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COMPARATIVE EVALUATION OF ANTIOXIDANT ACTIVITY BETWEEN NATIVE AND PROCESSED HONEY AVAILABLE IN NEPAL

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ABSTRACT
Antioxidant activity is a value added factor of honey to accept it as an essential food for human. Evaluation of antioxidant activity between native and processed honey is essential to select further types of honey. Among different types of constituents, phenolic compounds are mainly responsible for antioxidant activity in honey.To compare antioxidant activity between native and processed honey, total 10 honey samples (5 native and 5 processed) were analyzed in order to assess their total phenolic content by Folin-Ciocalteu method and their antioxidant activity using UV-Spectrophotometer. The antioxidant activities of native and processed honey were compared and, total phenolic contents were also compared in between them.The results of the study showed that antioxidant activity and total phenolic contents were found to be notably higher ($p < 0.05$) in native honey samples than that of processed honey. Furthermore, antioxidant activity of native honey was found to be considerably positively interrelated ($r = >0.5$, $p < 0.05$) with the respective processed honey. Similarly, total phenolic content of native honey was also found to be considerably positively interrelated ($r = >0.5$, $p < 0.05$) with the respective processed honey.

KEYWORDS: Antioxidant activity, carbohydrate, native honey, processed honey, total phenolic content.

INTRODUCTION
Honey has been used as a therapeutic agent from an ancient period in healing purposes; numerous studies have demonstrated that the honey is a source of energy and may be used for the treatment in many diseases such as colds, skin wounds and various gastrointestinal diseases.[1] As the only available sweetener, honey has been an important food for humans since very beginnings. Indeed, the relation between bees and humans started as early as Stone Age.[2] It is a natural food product produced when the nectar and sweet deposits from plants are collected, qualified and stored in the honeycomb by honey bees *Apis* species. The definition of honey stipulates a pure product that does not allow for the addition of any other substance.[3]

The main ingredients of honey are sugars (fructose 38 %, glucose 31 % and sucrose not more than 5 %), water amount is less than 20 %, while the acids are approximately 0.08 %, and the content of mineral substancesis approximately 0.18 %. Beside these, wide variety of other substances are also present in small concentrations like catalase, glucose oxidase, phenolic acids, ascorbic acid, flavonoids, carotenoid derivatives, organic acids, Maillard reaction products, amino acids and proteins.[4, 5, 6, 7, 8, 9, 10] Composition of honey varies depending on many factors, such as pollen in honey and also from climate, environment and honey processing. [11, 12, 13]

Among different biological properties, honey shows an antioxidant property which mainly depends from the contents present in honey; phenolic compounds, amino acids and vitamins. The basic sources of phenolic compounds are pollen, propolis and wax.[14, 15] Generally, there are two types of phenolic compounds; simple phenols like phenol acids and poly-phenols like flavonoids are found in honey.[12, 16, 17] Furthermore, antioxidant capacity of honey depends on the floral and geographical origin, climatic conditions and processing, storing and handling of honey.[5, 9, 18, 19]

By taking antioxidants from high-antioxidant foods, humans partly protect themselves from the harmful effect of free radicals. The mechanism of free radicals formation as by-products in the body depends upon the oxidation process in human aging and illness, even the oxidation is vital to life. The free radicals travel through the cell, disrupting the structure of other molecules and resulting in cellular damage. Such damage is believed to contribute to aging and various health problems.[20, 21, 22] The antioxidant content of honey may act as a natural

source of free radical scavenging compounds. Since some of diseases have been recognized as being a consequence of free radical damage, it seems that part of the therapeutic role of honey is due to its antioxidant activity.[3, 23] Many studies have shown that antioxidant activity is strongly correlated with total phenolic contents, as well as that the antioxidant activity.[4, 5, 19, 22]

Available literature indicates that until now there have been just few researches to determine the total phenolic content and antioxidant activity of native and processed honeys.[22] In this study, we compared an antioxidant activity between native and processed types of honey to see whether the antioxidant values in different types of honey might be varied. Additionally, correlation between native and processed honey was also evaluated with their antioxidant activity and total phenolic content individually.

MATERIALS AND METHODS

Sample Collection

A total of 10 honey samples (5 native and 5 processed) were collected from different locations across Nepal. The honeys were stored at room temperature analysis.

Methods

To assure the physical characteristics and sweetness of the honey, total carbohydrate content in the honey samples was determined by standard Molisch's test. Total phenolic content (TPC) of honeys was estimated by the modified method.[24] At first, honey samples were diluted with distilled water 1:10 (w/v), centrifuged (2000 rpm) and supernatant were collected. Then, the reaction mixture was prepared which contain 0.5 ml 50% Folin-Ciocalteu reagent, 2.5 ml 20% (w/v) sodium carbonate solution, and 1.0 ml gallic acid solution or sample extract. The mixture was placed in the dark for 20 minutes and the absorbance was recorded at 750 nm against a blank with a spectrometer (UV-1800 Shimadzu spectrophotometer).

The calibration curve for total phenolic content determination was prepared using gallic acid (2 to 10 µg/ml). The total phenolic content was expressed based as mg gallic acid equivalent mgGAE/100gm of honey from a calibration curve using the equation: $y = 0.099x - 0.019$ ($R^2 = 0.955$). All samples were analyzed in duplicate.

For the determination of antioxidant activity of honeys, the modified method was followed.[25] In which,1.0 ml standard gallic acid solution or 1.0 ml diluted honey sample (1:10 w/v) was mixed with 3.0 ml of the reagent solution containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. Then, the mixture was placed in the dark for 30 minutes and the absorbance of the solution was measured at 725 nm.

Subsequently, the calibration curve for antioxidant activity was prepared using gallic acid (2 to 10 µg/ml). The antioxidant activity was expressed based as mg gallic acid equivalent mgGAE/100gm of honey from a calibration curve using the equation: $y=0.006x - 0.009$ ($R^2 = 0.908$). All samples were analyzed in duplicate.

Statistics

Data obtained from the analysis were analyzed in MS-Excel (window 7) and SPSS (version 21). Statistical analysis; one tailed and one type student's t-test were applied to find out the significant difference in antioxidant activity or total phenolic content between native and processed honey. In addition, Pearson's correlation analysis was carried out to establish link between antioxidant activity and total phenolic content of native and processed honey.

RESULTS AND DISCUSSION

Visual assessment of honey samples and total carbohydrate content of honey samples were carried out to see whether the native and processed honey could be distinguished with their appearances and sweetening taste. Table 1 shows colour, crystals and total carbohydrate content (g/100g honey) of native and processed honey samples.

Table 1*:* Characteristic of honey samples

Sample no.	Colours		Crystals		Carbohydrate (g/100g honey)	
	Native	Processed	Native	Processed	Native	Processed
1	Dark amber	Amber	-	+	11.01	12.09
2	Dark cream	Cream	+	-	15.35	14.59
3	Dark Brown	Brown	-	-	12.99	11.78
4	Amber cream	Cream	-	-	16.19	15.59
5	Cream	Pale yellow	+	+	14.13	14.99

+: Presence, -: Absence

In practice, most of the workers notice a relationship between colour and antioxidant activity of honey samples. Here is also a general observation that the dark honeys (native honey) were found with considerably higher antioxidant activity and total phenolic content than the light coloured honeys (processed honey). Similarly it was also observed that crystals present in particular honey are related with higher carbohydrate content; however sample no. 4 with no any crystals showed higher carbohydrate content. This trend was generally similar to the relationship found for some Slovenian, Burkina Faso and Italian honeys.[6,14,16]

The antioxidant activity and total phenolic contents were investigated using *in vitro* methods. Based on the standard calibration curve, each analysis was evaluated for its efficiency and also the standard deviation between the duplicates was studied to ensure robustness of the data for antioxidant activity and total phenolic content. Furthermore, the mean of antioxidant or total phenolic content data from five native and five processed honey samples were taken with their standard deviation (SD). For native honey, the mean of antioxidant activity and the mean of total phenolic compound were found to be 66.624±26.24mgGAE/100g honeys and 8.03±2.48mg GAE/100g honeys respectively. Similarly, for processed honey, the mean of antioxidant activity and the mean of total phenolic compound were found to be 48.04 ± 11.44 mgGAE/100g honeys and 5.71 ± 1.26mgGAE/100g honeys respectively. **Figure1** shows that total phenolic content in native honey was also found to be notably higher (p=0.0125; p<0.05) than processed honey and likewise, antioxidant activity in native honey was found to be notably higher (p=0.033; p<0.05) than the processed honey.

Furthermore, to see whether antioxidant activity or total phenolic content of native honey is associated with that of processed honey, the statistical relationship between native (n=5) and processed honey samples (n=5) was tested by performing Pearson's correlation. **Table 2** shows that, both total phenolic content and antioxidant activity of native had a strong positive significant correlation (p<0.05) with that of processed honey samples.This correlation was in agreement with the findings of other honey samples also found that correlation between radical scavenging activity and the total phenolic content was at a level of p=0.5, while other authors found it stronger.[5, 6, 14, 16]

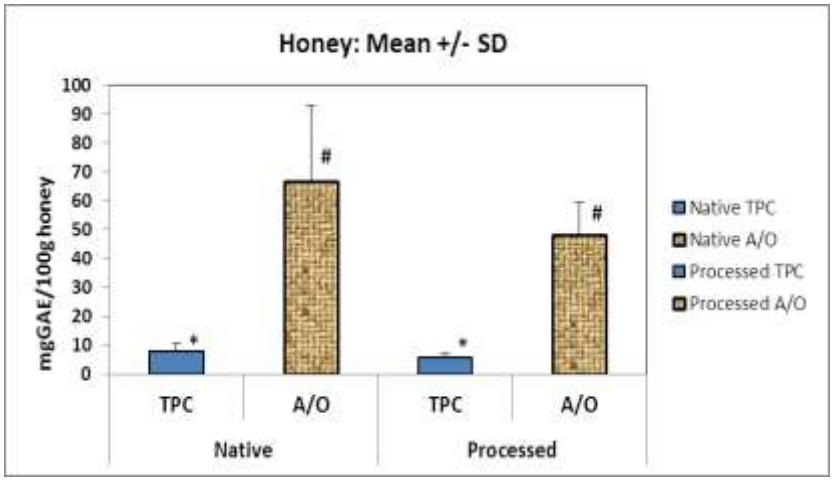

Figure 1: Mean values of antioxidant activity(A/O) and total phenolic content (TPC) of honey.

*The data are represented as the mean ± SD of antioxidant activity or total phenolic content in native and processed honey samples. The symbols # (antioxidant activity)and *(total phenolic content)indicates significant difference (one tailed student's t-test, p<0.05) between native and processed honeys.*

Table 2: Correlation analysis between native and processed honeys

Parameters	Pearson's Correlation (r)	*p*-value	Strength of relation
Total phenolic content	0.890	0.022	Strong positive
Antioxidant activity	0.908	0.016	Strong positive

If r=>0.5 to 1.0; high correlation, r= >0.3 to 0.5; moderate correlation and r= >0.1 to 0.3; low correlation. The negative number indicates same strength with negative correlation p<0.05, significant correlation.

CONCLUSIONS

In this study we observed that all the honey samples contained phenolic compounds and antioxidant activity. The total phenolic content and antioxidant activity varied between honey types (native and processed). The antioxidants properties of honey are characterized by content of some antioxidant compounds, although there is a wide spectrum of antioxidant types, phenolic contents predominate in many honeys.

The total phenolic content and antioxidant activity were found significantly higher in native honey. A significant positive correlation was found between the total phenolic content and antioxidant activity of native honey with processed honey.

For future perspectives, the antioxidant activity of honey needs to evaluate in *ex-vivo* or *vivo* condition which could also be interesting radical scavenging food for many diseases. Additionally, antioxidants other than phenolic compounds can also be compared between native and processed honeys.

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