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Raw and Gamma-Irradiated Honey Samples from *Apis mellifera* and *Trigona biroi:*Its Physico-chemical Properties, Total Phenolic and Total Flavonoid Contents

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ABSTRACT
The purpose of this study was to determine the physico-chemical properties, total phenolic and total flavonoid contents of honeysamples ofApis mellifera and TrigonaBiroi collected from Isabela State University- San Mariano Campus, Isabela, Philippines. The raw honeys were gamma-irradiated at 25 kGy. Results revealed that the moisture content, pH values and ash contents of the honeys are within the normal range indicated by the International Honey Commission, Codex Alimentarius and Philippine National Honey Commission. Raw and gamma-irradiated honey of Trigonabiroi has the highest phenolic and flavonoid contents. The phenolic and flavonoid contents of the raw honeys in this study were not greatly affected when subjected to gamma-irradiation. The floral sources of honey, seasonal and climate factors, and storage and processing conditions influenced the physico-chemical properties, phenolic and flavonoid composition of honeys. For further study, storage duration and lower doses of gamma-irradiation can be considered in assessing its significant differences to other properties of local honeys. The results of the study support the many beneficial effects of honey to human health especially the honeys produced in the locality.
Keywords*: physico-chemical properties, total phenolic, total flavonoid*

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INTRODUCTION

Honey has been contributing to human health since the ancient times because of its organic and natural sugar produced from the nectar of plants making it a complex natural liquid. Due to the process of regurgitation and evaporation by honeybees [43], honey contains complex mixture of sugars making fructose and glucose as the main ingredients. It is also a functional food because of its nutritive value and different biological properties.

With this, honey types produced by different honeybee species were subjected to analysis and evaluation such as physico-chemical, total phenolic and total flavonoid contents. The conduct of physico-chemical analysis of honey was to determine the variations of different honey samples and its qualities needed for storage and commercialization purposes [2, 19, 33].

Phenolic compounds are found in honeys because these are collected by the honeybees from the plants [27]. Beretta, *et.al*., [9], Hussein *et al* [25] and Meda*, et.al*. [31] observed that the total phenolic contents of honey are strongly correlated to its antioxidant activities, thus making total phenolic contents as one of the reliable parameters in indicating antioxidant activities and other biological properties [10, 11, 27]. In addition, results of darker honey samples had higher amounts of phenolic compounds, flavones, and flavonols and increased antioxidant activity [34, 40, 43, 41, 32]. Studies of Silva, Silva *et al* [44] found out that the antioxidant activity was higher in the samples that contained higher quantities of phenolic compounds. However, Satarupa and Subha [43] reviewed that the antioxidant properties of honey are not always positively proportional to its level of phenolic compounds because it may be due to the presence of various polyphenols that provides variable scavenging activity.

Flavonoids as another phenolic compound of honey contributes to the aroma, flavor and color of honey [43]. Flavonoids are oxidized by radicals, resulting in a more stable and less reactive radical. With this, flavonoids stabilize reactive oxygen species by neutralizing with the reactive element of the radical. Consequently, the antioxidant potential of honey can be associated with flavonoid concentrations [33]. Studies also shows that with the increasing honey concentrations, the total flavonoid contents of honey also increases [1, 2, 25, 34].

The flavonoids and phenolic compounds do not only influence the appearance [35], but also the functional properties and botanical origins [4] of honey. In addition, the total flavonoid and phenolic contents may vary from the different honey samples.
Food contamination is caused by microorganisms acquired during harvesting and packaging. Therefore, the food and drugs approved the irradiation of certain food including honey to reduce or eliminate disease-causing microorganisms while the nutritional value is essentially unchanged and at the same time, the food does not become radioactive. The process of irradiation lowers the level of microbiological contamination [8]. Irradiated honey is sterilized and can be used for creating further medicinal processes and used to lower the level of microbial contamination.. Moreover, gamma-irradiation can be used into contaminated tools and parts of the beehive [6].
As observed by Bera *et al* [8], the physical–chemical results obtained for honeys irradiated at 10 kGy when compared to their respective control, Hydroxymethylfulfural(HMF) values for irradiated samples were lower indicating favorable behavior of honey samples when subjected to gamma radiation. Therefore, the use of gamma-irradiation is a useful method of sterilization especially when products are used in medical research and development. Hence, raw honey samples in this study were also subjected to gamma-irradiation.
Honey samples that are available commercially differ in quality because various factors like seasons, packaging and processing conditions, floral source, geographical origin, and storage period. In addition, considerable differences in the various parameters between monofloral and multifloral honeys can also be observed.
In the province of Isabela, there are natural and exotic plant species making it a good dwelling place for beekeeping. However, properties of honey locally produced at Isabela State University- San Mariano Campus have not been well documented therefore, further assessment is needed. Moreover, this study also meant to enhance and maximize the use of natural honey towards increasing honey production. Since beekeeping industry is considered by the government as a necessary part of the agricultural industries [39] this will also strengthen the understanding of the significant health benefits of the community making this industry a good source of revenue. The results will also serve as a strong background in conducting further studies to other parameters and laboratory analysis of various honeybee species in the country.

MATERIAL AND METHODS

Collection of Honey Samples

Honey samples were collected using a mechanical extractor for *Apis mellifera* while dripping method was done for *Trigonabiroi* at Isabela State University- San Mariano Campus, Isabela Philippines. Honey samples are placed in sterilized and clear glass bottle.

Gamma-irradiation

The honey samples were irradiated at 25 kGy dose by exposing the samples to a Cobalt-60 source using the PHI-5030 gamma irradiator of Philippine Nuclear Research Institute (PNRI) Multipurpose Irradiation Facility at Diliman, Quezon City. Samples were placed at 20 cm from the source shroud, 20 cm from the centerline using 2-sided irradiation.

Determination of Physico-chemical Properties

Color Analysis. The honey samples filled the petri dish/cuvette and was placed into the sample analyzer using the Konica Minolta Spectrophotometer CM-5. Using the Minolta chromameter, the color of honey samples was expressed as mean ± standard deviation of parameters L*, a*, b*. The measure of the brightness (lightness) from black (0) to white (100) is represented by L* parameter. The a* parameter is the function of the red-green difference, where a positive value indicates red and a negative one represents green (−100/+100). The green–blue difference is the function of the b* parameter where a positive b* value indicates yellow while a negative value represents blue (−100/+100). Moreover, the perception of the color difference to an observer is equal to the units within the L*, a*, b*.
Moisture Content. Accurately weighted sample was placed in aluminum dish and dried at 100^0C in an oven for one hour. The dish with residue was weighted to determine weight loss as moisture following Official Method of Analysis 925.40.
Ash Content. Suitable amount of sample was charred on a hotplate and burned to ash in a muffle furnace at 600^0C until the residue is white or nearly white (OMA-AOAC 950.49).
Total Fat Content. Suitable amount of dried sample was extracted with solvent using Soxhlet for 4 hours at solvent condensation rate of 5-6 drop/s. solvent used was evaporated and fat residue was dried at 100^0C for 30 minutes, called and weighted following Official Method of Analysis 948. 22.
pH Analysis. The pH was determined using pH/Conductivity meter (APHA-AWWA-WEF Standard Methods for the Examination of Water and Wastewater, 22^{nd} ed. Method No 4500-H^+ B)

Soluble Solid Content. The % Brix of the sample was determined using brix refractometer.

Determination of Phenolic Content

Total Phenolic Content was done based on the modified Folin-Ciocalteau reaction by Singleton *et al* [45] with some modifications from Gajula *et al* [22]. Increasing concentrations of Gallic acid were used as standards and aliquots of the samples in 100mg/1mL solutions were treated with Folin-Ciocalteau reagent and Na_2CO_3. The mixtures could stand at room temperature for 90 minutes and the absorbance was measured at 750 nm wavelength using a microplate reader. The total phenolic content of the sample was measured against the gallic acid standard calibration and the results were expressed as milligram Gallic Acid Equivalence (GAE) per gram of sample.

Determination of Flavonoid Content

Total Flavonoids were estimated using the method of Woisky and Salatino [52] with some modifications. 100mg/1mL water solution of the honey sample extract was made. Equal volumes of sample and 2% AlCl3 were added in wells of a 96-well plate. After 1hr at room temperature, the absorbance was measured at 420nm using a microplate reader. Quercetin served as the standard and ultra-pure water served as a blank. Total Flavonoid contents were expressed as milligram Quercetin Equivalence (QE) per gram of sample.

RESULTS AND DISCUSSION

Physico-chemical properties of raw and gamma-irradiated honey samples

On Color Values

Using the L* parameter, the honey samples can be classified as light and dark. High values of L* present light honey while the lowest values of this parameter show dark honeys [24, 47, 48]. In this study, raw honey samples from *Trigonabiroi*(dark honey) got the lowest value of L* while raw honey of *Apis mellifera* (light honey) has the L* highest value. A slight increase of L* values were observed for gamma-irradiated honey samples from *Apis mellifera.* Therefore, it is observed that honey samples produced by *Trigona biroi* were darker than those honeys from *Apis mellifera*.

Color variations of honeys can be attributed to the nectar origin, processing and handling, and circumstances and duration of the storage of honeys [16, 17, 48]. As assessed by Da Silva, *et.al.* [16], dark honeys are mostly present in some parts of Europe like Greece, Tunisia in Africa, and in Asia.

On Moisture Content

The moisture content of raw and gamma-irradiated honey samples were within the standard ranges of moisture content (10.28 g/100g- 17.58 g/100g). A decrease in moisture content were also observed when the honey samples were gamma-irradiated. In storing and commercializing honey, it is important to maintain its moisture content below 20g/100g [12-14, 26] or not more than 20% (International Honey Commission) to avoid fermentation that could accelerate crystallization in certain types of honey and increase its water activity to values where certain yeasts could grow [1].

An increase of moisture content is also an indicative to adulteration of honey [23]. In ensuring the better shelf-life of honey, lower moisture limits should be maintained [12-14].

These variations of moisture content may also be attributed to the floral source of nectar, climatic factors, degree of maturity reached in the hive and climate [15].

pH Value

The results of the pH of all the honey samples collected were compared with the Codex Alimentarius and European Standards indicating that the normal pH range of honey is 3.42 to 6.10, respectively. It was observed that the pH range of the honey samples are between 3.6 to 3.8 and that is within the normal range however, honey samples from *Trigona biroi* were less likely acidic when gamma-irradiated. The variations of honey pH values are affected by floral and geographic origins. pH values influence the texture, stability and shelf-life of honey. Low pH values of honey hinder the growth of microorganisms making it very important during the extraction and storage [23, 30, 51].

Ash Content

Ash content as one of the parameters reflects the total inorganic minerals that are present in a honey sample after incineration and a quality criterion of botanical origin and floral mix [1, 26, 50]. Species of honeybee, seasons, color and geographical zones are also variables of the mineral contents of honey that is attributed to ash content [29]. Moreover, mineral content of honey affects also the variations of aroma, flavor, medicinal value and qualities of honey [29].

In this study, the ash contents of the honey samples range from 0.05g/100g to 0.9g/100g. Honey samples from *Apis mellifera* has lower ash contents than honey from *Trigonabiroi*. However, there is an observed decreased of ash content of honey samples from *Trigonabiroi* when irradiated. The variability in ash content of honey was influenced by the chemical composition of nectar that varies according to the different botanical sources involved in honey formation [1, 21].

Total Fat

Low fat content will likely decrease the susceptibility of rancid spoilage during storage [34]. The fat contents of honey samples investigated in this study fall within the ranges of 0.06g/100g to 0.2g/100g. Honey samples of *Apis mellifera* both raw and gamma-irradiated has lower fat content than the honey samples from *Trigonabiroi*.

Low fat content on honey samples were also reported such as those from Malaysian honey [10, 11] and honeys from Nigeria [9]. Moreover, the presence of free fatty acids like palmitic, oleic and linoleic acids are also observed in white clover honey [34]. As cited from Ndife Abioye, and Dandago, [36], Khalil, Sulaiman and Boukraa [28] reported that the total fat contents of honey indicate that honey contains very little amount of lipid and is not considered a good source of lipid. S

Total Soluble Solids (% Brix)

Sugars constituted most of the total soluble solids of honey and is also related to moisture content [38]. The proportion of sugar contents depends on the sources of nectar affecting its color, flavor and aroma [15]. According to Ndife, et. al. [36], the measure of dissolved solids in the honey samples were generally more than 80% and upon storage, it can be considered as high grade and highly stable [49].

The % Brix of the honey samples ranges from 8.6-10, respectively. The values can be compared to the minimum brix level for reconstituted fruit juices and puree [13] making the honey samples as a good source of sugar for food nutrition.

Table 1. Result of the physico-chemical parameters of raw and gamma-irradiated honeys

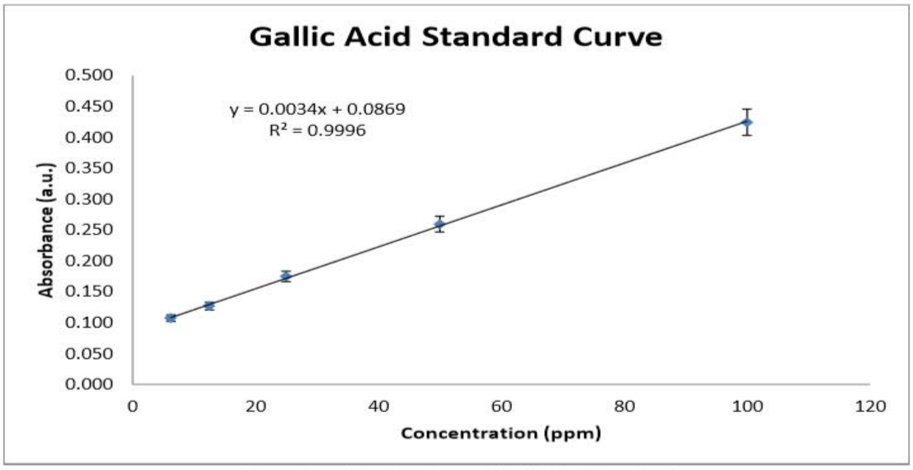
Honey by Species and Type	Parameters						
	Color Values	Color Description	Moisture (g/100g)	Ash (g/100g)	Total Fat (g/100g)	pH @ 21.0 ^{0}C	% Brix
Raw Honey							
Apis mellifera	L*=81.12 +0.15 a*=4.54 + 0.04 b*= 49.97 + 0.06	Light Honey	12.69	0.05	0.06	3.6	9.0
TrigonaBiroi	L*= 24.16 + 0.01 a*= 38.77 + 0.02 b*= 41.66 + 0.04	Dark Honey	17.58	0. 90	0.20	3.4	9.4
Gamma-Irradiated							
Apis mellifera	L*=81.23+ 0.06 a*=3.433 + 0.02 b*= 65.21 + 0.01	Light Honey	12.57	0.05	0.06	3.6	8.6
TrigonaBiroi	L*= 31.39 + 0.08 a*= 44.12 + 0.09 b*= 54.11 + 0.14	Dark Honey	10.28	0.88	0.07	3.8	10.0

L*-measure of brightness/lightness from black (0) to white (100)

a*-red-green difference; b*-green-blue difference

On Total Phenolic Content of Honey

The total phenolic contents of the honey samples were measured against the gallic acid standard calibration as shown in Figure 1. Within the gallic standard curve (R^2=0.9996), the presence of total phenolic contents of the honey samples were determined.



Graph1. Calibration Curve of Gallic Acid Standard

The phenolic contents of honey samples from *Trigona biroi* is higher than the honey samples from *Apis mellifera* both raw and gamma-irradiated. It was also shown in table 2 that phenol content among the raw honeys from *Trigonabiroi* increases when gamma-irradiated. Cited from Hussein, *et al*. [25], an increase of phenols could be due to radiolysis of phenolics like Gallic Acid, Caffeine, etc. in an aqueous solution that led to their efficient degradation to a hydroxylation effect.
But, it was also observed that raw honey samples from *Apis mellifera* decreases when gamma-irradiated. Deghan, *et al* [18] explained that the effects were attributed to the different phenolic compounds present in the various plant materials. It was also considered that other materials have considerable amounts of hydrolysable compounds, which may be more susceptible to gamma-irradiation compared to the condensed compounds present in other products. Floral sources of honey, seasonal and climate factors, and storage and processing conditions influenced the phenolic composition of honey [5, 33, 34].

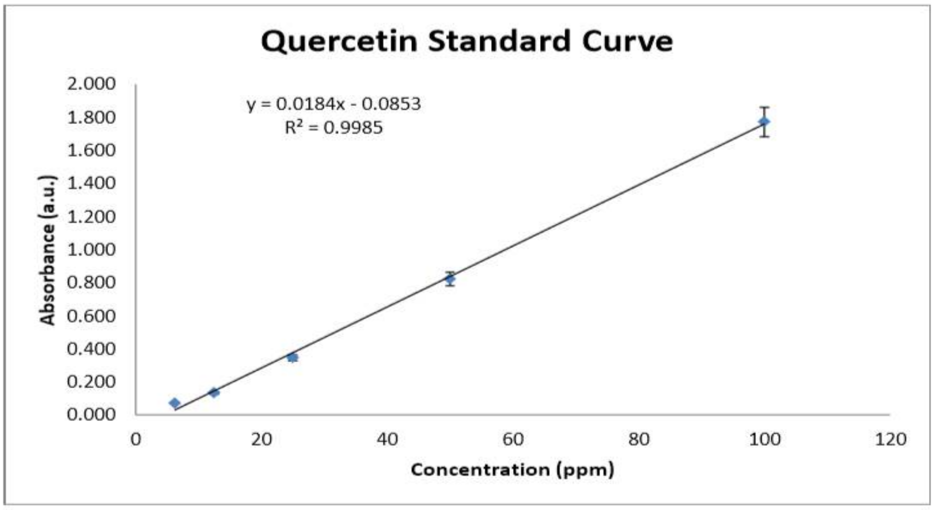
Table 2. The result of the average total phenolic content of raw and gamma-irradiated honey samples.

Honey by Species	Raw	Gamma-Irradiated
	mg GAE*/g sample	mg GAE*/g sample
Apis mellifera	0.3030	0.2850
Trigonabiroi	0.8320	4.4700

*Gallic Acid Equivalence

Total Flavonoid Contents of Honey

In this study, a calibration curve shown in Figure 2 was created using a standard solution of quercetin. In the quercetin standard curve (R^2=0.9985), the presence of total flavonoid contents of the honey samples were determined.



Graph 2. Calibration Curve of Quercetin Standard

In shown in table 3, sample honeys from *Trigonabiroi* both raw and irradiated has higher flavonoid content than the honey samples from *Apis mellifera*. There is also slight changes on the flavonoid content when the honey samples were gamma-irradiated.

Table 3. Average total flavonoid content of raw and gamma-irradiated honey samples.

Honey by Species	Raw	Gamma-Irradiated
	mg QE**/g sample	GAE/g
Apis mellifera	0.0120	0.0124
Trigonabiroi	0.1520	0.1500

**Quercetin Equivalence

Flavonoids are also responsible for the aroma and antioxidant properties of honey because of its low molecular weight phenolic compounds [2, 33]. It also contributes to honey flavor and color [43].
Alike from the study of Nweze, *et al*.,[37], higher flavonoid content was also observed from honeys of stingless bees from Nigeria when compared to honeys of *Apis mellifera*. Stingless bee honeys from Amazon also has high flavonoid content that affect its nutritional value [7]. Vit, *et al*., [50] remarked that stingless bee honeys showed to have more flavonoid types compared with honey bee honeys from Venezuela. In addition, Fadzilah, *et al*. [20] investigated that bee pollens collected by the three types of Malaysian stingless bees has high flavonoid contents because of the diversity and complexity of the bee pollen which is also important in promoting health effects of honeybee products.
Flavonoid contents of the honeys varied significantly with the floral type, the regions it was obtained and what type of honeybee species collected these honeys [42, 19].

CONCLUSION

The physico-chemical properties of raw and gamma-irradiated honey samples from *Trigonabiroi* and *Apis mellifera* produced in Isabela, Province are within the normal range indicated by the International Honey Commission, *Codex Alimentarius* and Philippine National Honey Commission. Moreover, total phenolic and total flavonoid contents were also present to both raw and gamma-irradiated honey samples. Irradiating the honey samples also has impact to the honey contents. However, other compositions of honeys should also be considered since the sample is a complex substance of various compounds and components.

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