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COMPARISON OF ANTIOXIDANT ENZYMES IN NATURAL AND COMMERCIAL HONEY

Braihna Mir¹, Ayesha Mushtaq^{1*}, Sabeena Rizwan¹, Farrukh Bashir¹, Uzma Jabeen¹, Farida Behlil¹, Saba Afzal¹

¹Department of Chemistry, Sardar Bahadur Khan Women's University (SBKWU), Quetta, Pakistan

*Corresponding Author: Dr. Ayesha Mushtaq. E. mail: ayeshamushtaq2000@yahoo.com



Abstract

Honey is popular all over the world and used commonly in medicines and food. This study was designed to compare the natural and commercial honey for their antioxidant enzymes. In this study, 11 different honey samples were collected from different areas of Balochistan. The activity of different antioxidant enzymes like superoxide dismutase, peroxidase and catalase enzymes were measured and compared by standard curves of pure enzymes. Moreover, protein content was also determined by using Bradford Assay. Results showed that highest protein content was found in one of the natural honey samples (4.42 ± 0.02 U/mg). Enzyme's superoxide dismutase showed highest activity of (4.93 ± 0.06 U/mg), whereas secondly peroxidase activity of (0.34 ± 0.02 U/mg) and thirdly catalase activity of (0.22 ± 0.01 U/mg) was observed in different natural honey samples. The current study demonstrated that higher concentration of protein content and antioxidant enzymes were found in natural honey which means they are good source of antioxidants. Therefore, antioxidant enzymes can be used as markers for determining the purity and quality of honey.

Keywords: Antioxidants, Catalase, Honey, Peroxidase, Superoxide dismutase

INTRODUCTION

Honey is consumed in almost all the countries of the world in the form of medicine and food. Naturally honey is a flavorsome and sweet product (1, 2). Honey bees produced it from flower nectar or from living plant secretions which is then assemble, converts and store in honeycombs. It is produced by *Apis mellifera* (Honey bees) and is a good source of energy (3). Honey usage is extremely increasing because of its antibacterial and antioxidant properties. The texture, odor and consistency rely on flowers from which it is extracted (4).

Honey is an intricate natural liquid that comprises at least 181 constituents. Its composition is somewhat mutable and mainly depends on the floral source though certain external factors are involved such as processing, ecological and seasonal changes. Honey bees naturally add enzymes in the honey during the ripening process. A unique characteristic of honey which makes it different from other sweetener is the presence of enzymes and these enzymes comes from various sources like bees, pollen, nectar, microorganisms and yeasts. Basically, enzymes are protein in nature, so they bring about biochemical variation under mild environments (5).

Honey is a nutritional antioxidant that is naturally processed by Honey bees. Different polyphenols, flavonoids, p-coumaric acid and kaempferol have been isolated from honey, which are responsible for antioxidant activity of honey (6). The commercial honeys are sometimes synthesized artificially and often adulterated with cheap sweeteners like corn sugar, beet sugar or corn syrup (7).

The characteristic of honey that make it beneficial to human health is its enzyme content. The foremost enzyme in honey is diastase, glucose oxidase¹ and invertase. Other enzymes like catalase, amylase, acid phosphatase in honey comes from plant (8). The added enzymes are mainly produced in the honey bee hypopharyngeal glands (9-11). Honeys nutritional and healing properties since early beginning makes it an important component of traditional remedy (12-15). Current opinions suggest honey not only as nutritional supplement but also highlight its properties like non-peroxide dependent and antioxidant (16).



Antioxidants in the living organisms plays a significant role against the failing effects of free radicals and lack of these antioxidants leads to oxidative stress. The oxidative stress in biomolecules is tempt by these radicals that bring change and incite the death of cell (17). Primary antioxidants like Catalase, Peroxidase and Superoxide dismutase are present naturally in the living tissue which act as shielding agent against this oxidative stress (18).

Natural antioxidants in honey are valuable against heart disease, cancer, inflammation, coronary disease, aging, tumor, gastrointestinal and wound healing (19-24). Honey added in tea samples increase the phenol and antioxidant properties up to 57% particularly in pine honey when added at high temperature. Results of this study supported the fact that honey can be used as natural sweetener and make tea consumption healthier (25).

Variation in antioxidant potential of honey depends on topographical and botanical differences (26, 27). Certainly, the color of honey and water content are linked with the content of antioxidant. Pakistan's Zizipus honey is viewed as the most valued honey in the world.

Honey has many antioxidant properties which can combat against various diseases, and it has many health beneficial properties, so this study is planned to find antioxidant enzymes activity and protein content of honey collected from different areas of Quetta, Balochistan.

MATERIALS AND METHODS

SAMPLING

Eleven natural and commercial honey samples were collected from different areas and local markets of Quetta, a capital city of Balochistan situated in southwest of Pakistan. All honey samples were diluted with distilled H₂O.

PROTEIN CONCENTRATION BY BRADFORD ASSAY

The protein content was quantified by Bradford reagent (28). 5 mL of Bradford reagent which was prepared by using coomassive blue (0.1 g) dissolved in ethanol (50 ml) and distilled water (50 ml) then this reagent was added to each of the 6 test tubes having different dilutions of honey samples. Bovine serum albumin was used as a standard. The test tubes were incubated in dark for 20 min and absorbance was read at 595 nm. Protein concentration of honey samples were evaluated from the standard curve of protein.

SUPEROXIDE DISMUTASE ASSAY (SOD)

The superoxide dismutase activity was measured by the method of Giannopolitis and Ries, in which the reaction mixture contains the enzyme extract (50 µl) or honey sample (50 µl), nitro blue tetrazolium (50 µL) dissolved in ethanol, methionine (13 mM), riboflavin (1.3 µl), EDTA (75 mM) and 7.8 pH of phosphate buffer (50 mM). This solution was kept in fluorescent lamp and then its absorbance was read at 560 nm and one unit of SOD was used to cause 50% inhibition of the NBT reduction (29). SOD activity of honey samples was evaluated from the standard curve of SOD.

PEROXIDASE ASSAY (POX)

Peroxidase activity was measured by using the method of Chance and Maehly, for this activity the assay mixture containing 7.0 pH potassium phosphate buffer (0.9 ml), H₂O₂ (1 ml), guaiacol (20 ml), and honey samples with different dilutions were added in test tubes. Increase in absorbance was read at 470 nm after every 20 sec (30). A standard curve of purified POX was made from which POX activity of honey samples was determined.

CATALASE ASSAY (CAT)

The catalase activity was measured by using Chance and Maehly method, in which the assay mixture (3ml) containing 7.0 pH of potassium phosphate buffer (2 ml), H₂O₂ (900 µl), honey sample with

different dilutions were mixed in test tubes. Decrease in absorbance was recorded after every 30 sec, at 240 nm. A standard curve of CAT was made, which helped in quantification of CAT activity in honey samples.

RESULTS AND DISCUSSION

PROTEIN ANALYSIS

Several proteins are also present in honey (31). About 11 honey samples were analyzed which revealed the content of protein. Figure 1 showed that N6 had highest protein content 4.42 ± 0.02 U/mg while the lowest amount of protein was observed in C1 honey 4.26 ± 0.02 U/mg. Significant differences existed among all the honey samples, when analyzed by SPSS (Table I).

Table I. Values of protein, SOD, CAT and POD in honey

Biochemical Parameters	Minimum	Maximum	Mean	Std. Error	Std. Deviation	P Value
CAT	0.140	0.220	0.168	0.008	0.028	0.006*
POD	0.180	0.340	0.256	0.020	0.065	0.000*
SOD	4.420	4.930	4.609	0.056	0.186	0.000*
Proteins	4.260	4.420	4.347	0.020	0.066	0.000*

*Analysis of Variance showed that all results are significant at $P = 0.05$ level

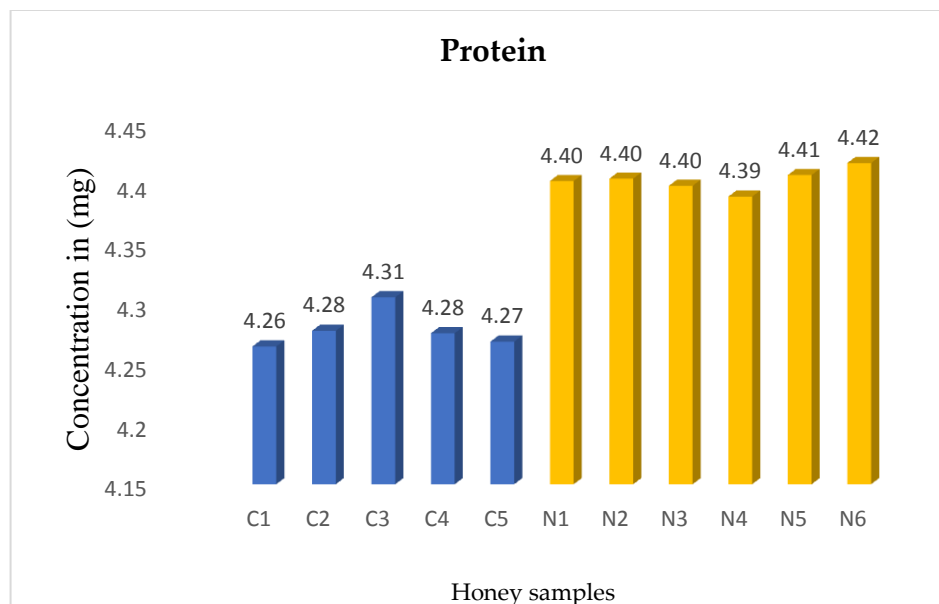


Fig. 1. Concentration of protein in different honey samples

Protein level of natural honey samples were much higher as compared to other reported data from various countries like French (0.37 ± 0.0 to 0.940 ± 0.01 g/100g) (32), Turkish (0.06 ± 0.006 to 0.170 ± 0.009 %) (33), Indian (0.048 ± 0.001 to 0.23 ± 0.0023 %) (34). Protein load of natural Pakistani honeys collected from different locations was (0.96 ± 0.25 to 1.70 ± 0.10 g/100 g) so protein content of commercial honey was less than natural honey (35) and same was examined in the present work.

ANTIOXIDANT ENZYMES ACTIVITY

SUPEROXIDE DISMUTASE ASSAY

Noor, 2015 also reported that natural honey is rich in antioxidant profile as compared to commercial honey. Fig. 2 showed the SOD activity of natural and commercial honey. N1 showed maximum activity of 4.93 ± 0.06 g/100 g while lower activity was observed in C4 that was 4.42 ± 0.06 g/100 g (Table I).

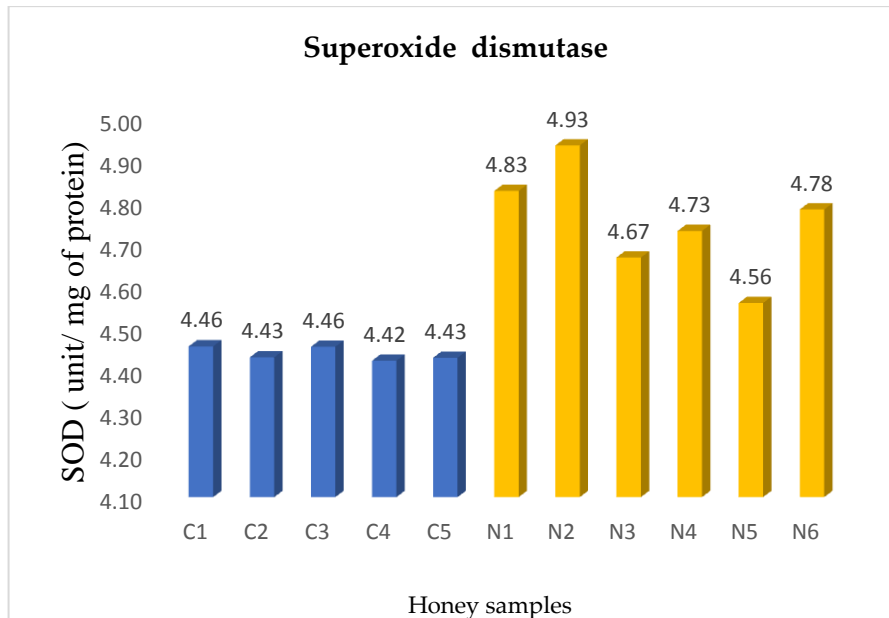


Fig. 2. Concentration of superoxide dismutase in honey samples

PEROXIDASE ASSAY

Fig. 3 showed variation in peroxidase activity in all samples of honey both natural and commercial. Natural honey samples such as N2, N6, and N4 showed higher peroxidase activity as compared to commercial honey samples and the POX activity values in natural honey were in the range of 0.33 ± 0.02 g/100 g. Among commercial honey samples C4 showed lower activity of 0.17 ± 0.02 g/100 g (Table I).

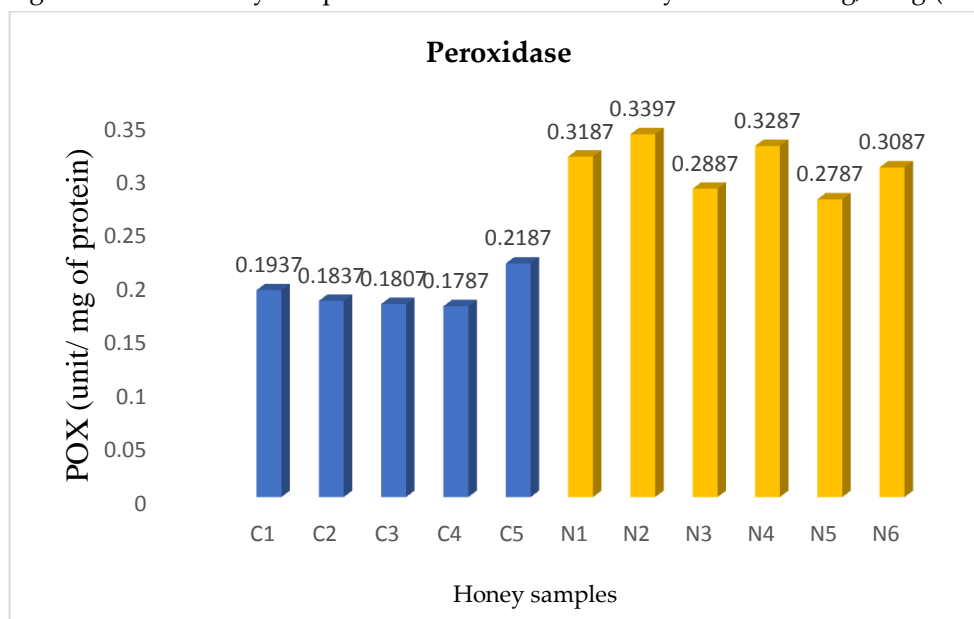


Fig. 3. Concentration of peroxidase in various honey samples

CATALASE ASSAY

Fig. 4 showed catalase activity in all honey samples in which like other two antioxidant enzymes SOD and POX, catalase also shows higher activity in natural honey as compared to commercial honey. Higher activity can be seen in N2, N4, and N6 while commercial honey samples C2 and C4 showed lower activity. The higher activity was shown by N2 about 0.22 ± 0.01 g/100 g while in commercial honey the lower activity of CAT was shown in C2 and C4 about 0.14 ± 0.01 g/100 g.

In a previous study, activity of catalase in 28 samples of honey was examined by spectrophotometric technique. Results obtained were related to earlier data of same samples on diastase and peroxide accumulation. Then these results were analyzed statistically which showed that peroxide and catalase accumulation were inversely related, and diastase and catalase amounts were directly related (36).

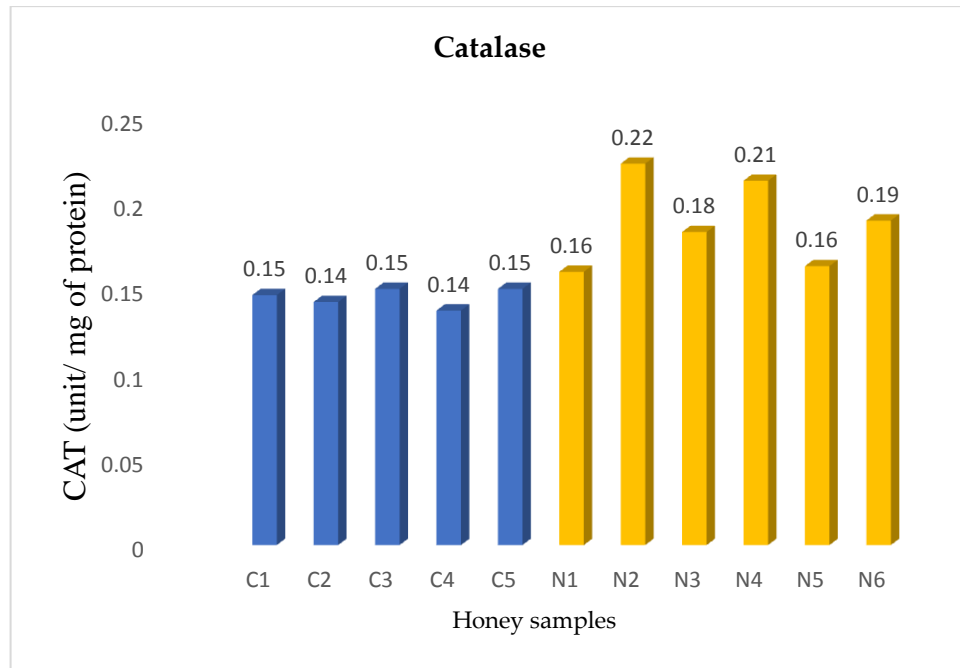


Fig. 4. Concentration of catalase in different honey samples

Many researchers informed about the occurrence of catalase in honey which plays an important role in inhibition of peroxide. Within a second million of H_2O_2 can be decompose to Oxygen and water by the catalase. From various foodstuff and products H_2O_2 is removed by using catalase. To rise reducing potential and antioxidants in human, honey can be use (34). In order to differentiate in honey, antioxidant activity has a valuable impact in this regard especially in forest honey because they show more antioxidant activity as compared to floral honey while both showed same level of phenolic content (37). It has been recently reported that the phenolic extracts of honeys had better antioxidant and enzyme inhibitory activities as compared to the entire honeys, regardless the monofloral honey type (38).

The overall results in fig. 5 showed that superoxide dismutase showed highest activity, second highest value was shown by protein while peroxidase and catalase also showed their activity at lower level. There can be many reasons for the difference in the values of these enzymes and protein in natural and commercial honey.

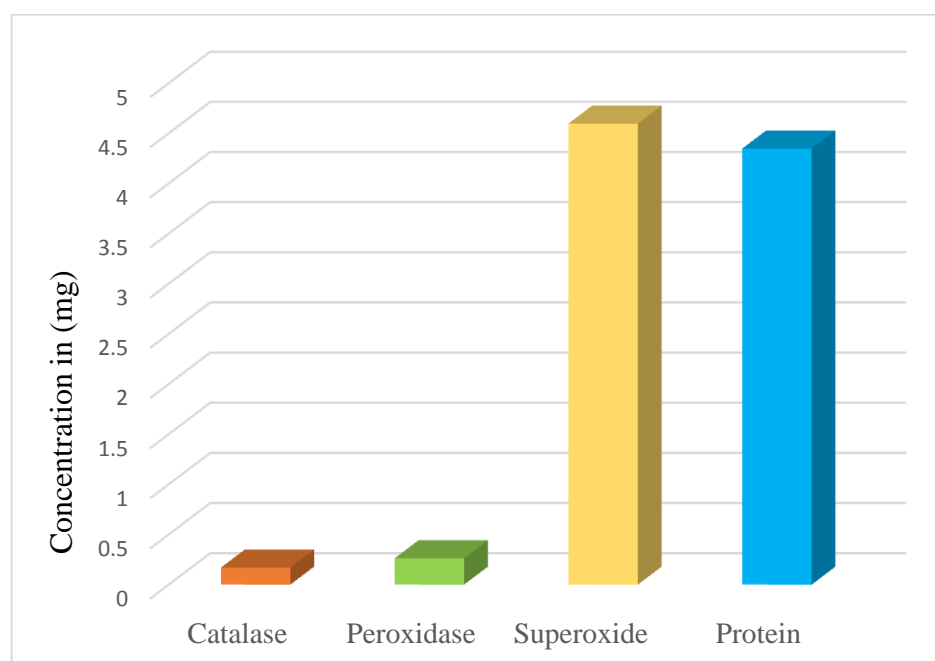


Fig. 5. The overall values of catalase, peroxidase, superoxidase and protein

The present study compared natural and commercial honey samples collected from different areas of Quetta, Pakistan from which it is concluded that high quality honey is usually rich in antioxidants and natural honey showed higher concentrations of antioxidant enzymes compared to commercial honey. All the results of this study are significant at $P < 0.05$.

The adulteration in natural honey is done for unscrupulous economic gain, commonly by corn syrup, cane sugar, or beet sugar. These adulterants can be detected by various techniques, simply by fluorescence spectroscopy (7). Similarly, the antioxidant enzymes can also be used as markers for the detection of adulteration in honey.

CONCLUSION

In the present work it is shown that all the natural honey samples contain protein and antioxidant enzymes, but the protein content and antioxidant enzymes varied among the honey samples. The highest antioxidant enzymes and protein content was determined in natural dark colored honey samples that showed great results, while the commercial honey showed least content of protein and antioxidant enzymes. Thus, high quality honey is full of antioxidants and protein, honey can serve as a natural food antioxidant.

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