended range (lower than 0.8 mS/cm) as suggested in the EU directive. All the raw honeys varied significantly in their electrical conductivity at $p < 0.025$. In a previous study by Ouchemoukh *et al.*, (2007) the EC values of some Algerian honeys were reported to be higher (0.21-1.61 mS/cm).

The EC value depends on the ash and acid content in honey, the higher the content, the higher the resulting conductivity. This

parameter was recently included in the international standards, replacing the determination of ash content (Alimentarius, 2001). It could be concluded that, among raw and processed honeys analyzed, Ad honey had the highest electrical conductivity which signifies the higher quantity of ionisable organic and inorganic substances present in it.

Ash content

Among the raw honeys analyzed *Apis florea* and *Apis dorsata* had higher levels of ash content of about 1.07% and 1.02% respectively. The *Apis cerana* honey had the next highest amount of ash content with 0.22% and on processing it had reduced to 0.06%. Ad honey had lower ash content on processing of 0.09%. The raw *Apis mellifera* (Am) honey and *Trigona* (Ti) honey had similar levels of ash content of 0.16% and no significant difference observed between the raw Ad and Af.

An increase in ash content was found to be accompanied by an increase in the electrical conductivity. The high electrical conductivity generally indicates the presence of minerals in honey (Silva *et al.*, 2009). This was proved to be true in the present study as the raw Ad honey had higher ash content likewise the electrical conductivity was also found to be higher.

Specific gravity

The *Apis mellifera* honey in the raw as well as processed form had the maximum specific gravity of 1.40 and 1.38 respectively, followed by *Apis dorsata* honey with 1.37 of specific gravity in raw variety and 1.36 of specific gravity in processed variety.

The *Apis cerana* honey had similar specific gravity levels in both raw and processed form of 1.36. The raw *Trigona* honey had a specific

gravity of 1.32 which had reduced to 1.30 on processing. The *Apis florea* honey had the lowest specific gravity of 1.28 among the raw honeys analyzed. Significant difference observed among all the raw honeys at $p < 0.025$ level.

Water activity

The aw of *Apis florea* honey was found to be 0.63 followed by *Apis cerana* honey (0.54) and *Trigona* honey (0.51). The least water activity was noticed in *Apis dorsata* (0.47) and *Apis mellifera* (0.44) honeys. On processing there was an increase in the water activity, it was highly evident in *Apis dorsata* honey where the aw had increased from 0.47 in the raw Ad to 0.61 processed Ad honey. The processed *Trigona iridipennis* honey also exhibited an increased aw of 0.52. These a_w values permit to classify honey as an intermediate moisture food, and thus, they are shelf stable for a reasonable period of time.

Free acidity

The acidity for the raw honeys was recorded as 0.68% (*Apis florea*), 0.31% (*Apis mellifera*), 0.2% (*Apis dorsata*), 0.16% (*Apis cerana*) and 0.15% (*Trigona iridipennis*). All the raw honeys analyzed exhibited an increased rate of change in the acidity after processing.

Acidity is influenced by chemical properties of the organic and inorganic acids, *e.g.* the tridimensional molecular structure and the ionization rate in the honey samples and also by the amino acids content provided by the nectar and salivary enzymes of *A. Mellifera* (Crane, 2013). This might be a possible reason for the variation in the acidity levels among the five different bee species analyzed in this study. The differences in the formic acid percentage among the honeys analyzed were also statistically significant at $p < 0.025$ level.

pH

The pH for all honeys studied was in range of 3.43 to 4.45. The highest pH was viewed in raw *Trigona* honey (4.45) and the lowest was in processed *Apis dorsata* honey (3.43). Out of the five raw honeys examined three of them had pH level < 4 viz., *Apis mellifera* (3.83), *Apis cerana* (3.55) and *Apis dorsata* (3.49) and the rest had pH > 4 namely *Apis florea* honey followed Ti honey with a pH of 4.35. The acidic pH of honey is desirable since acidification has been shown to promote healing by causing oxygen release from hemoglobin. The pH of honey is low enough to prevent the growth of many species of bacteria. This could be a contributing factor for the extended shelf life of the honeys under the investigation with negligible microbial proliferation.

Total soluble solids

The total soluble solids of the honeys analyzed had ranged from 75.5 to 79 ° Brix. Among the raw honeys studied the highest TSS content was observed in *Apis dorsata* honey of 79° Brix followed by *Apis florea* honey with 77.5° Brix. In the case of *Apis mellifera* and *Trigona* honey the total soluble solids was found to be equal with 76° Brix and for *Apis cerana* honey the TSS was 75.5° Brix.

There was significant difference in TSS among the raw honeys analyzed except for *Apis cerana* honey, *Apis mellifera* honey and *Trigona iridipennis* honey. The TSS of processed honeys ranged from 79 to 77° Brix. The TSS of *Apis dorsata* honey was 79° Brix and 77° Brix in *Trigona* honey. The processed *Apis cerana* and *Apis mellifera* honeys had similar levels of total soluble solids of 77.5° Brix. Most of the total soluble solids for honey are sugars. These account for about 80% or more of solids by weight. All the

honey samples analyzed in this study had soluble sugars lesser than 80%. The findings in this study on soluble sugars are in agreement with their total soluble solids content. As there were lesser soluble sugars in the honeys analyzed their TSS also tends to be lower.

Hydroxymethyl furfural content

The raw *Trigona* (3.4mg), *Apis florea* (10.4mg) and *Apis dorsata* (7.16mg) honeys had lower levels of hydroxymethyl furfural content, while higher levels were detected in *Apis cerana* (30.01mg) and *Apis mellifera* (19.64mg). The hydroxymethylfurfural content of processed honeys confirmed that all the honeys had undergone a specific amount of thermal processing. The HMF content for the processed honeys ranged from 6.36 to 51.87mg/Kg of honey. The highest HMF was noticed in *Apis cerana* honey of 51.87mg followed by *Apis mellifera* honey with 27.23mg. A moderate elevation of HMF was viewed in *Apis dorsata* honey with 12.09mg. Among the processed honeys the lowest HMF was obtained for Ti honey with 6.36mg. The hydroxymethylfurfural content varied significantly at 1% and 5% among all the honeys analyzed and also specifically between the raw and processed varieties of each honey. It is usual for HMF to be below 10 mg/kg in fresh extracted honey. In the present study out of the five raw honeys analyzed two of them (Ac and Am) had higher levels of HMF. Levels higher than this may indicate excessive heating during the extraction process.

Even though *Apis cerana* and *mellifera* bee honeys had higher levels of HMF than the usual natural honeys they were within the limits prescribed by European Union (1974), Codex (1993) and QSAE (2005). The international food standards requires that the hydroxyl methyl furfural content of honey

after processing and/or blending shall not be more than 80 mg/kg. This was followed by all the four processed honeys taken for the study.

Diastase activity

The diastase activity of raw honeys ranged from 36.7 to 57.5 DN. The lowest DN was observed in *Apis cerana* honey (36.7) DN followed by *Apis dorsata* honey (48.19) DN. All the other honeys had DN value > 50 among them the highest diastase activity was viewed in *Apis florea* honey with 57.5 DN, *Trigona* honey with 55.45 DN and *Apis mellifera* honey with 52.7 DN. On the other hand the diastase activity of the processed honeys ranged from 27.32 DN to 54.26 DN. Similar to the raw Ac honey the lowest diastase activity was obtained by *Apis cerana* honey among the processed honeys with 27.32 DN. The diastase activity decreased significantly (1%, 5%) in all the honeys on processing.

It is to be noted that the use of enzyme activities as indicators of honey freshness is often criticized as the enzyme activities in honey depend on the intensity of the nectar flow and the amount of nectar processing by the honey bees (White, 1994). Therefore honey from very rich nectar sources e.g. often show high natural enzyme activities which could be correlated with the results obtained for the Ti honey as it is a multiforaging bee with access to multifloral nectar could also leads to its high diastase activity (55.45)DN.

Correlation of physicochemical characteristics

The individual differences for each honey might be due to the presence or absence of one physico chemical parameter that can affect the presence or absence of the other which eventually have an impact on the quality of honey. In fact there can be positive

as well as negative association between the parameters that determine the quality of honey to be better or worse. Hence a correlation analysis was carried out to know the influence of individual parameters on the overall quality of honey. It was readily depicted in the correlation matrix that positive correlations exist between the pH and moisture, pH and acidity, acidity and ash. A very significant correlation was noticed in pH and moisture (0.577**) and acidity and ash (0.829**) while a positive correlation exist between pH and acidity (0.389[†]) significant at 10% level. Significant difference (> 0.44) was noted in specific gravity and electrical conductivity of 0.477*. A strong negative correlation were observed between total soluble solids and moisture (-0.755**) and diastase and hydroxymethyl furfural (-0.749**) significant at 1% level.

Thus it could be concluded that as the pH of honey increases the diastase activity will also be higher. The hydroxymethyl furfural content and diastase are the two physico chemical parameters that are affected by thermal processing of raw honeys. On thermal processing the diastase content in the honey tends to decrease and the HMF content tends to increase and hence they are negatively correlated.

Sugar profile of bee honeys

The reducing sugar of raw honeys in the present study ranges from 33.03% to 67.6%, the highest was acquired by *Apis cerana* honey (67.6%) followed by *Trigona* honey with 63.8% and *Apis dorsata* honey with 62.1% by mass. The *Apis florea* honey differed significantly from other raw honeys with least reducing sugars (33.03%). An average amount of reducing sugars was present in *Apis mellifera* honey of 59.5% by mass. Processing had decreased the reducing sugars in the honeys analyzed. Dimins *et al.*,

(2006) determined the reducing sugars and reported the values in the range of 73.7 to 82.8 per cent which is much higher than the values reported in the present study.

Among the raw honeys highest total sugars was recorded in *Apis mellifera* honey with 72.14% by mass followed by *Apis cerana* and *Trigona* honey having 71.66% and 71.61% by mass of total sugars. The lowest sugars were obtained by *Apis florea* honey of 54.98% followed by *Apis dorsata* honey with 68.75% by mass and significant difference was noted between these honeys at $p < 0.025$. The obtained result is similar to that reported for Indian and Bangladesh honey samples having relatively higher total sugar contents (62.88-78.32% and 42.80 to 60.67 %).

The higher sucrose content was in raw *Trigona* (19.73%) and *Apis florea* (16.08%) honeys. The lowest sucrose content was noticed in *Apis cerana* honey (3.80%) followed by *Apis dorsata* honey (5.31%), while the raw *Apis mellifera* (11.89%) had moderate levels of sucrose. Significant difference noticed among raw Am, Af, Ti and processed Am and Ti honeys respectively.

The legal limit for sucrose should be less than 5% on mass basis as stated in the Codex Alimentarius Commission (2001). Honey that

adulterated with high fructose syrup is usually high in sucrose level. However the higher levels of sucrose in certain honeys analyzed in the present study can also be due to the lack of conversion of sucrose by the bees.

Microbial contamination of bee honeys

Apart from the above discussed physicochemical parameters the occurrence of microbial contaminants if any, in the collected honeys were recorded under the study with special reference to detect the presence of bacteria, fecal coliforms, actinomycetes, moulds and yeasts. The raw honeys *Apis cerana* and *Trigona* did not show any signs of microbial growth whereas honeys viz., *Apis mellifera*, *Apis dorsata* and *Apis florea* had the presence of microbial strains. Yeast colonies were detected in raw *Apis mellifera* and *Apis florea* honeys at a rate of 1×10^{-2} cfu/g. The raw *Apis dorsata* honey was noticed to have 2×10^{-5} cfu/g of bacterial strains. Among the processed honeys apart from *Trigona* honey all the other honeys had fungal and yeast infestations although negligible. Yeast colonies were detected in the processed *Apis cerana*, *Apis mellifera* and *Apis dorsata* honeys at a rate of 2×10^{-2} cfu/g simultaneously, processed *Apis mellifera* and *Apis dorsata* honeys had an occurrence of fungal colonies at a rate of 1×10^{-3} cfu/g.

Table.1 Moisture, EC, ash and specific gravity of bee honeys

Specific gravity (g)		Ash content (%)		Electrical conductivity (mS/cm)		Moisture content (%)		Species
Processed	Raw	Processed	Raw	Processed	Raw	Processed	Raw	
1.36	1.36	0.06	0.22	0.24	0.41	14.7	16.6	Ac
1.38	1.40	0.14	0.16	0.13	0.31	13.3	14.38	Am
1.36	1.37	0.09	1.02	0.34	0.56	15.01	10.03	Ad
--	1.28	-	1.07	-	0.26	-	11.7	Af
1.30	1.32	0.16	0.16	0.33	0.35	18.34	19.79	Ti
0.030	0.003	0.003	0.061	0.030	0.030	0.572	0.770	CD

Table.2 Water activity, free acidity, pH and TSS of bee honeys

TSS (°Brix)		pH		Free acidity (formic acid %)		Water activity (a _w)		Species
Processed	Raw	Processed	Raw	Processed	Raw	Processed	Raw	
77.5	75.5	3.56	3.55	0.19	0.16	0.56	0.54	Ac
77.5	76	3.73	3.83	0.32	0.31	0.53	0.44	Am
79	79	3.43	3.49	0.22	0.2	0.61	0.47	Ad
-	77.5	-	4.35	-	0.68	-	0.63	Af
77	76	3.77	4.45	0.16	0.15	0.52	0.51	Ti
0.003	0.662	0.030	0.090	0.003	0.210	0.023	0.027	CD

Table.3 Correlation matrix of physicochemical characteristics of bee honeys

Diastase activity	Acidity	HMF	Moisture	Total ash	TSS	pH	Electrical conductivity	Specific gravity	
									Specific gravity
								0.47*	Electrical conductivity
							-0.89*	-0.68**	pH
							0.53*		TSS
						0.55*	-0.55*	-0.79**	Total ash
					-0.75**	0.57*	-0.48*		Moisture
					-0.52*			0.42	HMF
			-0.37	0.82**		0.38	-0.46*	-0.50*	Acidity
	0.48*	-0.74**		0.57**		0.68*	-0.55*	-0.47*	Diastase activity

Table.4 Sugar composition of bee honeys

Non-reducing sugars (%)		Total sugars (%)		Reducing sugars (%)		Species
Processed	Raw	Processed	Raw	Processed	Raw	
1.09 (1.26)	3.80 (2.07) [†]	61.45	71.66	60.3	67.6	Ac
8.53 (2.99)	11.89 (3.50)	63.76	72.14	54.34	59.5	Am
2.3 (1.63)	5.31 (2.37)	59.35	68.75	57.16	62.1	Ad
-	16.08 (3.87)	-	54.98	-	33.03	Af
18.39 (2.66)	19.73 (2.78)	70.06	71.61	62.9	63.8	Ti
0.431	1.084	0.770	1.566	2.528	4.277	CD

Fig.1 HMF content of bee honeys

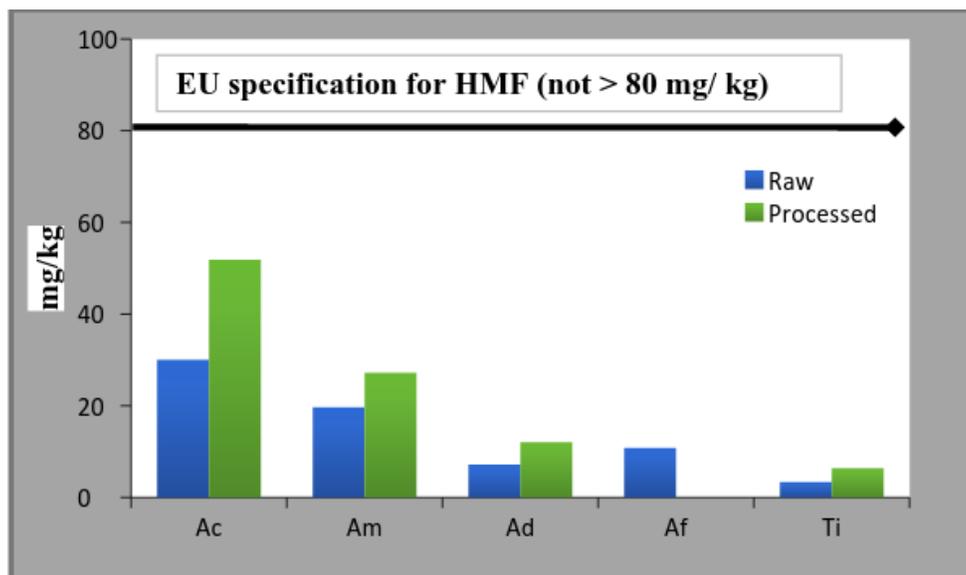
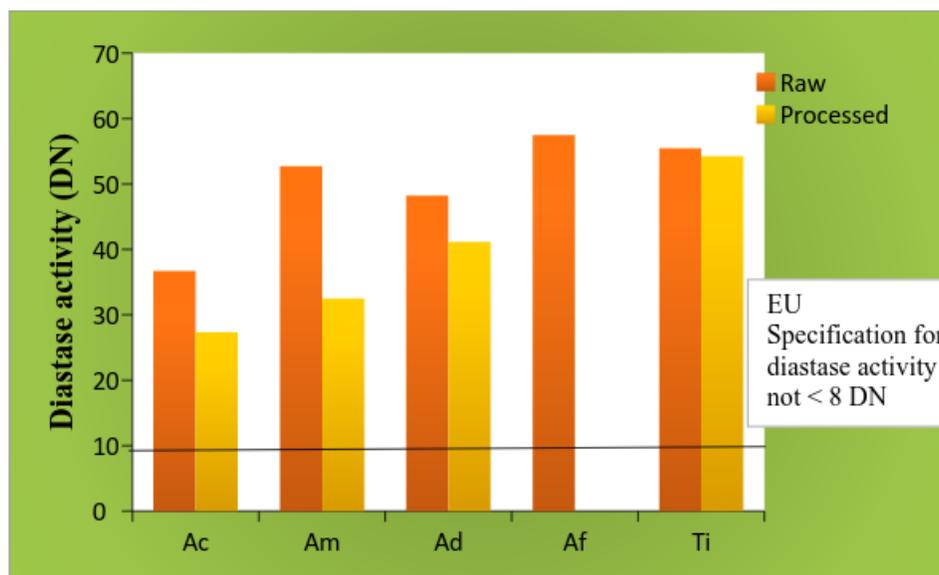


Fig.2 Diastase activity of bee honeys



Evidence based data clearly indicated the raw as well as processed *Trigona* honeys were free of microbial infestation throughout the study. The microbial analysis revealed the presence of fungal and yeast colonies in negligible levels, while pathogenic organisms were absent in all the honeys analyzed. Levels of quantification for commercial quality

parameters with respect to the presence of aerobic bacteria, moulds and yeast in the analyzed processed honey samples were lower than those reported by other authors. Iurlina and Fritz (2005) found higher levels of contamination for both bacteria (average 244cfu/g) and mould and yeast (34 cfu/g) counts. The absence of fecal coliforms also

enforced the sanitary quality and safety of the honeys analyzed in the current investigation. In contrast, Finola *et al.*, (2007) reported that 70% of 23 honey samples analyzed in their study were contaminated with coliforms and clostridia.

In conclusion, all the honeys analyzed in the present study were established to have exportable quality with all the physicochemical characteristics in the acceptable range and complies with the requirements of the current European Union Directives. However it should be noted that raw honeys have better characteristics when compared to their thermally processed counterparts, as high rate of influence were observed on the characteristics like hydroxymethylfurfural content, diastase activity, moisture content, total ash and electrical conductivity. Hence it could be concluded that there is a need for reformulating the traditional practices of harvesting and storage of honey with novel scientific techniques among the local apiculturists so as to improve the therapeutic potency and economic value of the honey in the international markets.

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