

## Exploring enzyme variation in honey as a marker for quality

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The physicochemical properties of honey, including enzyme activity, moisture content, sugars, and pH, are crucial for understanding its quality, authenticity, and potential uses. This understanding is essential for setting standards to distinguish pure from adulterated honey, fostering consumer trust, and ensuring regulatory compliance. While previous research has often focused on individual factors influencing honey quality, a comprehensive, multifactorial analysis of enzymatic activity across different bee species has been less explored in response to varying heat treatments and adulteration levels. This study specifically investigated the combined impact of bee species, adulteration, and processing on honey's enzymatic activities. It was found that stingless bee honey consistently exhibited the highest enzyme values, with remarkable levels of diastase (58.533 DN), invertase (49.353 IN), glucose oxidase (23.057 U/g), and catalase (21.870 U/g). The findings also clearly show that heat treatment leads to enzyme inactivation, with almost complete loss of activity at 120°C. Furthermore, adulteration with 50% sugar syrup significantly altered honey properties, increasing moisture and pH while dramatically reducing enzyme activity. These results strongly emphasize enzyme activity as a critical and highly indicative measurement of honey's richness and authenticity, influenced by honey type, purity, and quality parameters. This research confirms the reliability of enzyme activity as a key indicator for honey quality and authenticity, which has direct implications for consumer protection and regulatory oversight. To further ensure global honey quality, future investigations should delve into the specific mechanisms of enzyme denaturation under various processing conditions. Developing rapid, on-site detection methods for adulteration based on enzymatic profiles would also be a valuable contribution.

**Keywords:** Adulteration, Catalase, Diastase, Enzyme activity, Glucose oxidase, Invertase

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### Introduction

Honey, a natural food substance, is produced by honey bees primarily from floral and extrafloral nectars, and sometimes from honeydew. This complex natural product contains a minimum of 181 identified components. These include essential macronutrients like carbohydrates and water, and a diverse array of micronutrients and bioactive compounds such as minerals, vitamins, amino acids, phenolic acids, organic acids, flavonoids, enzymes, and other phytochemicals<sup>1</sup>. From ancient times, after considering its antioxidant and antimicrobial properties, honey has been widely used in therapeutics for the treatment of cardiovascular diseases, cancer, cataracts, and several inflammatory diseases, as well as in wound healing<sup>2</sup>. Depending on geographical region, season, harvesting, and storage, the composition of honey varies<sup>3</sup>.

Honey contains 0.2 per cent protein, which mainly originates from bees, and predominant among them are enzymes<sup>4</sup>, which play a crucial role in honey formation. Major enzymes found in honey include diastase, invertase, glucose oxidase, catalase, and  $\beta$ -glucosidase<sup>5</sup>. Enzymatic reactions include starch breakdown, sucrose hydrolysis, production and degradation of hydrogen peroxide, breakdown of  $\beta$ -glucans, and degradation of protein, which either takes place inside honeybees during honey formation or in honey during processing and storage<sup>6</sup>. Enzyme activities of honey are also attributed to bees' geographical race and location. Honey enzymes originate from three major sources: plant nectars and secretions, honeybees, and excretions of plant-sucking insects<sup>7</sup>.

The enzymes glucose oxidase, invertase, and diastase are all naturally secreted by bees into the honey<sup>8</sup>. Invertase helps to break down the complex sugar known as sucrose into the simple forms of sugars - glucose and fructose<sup>9</sup>, while diastase is responsible for the breakdown of starch to maltose<sup>10</sup>. Another enzyme that bees produce in their

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hypopharyngeal gland is glucose oxidase, which transforms sugar into gluconate and hydroxyl peroxygen in the form of hydrogen peroxide ( $H_2O_2$ ). The enzyme particularly demonstrates a high activity in honey with high water activity and also in honey that is not completely ripe<sup>11</sup>. The main active substance, which accounts for the therapeutic or antibacterial action of honey, is believed to be  $H_2O_2$ , which is a byproduct of the enzyme glucose oxidase<sup>12</sup>. In honey, catalase, which decomposes  $H_2O_2$ , is contributed mostly through nectar, flower pollen, and microbes found in the honey itself<sup>13</sup>.

Other than enzymes, moisture content, electrical conductivity, reducing and non-reducing sugars, ash content, free acidity, and HMF are the factors proposed by the International Honey Commission (IHC), which determines the quality of honey<sup>14</sup>. The determination of honey's moisture content is of great importance in identifying honey adulterated with water<sup>15</sup>. FSSAI honey standards<sup>16</sup> strictly limit the moisture content in honey to a maximum of 20 per cent by mass. One of the characteristics that stands out about the antibacterial efficacy of honey is the presence of acidity, which has values in the range of pH 3.2-4.5<sup>17</sup>. Honey is subjected to heat treatment for several purposes, including extension of shelf life, delaying crystallization, decreasing viscosity, preventing premature fermentation, and eliminating microbes<sup>18</sup>. However, heating of honey at temperatures above 50°C leads to the formation of toxic substances like hydroxymethylfurfural (HMF), which reduce the honey quality<sup>19</sup>. HMF possess various negative impact on human health such as genotoxicity, organotoxicity, enzyme inhibitory, hepatorenal toxicity, decreased glutathione levels in the cells, inductions of neoplastic changes, irritation on the mucous membranes of the upper respiratory tract, eyes, skin etc.<sup>20</sup>. Also, the increased demand and high price of authentic honey leads to the production of commercially cheaper fake honey by adulterating it with low cost additives like sugar syrup, corn syrup, maple syrup etc.<sup>21</sup>.

In contrast to earlier research, which often isolated factors influencing honey quality, this study presents a comprehensive, multifactorial analysis of enzymatic activity. We explored its response to diverse bee species, varying heat treatments, and different adulteration levels. The current study thus adopts a systematic approach to ascertain the combined impact of these variables on honey's enzymatic activities.

## Materials and Methods

### Honey samples

Five samples each of raw honey belonging to the species *Apis indica* Fabricius, *Apis mellifera* Linnaeus, *Apis dorsata* Fabricius, and *Tetragonula travancorica* were collected for honey enzyme analysis from an apiary at Kadakkal, Kollam, with similar bee foraging resources. Kadakkal (8.8264° N, 76.9177° E) is approximately 3 m above mean sea level and has essentially a tropical climate with plenty of nectariferous vegetation, which provides a greater opportunity for bee farming.

Raw honey of the Indian bee was heated at 30, 60, 90, and 120°C, and the activity of diastase, invertase, glucose oxidase, and catalase was determined in these heat-treated samples. Along with this, samples of manually and machine processed (vacuum processing at temperature  $\leq 40^\circ\text{C}$  under 500 mm Hg pressure) Indian honey from All India Co-Ordinated Research Project on Honey Bees & Pollinators, Vellayani Center, were also analyzed to determine the effect of processing on enzyme activity. Processed western bee honey from different nectar sources (Multifloral, Sidr, Acacia, and Tulsi) collected from Kashmir was analyzed to study the effect of botanical origin on enzyme activity. 20 g of each raw honey sample was adulterated with 50 per cent sugar syrup (50 g sugar + 100 mL water, heated at 100°C for 30 minutes). Honey samples were mixed with sugar syrup in the ratio 2:1, and the mixture was kept at room temperature. The enzyme activity was determined up to 72 h in the adulterated samples at 24 h intervals. Moisture, TSS, pH, and colour of the samples were also determined after 72 h and compared with the raw honey.

### Diastase analysis

Activity of diastase in samples was determined using the Schade method and expressed in diastase number (DN). The method is based on the action of an enzyme in a standard solution of starch that generates a blue colour with iodine under a specific intensity range. Reduction in the intensity of blue colour was measured by taking absorbance at 660 nm using a UV/VIS Spectrophotometer (Systronics India Ltd, Ahmedabad, India) at regular intervals. Diastase number was calculated by dividing 300 by  $t_x$  (the amount of time needed to achieve an absorbance of 0.235).

### Invertase analysis

Activity of invertase in samples was determined using the Siegenthaler method and expressed in

invertase number (IN). The method is based on the breakdown of p-Nitrophenyl- $\alpha$ -D-glucopyranoside (pNPG) into glucose and p-nitrophenol. The amount of p-nitrophenol converted to nitrophenolate anion was measured by taking absorbance at 400 nm using a UV/VIS spectrophotometer. Calculation of invertase activity was done as per Kostic *et al.*<sup>22</sup>.

#### Glucose oxidase analysis

Glucose oxidase activity in the samples was determined using a kit for glucose oxidase analysis (200201-8) of Megazyme International Company. The procedure for determining glucose oxidase in honey was as per Alshareef *et al.*<sup>23</sup>. Activity of glucose oxidase in 1 g of honey was calculated using the Megacalc Excel Program provided by the company.

#### Catalase analysis

Activity of catalase in the samples was determined using a catalase analysis kit (200206-6) of Megazyme International Company. The procedure followed for catalase analysis in honey was as per Alshareef *et al.*<sup>23</sup>. Catalase activity in 1 g of the sample was calculated using the Megacalc Excel program.

#### Physicochemical characteristics

Moisture content and Total soluble sugar (TSS) of the samples were determined according to the harmonized honey analysis method of the International Honey Commission (IHC)<sup>24</sup> using a digital handheld refractometer (ATC) and expressed in percentage (%) and degree Brix ( $^{\circ}$  Brix), respectively. Similarly, pH was determined using a digital pH meter (Eutech Waterproof pHTestr 30).

The colour of the honey samples was determined using the Pfund scale by measuring the absorbance of the honey samples at 560 nm against a deionized water blank using a UV/VIS spectrophotometer. The obtained absorbance was multiplied by a factor of 3.15, and the colour was determined at a range of white to dark amber in accordance with the United States Department of Agriculture's<sup>25</sup> grades for extracted honey.

#### Statistical analysis

Analysis of variance (ANOVA) was conducted to calculate the statistical significance of each group, and the significance of the difference between the means was determined using a complete randomized design with a 5% level of significance. Pearson's correlation was used to calculate the correlations in the treatment means. All the statistical analysis was conducted using the web application KAUGRAPES by Gopinath *et al.*<sup>26</sup>.

#### Results

Results on variation in enzyme activities in honey based on its entomological origin, physicochemical parameters, processing methods, and adulteration are presented below.

#### Raw honey samples

Stingless bee honey possessed significantly higher activity of diastase, invertase, glucose oxidase, and catalase (58.53 DN, 49.35 IN, 23.06 U/g GLOx, and 21.87 U/g CAT), low TSS (71.5  $^{\circ}$ Brix) and pH (3.17) among the samples (Table 1). Whereas, Indian bee honey, which showed low enzyme activity (32.97 DN, 36.24 IN, 16.45 U/g GLOx and 15.86 U/g CAT), had a high amount of TSS (75.4  $^{\circ}$ Brix) and pH (3.58). All the samples followed the Codex Alimentarius honey standard<sup>27</sup> (DN  $\geq$  8). A study on physicochemical parameters of raw honey samples (Table 2) revealed that moisture content and TSS ranged between 23.67-24.5% and 71.5-75.4  $^{\circ}$ Brix, respectively. Although there was no significant difference in moisture content, rock bee honey showed significantly lower enzyme activity compared to stingless bee honey.

Pearson's correlation studies on all the parameters (Fig. 1) showed a significantly high positive correlation among all the enzymes ( $r = 0.98$ ). All the samples had amber colour in different intensities, where stingless bee honey was darker and Indian bee honey was lighter comparatively. Colour intensity was found to have a weak positive correlation with

Table 1 — Enzyme activity in raw honey samples

S. No	Sample**	DS	IV	GLOx	CAT
1	Indian bee honey	32.967 $\pm$ 0.021 <sup>d</sup>	36.243 $\pm$ 0.032 <sup>d</sup>	16.453 $\pm$ 0.032 <sup>d</sup>	15.863 $\pm$ 0.031 <sup>c</sup>
2	Italian bee honey	55.297 $\pm$ 0.015 <sup>b</sup>	44.633 $\pm$ 0.006 <sup>b</sup>	19.860 $\pm$ 0.026 <sup>b</sup>	19.143 $\pm$ 0.035 <sup>b</sup>
3	Rock bee honey	54.763 $\pm$ 0.021 <sup>c</sup>	40.537 $\pm$ 0.025 <sup>c</sup>	18.960 $\pm$ 0.030 <sup>c</sup>	19.133 $\pm$ 0.032 <sup>b</sup>
4	Stingless bee honey	58.533 $\pm$ 0.021 <sup>a</sup>	49.353 $\pm$ 0.025 <sup>a</sup>	23.057 $\pm$ 0.025 <sup>a</sup>	21.870 $\pm$ 0.026 <sup>a</sup>
	CD*	0.038	0.040	0.044	0.047

\*CD – Critical difference (p value<0.01) DS – Diastase activity (DN), IV – Invertase activity (IN), GLOx – Glucose oxidase activity (U/g), CAT – Catalase activity (U/g).

\*\* Activity of all the enzymes was determined in 1g of the sample.

Table 2 — Physicochemical parameters in raw honey samples

S. No	Sample	Mo	TSS	pH	Colour
1	Indian bee honey	23.850±0.265 <sup>b</sup>	75.425±0.377 <sup>a</sup>	3.583±0.477 <sup>a</sup>	Light amber
2	Italian bee honey	23.675±0.310 <sup>b</sup>	73.450±0.342 <sup>b</sup>	3.348±0.113 <sup>a</sup>	Amber
3	Rock bee honey	24.475±0.258 <sup>a</sup>	73.625±0.411 <sup>b</sup>	3.562±0.389 <sup>a</sup>	Amber
4	Stingless bee honey	24.500±0.222 <sup>a</sup>	71.575±0.330 <sup>c</sup>	3.175±0.212 <sup>a</sup>	Amber
	CD*	0.409	0.565	0.472	

\*CD – Critical difference (p value<0.01) Mo – Moisture (%), TSS – Total soluble sugar (°Brix)



Fig. 1 — Heat map of Pearson's correlation matrix for raw honey samples.

DN – Diastase, IN – Invertase, GLOx – Glucose oxidase, CAT – Catalase, Mo- Moisture, TSS- Total soluble sugar, Clr- Colour intensity. Green box – strong positively correlated, Pink box – strong negatively correlated, White box – slight to no correlation

enzymes (r = 0.5). TSS and pH exhibited a high negative correlation with all the enzymes analyzed (r = -0.99), whereas moisture showed a weak negative correlation (r = -0.19).

**Processed samples**

Indian bee honey samples treated at different heat levels revealed a reduction in the activity of all enzymes analyzed with increase in temperature (Fig. 2). Activity of all enzymes reduced drastically between 30 and 60°C with a percentage reduction of 59, 67, 45, and 58% in case of diastase, invertase, glucose oxidase and catalase, respectively. Three of the four enzymes (diastase, invertase, and glucose oxidase) were denatured and showed negligible activity at 120°C, with a reduction of almost 99% when the samples were caramelized. However, catalase showed minimal activity at 120°C, with a reduction of 97% among them. Among the processed samples of Indian bee honey, machine processed samples with vacuum processing showed significantly higher activity of all the enzymes (26.14 DN, 25.56

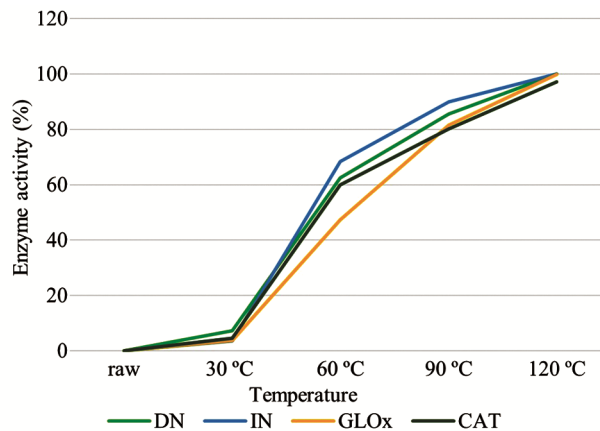


Fig. 2 — Percentage decrease of enzymes in heat treated Indian bee honey compared with raw honey.

DN – Diastase, IN – Invertase, GLOx – Glucose oxidase, CAT – Catalase.

IN, 14.33 GLOx and 13.74 CAT) compared to manually processed samples (Supplementary Fig. 1). Among the samples with different botanical origin, honey obtained from multifloral source was found superior in enzymatic activity (19.55 DN, 13.55 IN, 9.42 GLOx and 10.66 CAT), followed by tulsi honey (Supplementary Fig. 2). Diastase activity adopted as a honey quality parameter fulfilled the Codex Alimentarius honey standards<sup>27</sup> (DN ≥ 8) for all the samples.

**Adulterated honey samples**

Results obtained from the study on adulterated samples (Fig. 3) revealed reduced activity of all enzymes – diastase, invertase, glucose oxidase, and catalase. Activity of glucose oxidase and catalase in the samples was least reduced (<70%) compared with diastase and invertase. All four samples showed a sudden decrease in the activity of enzymes during the first 24 h (>40%), and the rate of decrease was reduced for the later hours. Among the samples, adulterated stingless bee honey showed least reduction (70% DN, 70% IN, 50% GLOx and 64% CAT) for all enzymes (Fig. 3d) whereas, rock bee honey was seriously affected with high percentage decrease (80% DN, 78% IN, 69% GLOx and 74%

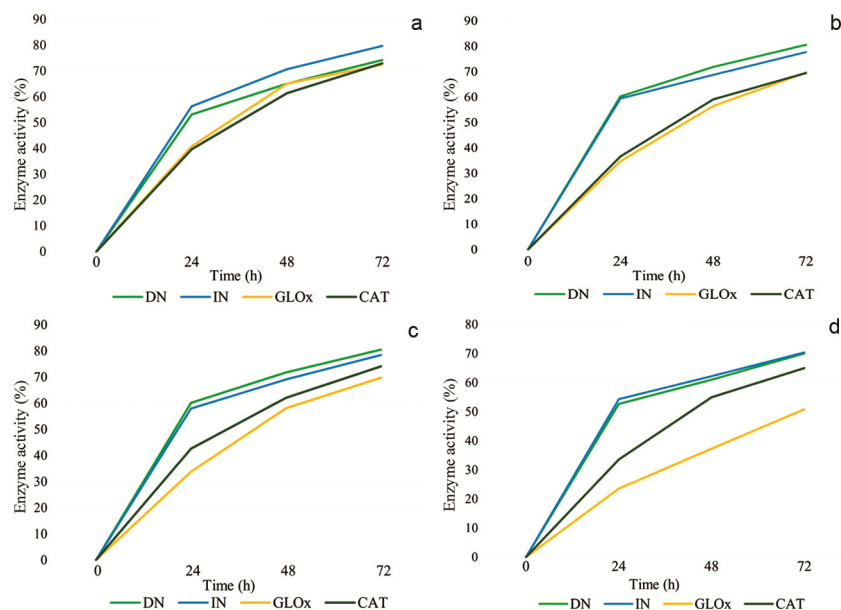


Fig. 3 — Percentage decrease of enzyme activity in adulterated samples a) Indian bee honey, b) Italian bee honey, c) rock bee honey, and d) stingless bee honey.

DN- Diastase, IN – Invertase, GLOx – Glucose oxidase, CAT – Catalase.

CAT) (Fig. 3c). Study on physicochemical parameters of adulterated honey samples showed a significant increase in moisture, TSS and pH in all the samples compared to the corresponding raw honey (Supplementary Fig. 3). Moreover, colour intensity of the samples decrease even though the colour remained in the range of amber.

## Discussion

### Raw honey samples

Honey, a natural food produced by bees, has been appreciated for thousands of years because of its taste, health, and nutrition. Honey has different flavours, colours, and consistencies depending on the plant source and the environment. Evaluation of honey's biochemical and physicochemical parameters, like enzyme activity, pH, moisture content, and sugar composition, gives a useful perception about the quality of honey. Enzyme studies on raw honey samples show stingless bee honey as superior, with the highest enzyme activity (58.53 DN, 49.35 IN, 23.06 GLOx, and 21.87 CAT). Findings of this study align with those of Krishnasree & Ukkuru<sup>28</sup>, which revealed the high diastase activity (55.45 DN) and moisture content (20%) in stingless bee honey. Elevated enzyme activity in stingless bee honey may be explained by increased enzyme secretion from the bee's hypopharyngeal gland. The floral source likely

influences the higher catalase activity, given that stingless bees predominantly forage on medicinal plants. The observed superiority of stingless bee honey is consistent with the results of Muhammad & Sarbon<sup>29</sup>, showing high acidity, antioxidant properties, enzymes, colour intensity, and minerals in stingless bee honey compared to honey from *Apis* spp. Similarly, Nweze *et al.*<sup>30</sup>, Tesfaye *et al.*<sup>31</sup>, and Alvarez-Suarez *et al.*<sup>32</sup> also revealed the superiority of stingless bee honey from different species (*Hypotrigena* sp., *Melipona* sp., and *Meliponula* sp.) over *Apis mellifera* honey.

A comparative study by Moniruzzaman *et al.*<sup>33</sup> revealed higher moisture content and pH in *A. dorsata* than *A. mellifera* honey. Significantly low TSS in stingless bee honey (71.5 °Brix) was also reported by Sousa *et al.*<sup>34</sup>, which was mentioned between the range of 71.1 and 74.7°Brix. A study by Wu *et al.*<sup>35</sup> on honey from China revealed negative correlation between diastase activity and physicochemical parameters like moisture content, pH and TSS, which was similar with the correlation analysis of current study (Fig. 1). Whereas, the result was not in harmony with the findings of Joshi *et al.*<sup>36</sup> which revealed positive correlation of invertase and glucose oxidase with moisture and pH. Discrepancies noted in this study likely arise from the inherent variability in bee genetics and behaviour across species such as

*A. indica*, *A. mellifera*, and *A. dorsata*, which impacts their enzyme secretion and nectar processing.

It's also critical to consider that enzyme activity is highly susceptible to environmental factors, the specific nectar source and flow, bee species, and colony age<sup>37,38</sup>. Beyond these biological and environmental influences, sample handling and storage protocols are known to exert a considerable effect on both enzyme activity and the overall physicochemical parameters of honey. Eleazu *et al.*<sup>39</sup> revealed the correlation of enzyme activity with sugar and colour of honey, as the darker honey contains less soluble sugar and possesses high enzyme activity compared to lighter honey, which agrees with the obtained results. A high positive correlation between diastase and invertase ( $r = 0.84$ ) was attained and was compatible with that obtained by Serrano *et al.*<sup>40</sup> in a study on Andalusian honey ( $r = 0.85$ ). The present study revealed a high negative correlation between colour and pH ( $r = -0.76$ ), whereas a study by Nayik & Nanda<sup>41</sup> showed a positive correlation ( $r = 0.66$ ) between them.

#### Processed samples

Enzymes are vital bioactive compounds that contribute to the distinctive properties of honey. While heating improves the texture and prevents crystallization in honey, it causes the denaturing of enzymes and diminishes their activity. Processed and heat-treated samples in the current study revealed a reduction in enzyme activity, which was consistent with the findings of Hasan<sup>42</sup>, who revealed the loss of diastase and invertase activity at temperatures above 55°C. Invertase was degraded more quickly than amylase while heating. This suggests invertase can be a more sensitive heat damage indicator than diastase activity<sup>43</sup>. The least percentage reduction (<70%) of glucose oxidase obtained at 30–40°C was similar to that obtained by Kretavicius *et al.*<sup>44</sup>, which showed negligible decline in glucose oxidase activity between 20–50°C. Similar findings were reported by Bucekova *et al.*<sup>45</sup> who stated that conventional thermal treatment at 45–55°C did not affect the activity of glucose oxidase. Bonvehi *et al.*<sup>46</sup> reported a decrease of invertase activity in 81 samples of processed honey with an increase in processing temperature and time. Comparatively least reduction of catalase at 120°C accounts for its multiple sources of origin, including nectar, pollen, and microbial content, which cause greater viability and partial stability even under heating. Yener *et al.*<sup>47</sup> stated that the caramelization of

honey and denaturing of honey enzymes above 90°C were similar to the findings of the current study.

The most important variable that influences enzyme activity level in honey is the botanical origin of nectar. Honey derived from various nectar sources exhibits a distinct enzymatic profile. High glucose oxidase and catalase (9.42 GLOx and 10.66 CAT) were found in multifloral honey than that derived from monofloral nectar sources in the current study, which was similar to the findings of Alshareef *et al.*<sup>23</sup> in honey from Saudi Arabia. Similarly, the dependence of diastase and invertase activity in the nectar source was highly visible in the study by Assia & Ali<sup>48</sup>, in *Apis mellifera* honey collected from Algeria. Also, the highest diastase activity was determined in the multifloral honey (26.17±10.39) as slow nectar flux enables bees more time to process the nectar<sup>49</sup>.

#### Adulterated honey samples

Variation in the physicochemical properties among honey produced by different bee species makes them variably susceptible to different types of adulteration. Honey adulterated with sugar causes changes in chemical and biochemical parameters like enzyme activity, TSS, pH, etc. A study by Abdel-Aal *et al.*<sup>50</sup> was consistent with the current study, which revealed a gradual reduction in diastase activity with an increase in percentage of adulterant concentration. Similarly, Lawal *et al.*<sup>51</sup> identified a loss in activity of invertase and also reported the increase in moisture content as an indicator of adulteration. Reduction in the activity of glucose oxidase and thus production of H<sub>2</sub>O<sub>2</sub> due to falsification causes a decrease in the antibacterial properties of honey<sup>52</sup>. Increase in pH, moisture and TSS (Supplementary Fig. 1) in the adulterated samples compared to the pure honey in the current study conformed with the findings of Ribeiro *et al.*<sup>53</sup>. Here also, the correlation studies after 72 h revealed high positive correlation among the enzymes and negative correlation between enzymes and physicochemical parameters (moisture, pH, TSS).

To summarize, stingless bee honey consistently exhibited superior enzymatic activity across all raw honey samples, indicating its notable bioactivity and resistance to adulteration. Our investigation confirmed that thermal processing induces irreversible enzyme degradation, particularly pronounced above 60°C, leading to complete inactivation at approximately 120°C. Regarding floral origins, multifloral honey demonstrated the most robust

enzyme profile. Collectively, enzymatic profiling represents a potent methodology that, when integrated with physicochemical parameters, provides critical support for consumer safety and regulatory frameworks aimed at identifying adulteration and upholding honey quality.

### Conclusion

Enzyme analysis confirms honey is rich in bioactive ingredients like diastase, invertase, glucose oxidase, and catalase, which are crucial for its nutritional and medicinal properties. However, thermal degradation and syrup adulteration detrimentally affect honey's biochemical properties, leading to a reduction in its overall quality. Current honey standards, as outlined by Codex Alimentarius (2019) and FSSAI (2020), mandate a minimum diastase content of 8 and 3 Schade units/g, respectively. The consistent diastase activity observed across our samples, a widely recognized honey quality marker under international standards like Codex Alimentarius, firmly grounds our findings in global benchmarks. While all samples examined conformed to these quality benchmarks, stingless bee honey warrants particular attention. Its superior enzyme concentration, coupled with enhanced stability under thermal processing and in the presence of adulterants, reinforces its significant value for potential medicinal applications and makes it a highly desirable option. Furthermore, our findings acknowledge the critical role of botanical origin, with multifloral honey demonstrating distinct advantages in determining enzyme activity. The physicochemical correlations of enzymes further shed light on honey's compositional integrity, emphasizing that enzymes are not merely health-promoting compounds but also serve as reliable indicators for honey authenticity and stringent quality control.

### Conflict of interest

The authors have no relevant financial or non-financial interests to disclose

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