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Enzyme activity of raw honey harvested from different localities of Kannad region, Aurangabad District (M. S.), India

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Abstract
The present study deals with the enzyme activity of honey samples harvested from three different locations of Kannad region of Aurangabad district (M. S.), India. Diastase and invertase activity were analyzed by using Schade *et al*., (1958) and Siegenthaler (1977) method respectively. Results clearly indicate that the honey harvested from agricultural and forest area shows highest enzyme activity than road side area. The values of enzymes in honey were varied from location to location. This study also clearly demonstrates thin viat honey harvested from Kannad region were fresh and unheated because the honey samples have more enzyme activity. The values of diastase and invertase were within the quality regulation limits proposed by Codex standards.

Keywords: Raw honey, diastase, invertase, enzyme activity, honey quality, Kannad region, etc.

Introduction
Honey is a sweet viscous food made by honeybees from the sugary secretions of plants such as nectar by regurgitation, enzymatic activity and water evaporation as well as store in wax structures called honeycombs [1]. Honey contains small amounts of different enzymes and the most important ones being diastase (α- and β-amylase), invertase (α-glucosidase), glucose oxidase, catalase and acid phosphatase, which comes from the nectar sources, salivary fluids and the pharyngeal gland secretions of the honeybee. The enzyme content in honey is one of the characteristics which make honey or its products different from other sweeteners.
The biochemical composition of honey varies greatly and it is mainly depends on the floral, regional and climatic conditions. Because of the great variation, lot of research has been carried out to classify and identify the origin of honey in relation to its physical and biological properties. To our knowledge, very limited study was carried on the relationship between the biochemical (enzymes) and nutritional components of honey. Often, the major concern of honey consumers, regardless of honey origin is the quality of honey. This relationship is very important for food processing industry, particularly for those industries using honey as ingredient in their food products [2].
The enzymes are closely related to the nutritional content and honey freshness. Even though enzymes are present in very small amount, they have significant effect on the quality of honey. This is because of the enzymes would significantly affect the protein content, free amino acid profile and acidity of honey samples. Mostly, enzymes found in honey samples were secreted from bee salivary fluids namely oxidases, catalases and amylases [3]. These enzymes would break down complex sugars into simple sugars such as fructose and glucose. The simple sugars might also be further catalyzed into alcohol and acetic acid under an appropriate amount of moisture content at the right temperature condition because of fermentation.
Besides affecting the pH value, the activity of enzymes might change the flavour and aroma of honey after fermentation [2]. Since enzymes are present in trace amount, many studies are likely to focus on sugar composition for the determination of honey origin [4]. Besides as macronutrient, the composition of monosaccharide and disaccharides as well as their ratios could be used to determine the degree of honey maturity. Usually, ripen honey samples have lower disaccharides such as sucrose and maltose content than those from honey harvested at an earlier stage. This is because most of disaccharides have been converted into monosaccharides by the action of enzymes. Hence, the predominant sugars and their ratios are crucial parameters for honey characterization [2].

1) Diastase

It is a common name for the enzyme α-amylase. Its activity indicates the honey quality which is used to determine if honey has been extensively heated during processing. It is found in the nectar and also added by the honey bee during the collection and ripening of nectar [5]. The diastase content of fresh unheated honey is known to vary over a wide range. Any type of honey possesses several kinds of enzyme that play both nutritional and analytical role in the product. It is most important honey enzyme that is capable to break down glycosidic linkages in oligo- and polysaccharides. The activity of this enzyme decreases with the time of storage and that of heating. This starch-digesting enzyme of honey used as a quality indicator of honey because of their sensitivity to heat treatment [6].

2) Invertase

It is an enzyme which is widely distributed among plants and microorganisms and that catalyzes the hydrolysis of the disaccharide sucrose into glucose and fructose. Invertase (α-glucosidase) is an enzyme that produced in the hypopharyngeal glands of honeybee and which is catalyst for one of the most important reactions in the transformation of nectar (sucrose) into honey (glucose and fructose) [7]. It is more sensitive than diastase to thermal treatment and storage of honey, therefore invertase is best parameter for characterization of thermal treatment and storage time [8]. Hence, invertase activity is the quality indicator for thermal treatment of honey and was used as a freshness indicator [9].

Therefore in the present study, the determination of invertase and diastase activity of blended raw honey samples harvested from three different bee species from three different localities of the Kannad region of Aurangabad district (M. S.), India were indicated.

Materials and methods

Study area: The total area of Aurangabad district is about 10.07 lakh hector. Out of which 8.12 lakh hector is under agriculture and 0.12 lakh is under forest area. Geographically, Kannad taluka of Aurangabad district is located at 20º 27' N 75º 13' E. The average altitude of this area is 633 meter above sea level. Honey samples were collected from three different locations of Kannad taluka of Aurangabad district.

Collection of samples: Honey samples were collected from three bee species (*Apis florea*, *Apis cerana indica* and *Apis dorsata*) from three different locations of Kannad region during October 2015 to September 2016. Total 23 different honey samples were collected: 9 from agricultural area, 9 from road side area and 5 from forest area. Area wise honey samples were blended in equal quantity (100g each) and honey samples were put in air tight sterilized plastic containers. They were labeled, brought to the laboratory and stored at 0 - 4º C until analysis.

Determination of Diastase and Invertase: The diastase and invertase activity of honey samples was determined according to the procedure of [10] Schade *et al*. (1958) and [11] Siegenthaler (1977), respectively.

Calculation for Diastase Activity

The classical method for determination of diastase activity is the method of [10] Schade *et al*., (1958). There was a very good correlation (r = 0.987) between the two measurements. Linear regression of y (diastase number) against x (ΔA_{620}) yielded the following relation:

$$DN = 28.2 \times \Delta A_{620} + 2.64$$

Calculation for Invertase Activity

The amount of p-nitrophenol in µM produced during the test corresponds exactly to the amount of substrate in µ utilized. Therefore, the honey invertase activity can be calculated from the absorbance measured at 400 nm and is indicated in Invertase Number (IN).

$$IN = 21.64 \times \Delta A_{400}$$

Results and Discussion

In the present study the diastase and invertase activity were determined in blended raw honey samples harvested from three different locations of Kannad taluka of Aurangabad district and obtained results were presented in the Table No.1 and Fig. No. 1 and 2.

The enzymes are important honey quality parameter and biological activity indicators. Honey naturally preserves small amounts of enzymes that have huge impact on human life processes. Enzymes like invertase and diastase are very sensitive to heat and storage, they are also acts as freshness indicator of honey [9].

1) Diastase activity in honey

Enzyme diastase (amylases) breaks down starch into simple sugars. The activity of diastase in honey is affected by storage and is sensitive to temperature, which is used as an indicator of storage time/freshness and controls during processing of the honey. Therefore proper heating and storage is of utmost importance to retain the market value of honey.

The obtained results in our study for the diastase activity were summarized in table no. 1. The mean values of diastase number in the blended honey samples harvested from different locations of Kannad region were within the range 5.35-7.91 (DN). Result showed that the mean diastase numbers in honey obtained from forest area are higher than agricultural area and road side area.

There was no significant difference in the values of diastase activity from species to species and locations to locations. All the collected honey samples were within the imposed limit of Codex [12, 13]. Similar investigations were carried out by many researchers.

Yilmaz and Kufrevioolu, (2001) reported the mean value of diastase was 14.6 (DN) from Eastern Antonia [14]. Terrab *et al*., (2002) reported the diastase activity of Moroccan unifloral honey ranges between 0.18 to 236 Gº [15]. Serrano *et al*., (2004) determined the chemical and physical parameters of Andalusian honey and found the values ranges from 1.47 to 49.42 (DN) [16]. Guler *et al*., (2007) studied biochemical properties of honey to discriminate pure and adulterate honey from Turkey and reported the mean value 16.50 (DN) [17]. Serrano *et al*., (2007) reported diastase activity of Andalusian honey in the range of 3.99 to 49.42 (DN) [18]. Silva *et al*., (2009) reported the values of diastase ranges from 3 to 38 (DN) from Luso region (Portugal) [19]. Aloisi (2010) observed the diastase activity of honey from Chubut (Argentinean Patagonia) ranges between 3.90 to 39.28 Gothe units [20].

Estevinho *et al*., (2012) assessed the diastase value in the range 13.9 to 16.4 (DN) with mean value 15.3 (DN) from Tras-Os-Montes regions of Portugal [21]. Buba *et al*., (2013)

determined the diastase activity of honey samples from North-East Nigeria ranges between 8.00 to 13.00 (DN) [22]. Iftikhar *et al*., (2014) mentioned the diastase activity of local and imported brands of honey samples available in the Rawalpindi and Islamabad markets Pakistan ranged from 0.00 to 17.0 (DN) [23]. Chakir *et al*., (2016) reported the diastase activity of some honey produced from different plants in Morocco ranges between 4.30 to 29.60 Gº [24]. Silva *et al*., (2017) observed the diastase activity of Portuguese honey from Castelo Branco region in the range of 5.2 to 15.8 (DN) [25]. Goncalves *et al*., (2018) reported the diastase activity of selected Portuguese commercial monofloral honey samples ranges between 6.4-13.3 (DN) [26]. Bouhlali *et al*., (2019) determined the diastase activity of eleven monofloral honey samples produced in Morocco in the range of 7.40 to 29.29 (DN) [27]. Sajid *et al*., (2020) comparatively studied diastase activity of fresh and branded honeys from Pakistan in the range of 26.97 to 43.46 (DN) in fresh honey and 5.95 to 10.35 (DN) in branded honeys [28].

Invertase activity in honey

Invertase activity is the quality indicator for thermal treatment of honey and is used as a freshness indicator [9]. Invertase activity was determined in blended raw honey samples from three different locations of Kannad region and obtained results were summarized in table no. 1

The mean values of invertase activity in the blended honey samples harvested from the study area were within the range of 7.89-18.4 (IN). The mean invertase values in honey obtained from forest area are higher than road side area as well as agricultural area. The variability in enzyme activity found in the different honey types was probably due to nectar collection period (consequently the physiological stage of the colony); abundance of nectar flow and its sugar content (a high flow of concentrated nectar lead to lower enzyme content); age of the bees (the glands of honeybees produce more digestive enzymes when they become a forager); pollen consumption, etc. [5]. The activity of invertase has a great natural variation; its use has been proven in honey quality control.

In our study the values of invertase activity clearly demonstrate the significant difference from locations to locations. The obtained values of invertase activity for all the collected honey samples were within the imposed Codex limit [12, 13]. The enzyme invertase is more sensitive to storage as compared to the diastase in thermal treatment and storage of honey. Therefore invertase is better parameter for characterization of thermal treatment and storage time [8].

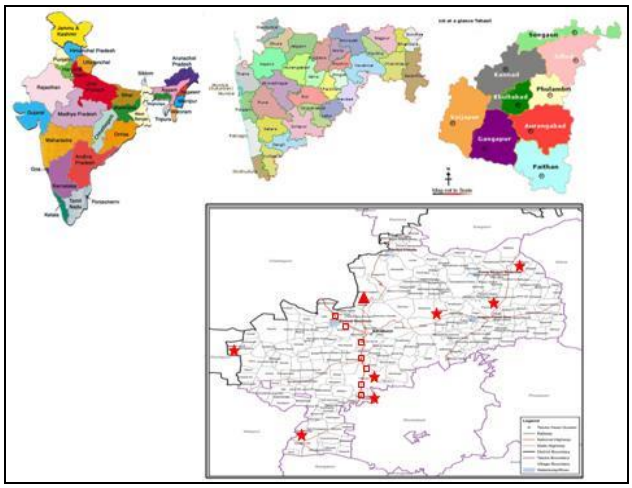
Many authors have expressed different opinions about the propriety of those parameters. Bonvehi *et al*., (2000) reported invertase activity in fresh and processed honeys and recorded the values of invertase ranges from 4.04 to 46.2 (IN) [29]. Vorlova and Pridal, (2002) determined the invertase and diastase activity in honey of Czech Provenience and reported the invertase values ranges from 0.8 to 25.9 (IN) with the mean value 15.7 (IN) [30]. Serrano *et al*., (2004) studied the chemical and physical parameters of Andalusian honey and found the invertase number (IN) ranges from 0.20 to 50.5 (IN) [16]. Serrano *et al*., (2007) reported diastase activity of Andalusian honey in the range of 1.2 to 36.8 (IN) [18]. Silva *et al*., (2017) observed invertase activity of Portuguese honey from Castelo Branco region in the range of 12.8 to 37.4 (IN) [25]. Boussaid *et al*., (2018) reported the invertase activity of six Tunisian honey samples from various floral origins ranges between 46.25 to 184.68 IU (International Units) [31]. Sajid *et al*., (2020) comparatively studied invertase activity of fresh and branded honeys from Pakistan in the range of 58.55 to 81.9 (IN) in fresh honey and 3.10 to 9.66 (IN) in branded honeys [28].

Table 1: Invertase and diastase activity in blended raw honey of three bee species harvested from three different locations of Kannad region of Aurangabad district.

Site of Collection	Diastase Activity (DN)	Invertase Activity (IN)
Agricultural Area	6.81±0.072	12.46±0.082
Forest Area	7.91±0.064	18.45±0.086
Road Side Area	5.35±0.041	7.89±0.063
Codex Alimentarius Standards, 1998 and 2019.	<8	6.5-17.7

± indicates the Standard Deviation

Location Map



= Road side area

= Forest Area (Gautala)

= Agricultural Area

Fig: Map showing the locations of honey sample collection.

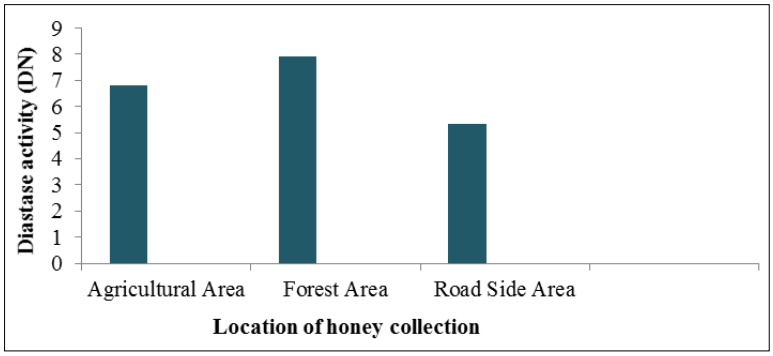


Fig 1: Diastase activity of blended honey harvested from three different locations of Kannad taluka

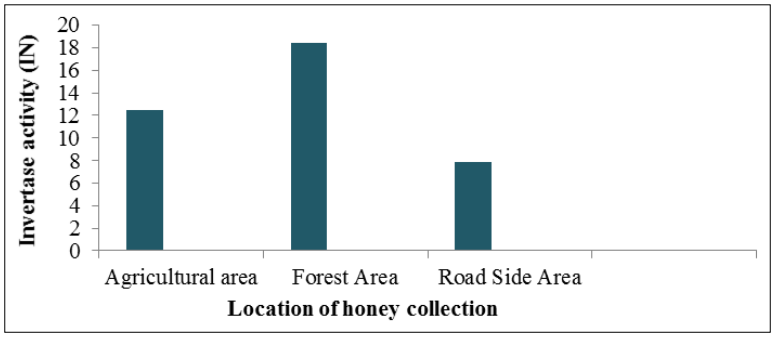


Fig. 2: Invertase activity of blended honey harvested from three different locations of Kannad taluka

Conclusion

Overall we conclude that the honey harvested from agricultural and forest area from the Kannad region shows higher invertase and diastase activity than road side area according to limits proposed by Codex Alimentarius Standards. Hence the honey harvested from agricultural as well as forest area has highest nutritional values than road side area. It is the indication of honey freshness and the honey was unheated which has great nutritional importance in human diet.

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