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Physicochemical determination and effect of storage temperature on diastase level in *Apis mellifera* honey produced from North Maharashtra region, India

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Abstract
The present study intends to determine the physicochemical properties and the effect of different storage temperatures on the diastase activity of *Apis mellifera* honey from five different sites of North Maharashtra Region, India. The analysed honey samples from all sites revealed that floral origin, foraging preference and nature of bee species significantly affects the physicochemical properties such as moisture content, pH, acidity, ash content, fructose, glucose and diastase number. For the assessment of diastase activity, samples were stored at six different temperatures (15^0C, 25^0C, 35^0C, 45^0C, 55^0C and 65^0C) for 60 minutes duration. It was noticed that, the diastase activity at room temperature (21.38±0.82 N), reduced with decrease in temperature (15^0C) in an average of 11.33% in the honey samples. However, the reduction of diastase activity with increased the temperatures (65^0C) in an average of 27.68% in honey samples. Therefore, it was conclude that the heating treatment at higher temperature cause diminution in diastase activity which may affect the quality and reduces the life of honey.

Keywords: Diastase activity, honey, *A. mellifera*, storage temperature, North Maharashtra Region (India).

Introduction

Honeybees known for the world for their sweet honey and bitter sting. Honey is a natural sweet substance prepared by worker bees when visiting the flowers and collect the nectar or honeydew secretions from certain hemipterans. It has high demand in the market due to high nutritional value and safe for human consumption. Honey is a mixture of sugar mainly glucose and fructose and many other compounds as pollens, enzymes, dextrin, waxes, organic acids, ethers, aroma substances and minerals[1].

Before introduced in the market, honey is processed conventionally by filtration and heating. Initially honey is preheated at about 40°C, then strained (filtration) to remove pollen grains, wax particles and other impurities. Finally, honey is heated at 60-65°C for 25-30 min. to reduce the moisture content and to remove the microorganisms responsible for deterioration of honey [2]. Many components present in the honey are heat sensitive which may decline the quality of the honey after heating. Among the honey components, many starch-digesting enzymes as invertase, amylase (diastase) and glucose oxidase are the major enzymes while catalase and acid phosphatase present in minute quantity may play the nutritional and analytical role in the product [3].

Diastase is one of the thermally stable enzyme which able to digest the starch and converted into maltose [4]. Determination of diastase activity is one of the main quality control used for evaluating the quality, freshness, overheating and storage of honey [4, 5, 6]. The diastase activity is usually expressed in Schade units or diastase number (DN). It is defined as the amount of enzyme that will convert 0.01 g of starch to the prescribed end-point in 1 h at 40^0C under the conditions of the test [7]. According to the International Regulatory Standards, the diastase activity must be >8 DN while it should be greater than 3 as per (India) standards [5, 8].

It was noted that the diastase activity may be influenced by certain physico-chemical factors (pH, Electric conductivity, sugar content) [9, 10], Honey processing, storage conditions and harvesting time of honey [11, 12]. There is a lot of information on the instability of diastase activity in honey due to the effect of variable temperatures at specific time duration observed in different parts of the world [6,13,14,15,16].

North Maharashtra Region of India is mainly covered by agro-forest area. As a part of economy, many tribal communities of this region rely on forest products as resins, timbers, honey, wild fruits and vegetables etc. In addition to this, many beekeepers provides the domestic bee boxes to the farmers for agriculture field while collected fresh honey get available in the local market at low cost. It is necessary to aware the tribes and beekeepers about the economic value of honey which may provide international platform for honey. Hence at preliminary level it is essential to study the physico-chemical parameters of honey with effect of heating treatment on diastase level of honey collected from the different floristic area of North Maharashtra region, India.

Material and methods

1. Honey sample collection sites

For this study, total 15 honey samples were collected directly from the colonies of *Apis mellifera* from the 5 selected sites, 2 samples (A & B) from Jalgaon and each sample from Dhule, Nandurbar and Nasik districts (C, D & E respectively) of North Maharashtra region, India during March 2022 to May, 2023 (Fig.1). All the honey samples were fresh, unheated and naturally pure collected directly from the combs and also from beekeepers (Fig. 2). The collected honey samples then brings in the laboratory, filtered through fine cloth and were stored in airtight sterilized plastic containers at room temperature (Fig. 3). As per pollen spectra present in honey, it was found that all the honey samples were multi-floral.

2. Physico-chemical analysis

The standards methods established by Association of Official Analytical Chemists [17] were applied for the analysis of moisture content, pH, acidity, ash content, fructose, glucose and diastase number from the collected honey samples [7].

3. Diastase activity

Diastase activity in honey samples were determined spectrophotometrically (UV-visible Spectrometer) [7]. The diastase activity is measured as diastase number (DN). The starch solution, acetate buffer and sodium chloride (NaCl) were prepared for the analysis of diastase activity. The honey samples collected from two domesticated bee colonies were kept at five different temperatures as 15^0C, 25^0C, 35^0C, 45^0C, 55^0C and 65^0C.

The diastase activity was determined by using 10 g of honey samples weighed in a 50 mL beaker and dissolve completely in 15 mL of distilled water and 5 mL of acetate buffer. Then add 3 mL of NaCl and the solution was diluted to 50 mL with distilled water.

The starch solution was calibrated by using an iodine solution at 660 nm. Pipette 10 mL of honey and starch solution into two separate 50 mL beaker. Both solutions were heated at 40^0C. After 15 minutes, added 5 mL of starch solution into the honey solution, mix properly and the stopwatch was started. At periodic intervals, an aliquot was taken every 5 min and was added rapidly 5 mL of iodine solution. Then it was added 11 mL of distilled water in each solution, mix well and recorded immediately the absorbance at 660 nm.

4. Statistical analysis

For each honey sample, the diastase activity were determined in triplicate. The data obtained from honey samples were compared by using t-test. Differences between mean values were considered significant at $P \leq 0.05$. Descriptive statistics was used to analyze the data generated through survey.

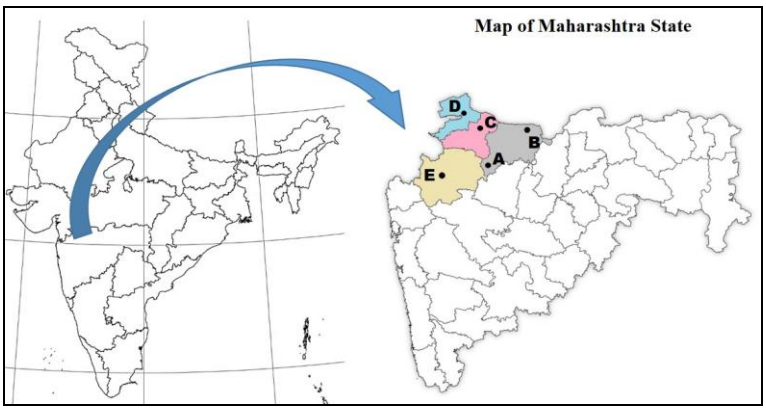

Fig 1: Collection of honey from the selected area of North Maharashtra Region

Fig 2: Inspection and collection of honey from *A. mellifera* colony

Fig 3: Collected honey samples from *A. mellifera* colony for the analysis of physico-chemical determination

Result and Discussion

All of the honey samples obtained from five different sites have been determined and showed variation in moisture content, pH, acidity, ash content, fructose, glucose and diastase number (Tables 1).

Table 1: Physicochemical properties of honey collected from *A. mellifera* colonies

Selected Sites	Moisture (%)	PH	Acidity (meq/kg)	Ash (%)	Fructose (%)	Glucose (%)	Diastase number (DN)
A	15.9±0.11	3.59±0.06	37.15±0.96	0.21±0.08	32.3±0.73	28.6±0.10	21.1±2.3
B	16.3±0.09	3.53±0.04	33.34±0.42	0.28±0.11	34.4±0.61	30.1±0.15	19.7±1.6
C	16.1±0.14	3.67±0.16	34.72±1.12	0.29±0.18	33.5±0.38	29.3±0.24	20.3±1.9
D	17.2±0.10	3.61±0.11	34.48±1.20	0.25±0.12	33.8±0.29	30.4±0.23	21.6±2.5
E	17.4±0.13	3.43±0.07	36.19±0.89	0.24±0.09	34.2±0.26	31.7±0.18	24.2±2.8
Mean±SD	16.58±1.07	3.57±0.82	35.18±1.28	0.25±0.77	33.64±1.47	30.0±1.13	21.38±0.82

Results are expressed as Means ± SD.

Number of honey samples from different sources (n = 15)

The natural enzyme diastase has the ability to digest starch into simple sugars i.e. maltose. It is well known that diastase is an indicator of freshness in honey. The activity of the enzyme is a honey quality parameter can be a used to measure of honey exposure to heat during processing or even storage conditions [4]. Honey samples of both domestic honeybees collected from five different sites were thoroughly analyzed for the determination of diastase activity. The diastase activities were determined after storage at six different temperatures (15^0C, 25^0C, 35^0C, 45^0C, 55^0C and 65^0C) are given in Tables 2.

The initial diastase activity in the honey samples varied from 19.7 to 24.2 mg/kg respectively at room temperature (Table 1). All the honey samples showed the values as per the regulation of FSSAI (India) *i.e.* greater than 3 DN and Codex Alimentarius and the European honey directive i.e. greater than 8 DN. According to Nayik and Nanda [20], the diastase activity in three different honey samples i.e. multifloral, acacia and pine honeydew found as 14.93 DN, 15.51 DN and 25.99 DN respectively. Similar results were represented by Meda *et al* [23] for Burkina Fasan honey, Saric *et al* [24] for Croatian honeys, Korkmaz and Küplülü [11] for Turkey honey, Nayik and Nanda [20] for Kashmir valley honeys.

Table 2: Variation in Diastase activity at different storage temperatures of *A. mellifera* honey samples[a]

Selected Sites	Honey stored at varied temperature for 60 minutes						DN Average reduction%[b]
	15^0	25^0	35^0	45^0	55^0	65^0	
A	19.6±0.95	20.1±1.32	20.5±0.84	19.2±1.21	17.8±0.69	15.7±1.10	25.59%
B	17.4±1.12	18.6±0.96	19.2±1.16	18.4±0.86	16.9±0.76	14.3±0.69	27.41%
C	18.4±0.88	19.1±0.74	19.7±0.94	18.2±0.93	16.6±0.79	14.3±0.78	29.56%
D	19.5±1.14	20.8±1.22	21.3±1.32	20.1±1.26	18.8±1.10	15.7±0.73	27.31%
E	21.1±1.26	23.0±1.43	23.6±1.26	22.9±1.48	20.5±1.31	17.3±0.85	28.51%

Results are expressed as Means ± SD.

[a] The analyses were performed in triplicate.

[b] Average percentage of DN calculated by comparing normal value with value at 65^0.

Variations in the diastase level in honey samples may be caused by biotic and abiotic factors, floral and geographic origins, the age of the bees and the bee colony [18, 19, 20, 14]. Certain physico-chemical factors (pH, Electric conductivity, sugar content) [9, 10, 21], Honey processing, storage conditions and harvesting time of honey may also influence the diastase activity [13, 11, 12, 15, 22]. The analysed honey samples from both bee colonies revealed that floral origin, difference in foraging preference according to the nature of bee species significantly affects the diastase activity of honey samples.

The diastase activity of honey samples stored at 15^0C, 25^0C, 35^0C, 45^0C, 55^0C and 65^0C were found between 17.4-21.1, 18.6-23.0, 19.2-23.6, 18.2-22.9, 16.5-20.5 and 13.6-17.3 mg/kg respectively (Table 2). In all the honey samples, it was observed that the diastase activity decline with increased the temperatures in a range of 25.59 to 29.51%. Similar results were noticed by earlier workers [6, 10, 14, 15, 16].

According to Tosi *et al* [15], the diastase activity in six honey samples was vary when applying transient as well as isothermal heating. During the transient heating, the reduction of diastase activity associated with an increase in temperature from 60 to 100^0C. During isothermal heating, diastase activity decreases at short heating times but it recovered in medium temperature treatments at longer times. The activity becomes zero at 100^0C for both transient and isothermal heating. Sajid *et al* [14] had revealed that Pakistani honey when heated at 4, 25, 35, 45 and 55^0C temperature for 4 and 8 hours affect the diastase activity. It was found that except low temperatures (4°C and 25°C), the higher temperature range (35, 45 and 55^0C) caused declining in diastase activity with increasing the duration of heating time. According to Ramirez Cervantes *et al* [6] where 61-70% loss of diastase activity was observed in honey samples heated at 55^0C for 3-15 minutes. Kamboj *et al* [10] revealed that with increase in temperature (45-55^0C) and time (5-15 min) diastase activity decreased significantly. However, fall in diastase activity also depends on the pH which shifted from 4.6 to 5.6. Similar results were noticed by earlier workers [25, 26, 27]. Diastase activity of honey is correlated to the plant taxa and environmental conditions of the region.

At the low temperature (15^0C), it was observed that the diastase activity also decline from 7 to 14 % and 12 to 22% in the samples of *A. c. indica* and *A. mellifera* respectively. Yilmaz and Frevüoúlu [16] was also noticed similar results that the diastase numbers in the honey samples after one year storage at 20±5^0C reduced from 14.6 to 10.7 DN (27% decrease in diastase activity). Moreover, a few honey

samples either didn't show any change or small loss in diastase activity at 25^{0}C also noticed by Tosi *et al* [15]. According to Hasan [13] (2013), short term heating applied to honey at 55, 65, 75^{0}C for 5,15,20,25 minutes does not affect the diastase activity.

Korkmaz and Küplülü [11] were noticed the temperature and time-dependent alterations affect the diastase activity in flower honey and honeydew honey samples when stored at 3 different temperature (10±2, 22±2 and 35±2^{0}C) for 3, 6, 9 and 12th month. According to findings, the diastase activities of the samples at 10±2^{0}C and 22±2^{0}C remains above the 8 DN as per the limit value for Turkish Food Codex but it decline at 35±2^{0}C at the end of 9th and 12th month. Similar results were also noticed by Castro-Vàzquez *et al* [28]. According Turhan [29] was determine the diastase values of 40 honey samples where the initial value (16.3 DN) decreased to 11.4 DN when the samples stored at 25^{0}C for a year. Sahinler [30] examined the diastase activities that changed in honey samples stored at room temperature for a year after keeping the samples at 55, 65 and 75°C for 15, 30, 45 and 60 minutes, respectively.

Kowalski *et al* [12] were conducted study to compare the diastase activity in honey by conventional process and after microwave treatment. Microwave heating were carried out at power level of 1.26 W/g of honey for heating periods 2, 4, and 6 min respectively. It was noticed that the inactivation of diastase is much faster under microwaves treatment than the conventional process. The microwave treatment at the initial stage up to 2 min had showed no any effect on the DN. Hence, the industrial processing of honey with microwaves may as a short and effective heating method. Hebbar *et al* [31] was also reported the reduction in the diastase activity of honey treated at different power levels of 6.3 W/g, 9.1 W/g, and 11.9 W/g, respectively at different heating times between 15 to 90 seconds.

The diastase is one of the enzyme which able to digests starch and converted to form maltose. It has heat resistant property which is helpful to indicate the quality and freshness in honey. The prolong honey storage and even heating may affect the variation in diastase activity in honey samples [4]. Therefore, it was conclude that the heating treatment at higher temperature cause reduction in diastase activity which may affect the quality and decline the life of honey.

Conclusion

As per the results, it could be concluded that the moisture content, pH, acidity, ash content, fructose, glucose and diastase number in *A. mellifera* honey depends on the floral origin, foraging preference and nature of bee species. The physico-chemical properties of all honey samples were in the range approved as per the regulation of FSSAI (India) and Codex Alimentarius. It was noticed that the reduction of diastase activity with increased the temperatures in an average of 27.68% in honey samples. However, the diastase activity reduced with decrease in temperature from 35^{0}C to 15^{0}C in an average of 11.33%. Overall it was conclude that the heating treatment at higher temperature and prolonged storage of honey cause diminution in diastase activity which may affect the quality of honey and reduces the life of honey.

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