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ORIGINAL PAPER

Evaluation of physical, biochemical properties and cell viability of gamma irradiated honey

Amina A. Aly[1] · Rabab W. Maraei[1] · Mohannad M. Abd-Allah[2] · Gehan Safwat[2]

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Abstract

Honey, rich with polyphenols, vitamins, proteins, organic acids and minerals, and is supersaturated solution of sugars. Gamma irradiation could be a physical food preservation technique which protects against food related-insects and microbial contamination and increases the shelf life of some products. The present research was investigated the effect of γ-irradiation (0.0, 2.5, 5.0 and 10.0 kGy) on the physicochemical possessions of the substances of honey; phenolic and flavonoids content in addition to the antioxidant activity by DPPH radical. Diastase No. is also identified with and without gamma irradiation. After γ-irradiation a minor decrease in moisture content was observed while color intensity was significantly increased by elevating irradiation dosage height mainly dose intensity 10.0 kGy which gave the premier color intensity compared with control. But there was no significant difference in the pH values and total soluble solids (TSS%) as well as sugars% of honey samples. Significant increases within the phenolic content were observed with the use of γ-rays particularly at dose 10.0 kGy which gave the main content relative to the control, while a trivial increase in the flavonoid content was obtained within the rise of the γ-irradiation dose. The findings have also shown that free radicals can be scavenged by honey and reveal high activity of antioxidants. Hydroxymethylfurfural is reduced by raising the irradiation dose, in converse diastase No. is enlarged by amplifying the level of the γ-rays. Also, the results showed that the inhibition zones varied according to the irradiation dose level, the dose level 10.0 kGy was more effective for inhibiting the growth of both Gram-positive and Gram-negative bacteria as well as fungal development. The lung cancer cells (A549) showed a decrease in cell viability and density, irradiated honey with dose level 10.0 kGy gave the lowest IC_{50} (6.08 mg/ml). Honey treated with a dose of 10.0 kg of gamma rays did not affect the physical and biochemical properties of honey and was more effective as an antimicrobial and anti-lung cancer, and the quality of honey remained unchanged upon radiation treatment.

Keywords Honey · Gamma irradiation · Phenolic compounds · Diastase number · Antimicrobial activity · Lung cancer (A549) cell line

Introduction

Honey is the most popular and economically important commodity of the colony of the honey bee (*Apis mellifera*). It's characterized as the natural sweet matter formed by bee's of honey, commencing the plant blooms nectars [1]. The honey characteristics and contents depend on its ecological flowery source, time of year, environmental attributes and concern of beekeepers [2]. Honey is known as including several of biological possessions, counting antioxidant effects; phenolic compounds are the principal antioxidants in this innovation [3]. There are a plenty of studies about prospective of honey such as antioxidants, antimicrobials, antivirals, antimutagenic, antiinflamatory, immune suppressants and cytotoxic activity [4]. The major sugars (fructose and glucose), are the main components of honey but they contains about 200 molecules such as proteins, vitamins, amino acids, enzymes, organic acids, minerals, ash, phenols and flavonoids substances which give significant contribution to their biological effect [5]. One of the major consistency criteria is the botanical origin of honeys. In recent decades, the medicinal prospective of honeys has resulted in increased concern in

✉ Amina A. Aly
aly_amina@yahoo.co.uk

1 Natural Products Department, National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, Nasr City, P.O. Box., 29, Cairo, Egypt

2 Faculty of Biotechnology, October University for Modern Science and Art (MSA), Cairo, Egypt

their nutritional benefits furthermore as antioxidant and antimicrobial activities [6].

Phenolic compounds are well-known partially responsible for honey's antimicrobial activity. Nevertheless, the content of hydrogen peroxide (H_2O_2), delivered by glucose oxidase (GOX)-intervened glucose renovation in honey, can add to the antibacterial properties of honey too [7]. The hypothesis of bacteria-killing mechanism depends on the oxidative destruction and DNA of bacterial deprivation. The important capability of honey for human wellbeing must be viewed as dependent on the assurance of its physicochemical assets just as its cell antioxidant and antimicrobial forthcoming. The activity of diastase (α-amylase) as one of the main compounds in honey that is added to honey through the gathering and ripening of bloom nectar by the bees. Several researchers also illustrate that honey shows majors differences in diastase content based on structure, pH value and floral resource. In fact, heating is not the only aspect affecting diastase content of honey, but it can also be changed by changing the honey's pH level. Consequently, the quantity of diastase is an inadequate marker for honey quality. Seow et al. [8] reported that heating treatment modifying the composition and properties of honey by decreasing the activity of the diastase enzyme as well as the pH in addition to increase the hydroxylmethylfurfural (HMF), free acids, lactone content and total acidity. In the same concern, Seow et al. [9] cited that the high diastase content in honey bee honey (HBH) showed a superior ability to hydrolyze amylopectin chains into smaller chains of low molecular weight. Thus the degradation of low molecular weight amylopectin gels is slower as compared to high molecular weight amylopectin gels.

Other study has documented the inhibition and antagonistic impacts of honey against approximately 60 bacterial strains counting Gram-negative, Gram-positive, aerobal, anaerobal and microorganism [10]. Likewise, Mokaya et al. [11] detailed that the antimicrobial activity of honey proved by smothering the development and endurance of pathogenic which can improve the healing process of infected wound-associated microbes. This is partly appropriate to existence of numerous antimicrobial factors like high osmotic pressure, high acidity, H_2O_2, phenolic substances, flavonoids, antibiotic-like derivatives, antibacterial peptides, and other non-characterized compounds.

Gamma irradiation where goods are subjected to gamma rays which is a physical process of sterilization or decontamination. Gamma rays, which are types of very short electromagnetic wavelengths radiation serves as an ionizing energy source that kills bacteria and pests. It increases the hygienic consistency of different foods and herbal products decrease the losses owing to microbial contamination and insect injure [12]. Earlier studies found that γ-rays at 10.0 kGy did not cause major physicochemical modifications in Brazilian honeys [13] whereas Molan and Allen [14] illustrated that γ-rays at 25 kGy was adequate toward accomplish sterility for honey and proved that γ-rays at 25 kGy was helpful for sanitizing the honey as well as improve the action of antioxidants and phenolic compounds in honeys of Gelam and Nenas [15]. Similarly, Khalil et al. [16] also recorded that, Malaysian Tualang honey had greater antioxidant activity following gamma irradiation at 25 kGy. Therefore, in this study, efforts were made to study the effect of gamma rays on the physical, chemical, antioxidant properties and antimicrobial activity of Egyptian clover honey as well as evaluation the cytotoxic effect of irradiated honey on lung cancer cells.

Materials and methods

Honey samples

Samples of clover honey were collected after ripening in May 2020 from honeybee (*Apis mellifera*) colonies situated in the area of Toukh, Qalyoubia Governorate, Egypt.

Chemical and solvents

Diphenyl-2-Picrylhydrazyl (DPPH), sodium carbonate, Folin-Ciocalteu's phenol reagent, gallic acid, aluminium chloride hexahydrate ($AlCl_3$. 6 H_2O), quercetin, DNSA, potassium ferrocyanide, zinc acetate, sodium bisulfite, penicillin G potassium, streptomycin and neutral red were obtained from Sigma-Aldrich (Sigma–Aldrich, Milan, Italy). Trypsin–EDTA Mueller–Hinton agar Gibco (Thermo Fisher Scientific, USA). Complete media of DMEM supplemented with fetal bovine serum (SeraLab-Bio-Connect B.V., Begonialaan, Netherlands). Sodium nitrite, gentamycin, nystatin, ethanol HPLC grade and sodium hydroxide were supplied by Roth Company (Overland Park, KS, United States).

Gamma irradiation process

Honey samples in closed glass jars were irradiated by a ^{60}Co source (Gamma cell 220- Canada) for γ-rays at dosages (0.0, 2.5, 5.0 and 10.0 kGy) and dose rate 1.2 kGy/h. The irradiation treatments were performed at the Egyptian Atomic Energy Authority, National Center for Radiation Research and Technology, Nasr City, Cairo- Egypt.

Honey samples were then kept within the dark at room temperature until use.

Moisture content

The usual-drying oven process was done as illustrated in AOAC. [17]. The content of humidity was measured by drying 1.0 g of honey samples in porcelain crucible for 3 h at 105 °C (or until a steady weight was acquired).

Determination of ash

Two grams of honey were weighed accurately and placed in to crucible porcelain and 4:5 drops of olive oil have been applied to avoid spattering. The blind was then thoroughly heated over a low flame until swelling ceased ignites in a muffle Furness till white ash was obtained. After cooling the obtained ash was weighted.

Determination of colour intensity

The intensity of the colour was determined using Beretta et al. [18] process. Honey solution 50% (w/v) was prepared at 45–50 °C with warm water then sifted to eliminate every vulgar molecules. Absorbance was measured using spectrophotometer (Jasco V530-Japan) as the difference between 450 and 720 nm absorbance, the findings were articulated as mAU.

pH determination

A pH meter was used (JENWAY, UK 3305P) to determine the pH of honey solution (10%) prepared (w/v) by distilled water [19].

Percentage of total soluble solids (TSS %)

A hand refractometer was used for determining the total soluble solids content as illustrated previously [17].

Reducing sugars

Content of reducing sugar was calculated utilizing 3,5-dinitrosalicylic acid (DNSA). In theory, the reducing sugar reduces DNSA to 3-amino-5-nitrosalicylic acid, bringing in about ruddy orange resolution, and as defined by Khalil et al. [20]. The obtained color was determined at 540 nm using spectrophotometer (Jasco V530-Japan). Outcome results w ere expressed as g Glu/100 g honey.

Sugars profile by HPLC

Sugars (glucose, fructose and sucrose) were calculated using HPLC (smart line Knauer, Germany), the column used was PhenomenexLuna NH_2 250 X 4.6 mm. Acetonitrile HPLC grade: water, 80:20, as mobile phase was detected by Refractive Index (RI detector), information combination by claritychrom programme and the temperature was held at 30 °C. From the peak areas of the sample, quantification was conceded against the corresponding standard graph. Obtained results have been recorded as g/100 g.

Total phenolic content

Total phenolic substance was defined by the Folin-Ciocalteu process as outlined by Singleton et al. [21] with some modifications. The spectrophotometer (Jasco V530-Japan) measured the absorbance of the reaction mixture at 725 nm. Total phenolic content was explained in mg of gallic acid equivalent (GAE) per 100 g of honey.

Total flavonoid content

Aluminum colorimetric approach of Marinova et al. [22] was used to measure the flavonoid content. The absorbance rates were estimated at 510 nm utilizing spectrophotometer (Jasco V530-Japan). Results were uttered as mg quercetin equivalent/100 g (mg QUE/100 g) sample.

Antioxidant activity

The method previously described by Gulluce et al. [23] was concerned with the determination of the ability of honey samples to neutralize the DPPH radicals.

Diastase determination

Diastase activity (α-amylase) is one of the major enzymes in honey that is included to honey during the processing and ripening of bloom nectar by the bee. Diastase number (DN) was determined following the International Honey Commission [24] Schade method. One diastase activity unit is classified as that amount of alpha-amylase that will convert 0.01 g of starch to the specified end-point at 40 °C in one hour. The obtained results are stated per gram of honey in Schade units named Diastase Number (DN).

Determination of hydroxylmethylfurfural (HMF) content

Sample of honey (5 g) of was dissolved in 25 ml of distilled water and transferred to a 50 ml volumetric flask to assess the HME content by white method [19]. At that point, 0.5 ml of Carrez solution I (15 g potassium ferrocyanide melted in distilled water and diluted to 100 ml) and 0.5 ml of Carrez solution II (30 g zinc acetate disintegrated in refined water and diluted to 100 ml) were added and the combination was made up to 50 ml with distilled water. After filtration utilizing filter paper (the initial 10 ml of the filtrate clarification was dismissed), 5 ml of the solution was moved into two test tubes. In one of them, 5 ml of refined water (sample solution); and to the next tube, 5 ml of sodium bisulphate solution 0.2% (reference solution) was added. A spectrophotometer (Jasco V530-Japan) was used to determine the absorption of the 284 and 336 nm utilizing a comparable recipe recommended by the International Honey Commission [24].

Antimicrobial activity of honey

The used bacterial and fungal strains were supplied by the Department of Microbiology, National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, Cairo, Egypt. The antimicrobial activity of the samples tested (*Escherichia coli, Klebsiella pneumoniae* and *Bacillus subtilis, Staphylococcus aureus* represent the Gram-negative and Gram-positive bacterial strains respectively and *Candida albicans* represent fungal strain) was evaluated by disc diffusion assay. For determination of antimicrobial activity, discs in diameter 6.0 mm, which made of Whatman No. 1 filter paper sterilized in an oven for 1 h at 80 °C dry heat were used. For fungal and bacterial strains, Sabouraud dextrose agar and Muller Hinton agar media were arranged, respectively. After sterilization it was decanted onto sterilized Petri dishes and sited aside to solidify, cultures were moped on the outside of medium plates utilizing a sterile cotton mop. The discs were filled by 20 µl of each sample and aseptically laid over containing medium plates. In addition to the samples analysed, Nystatin (100 µg) as antifungal disc and Gentamycin (10 µg) as antibacterial disc were taken as standards. For each study, three replicates have been preserved and the plates were incubated for fungal and bacterial strains at 28 °C and 37 °C for respectively. The inhibition zone was observed after 4 days and 18 h for fungal and bacterial strains, respectively. With filter paper discs dipped in methanol was used as control. The antimicrobial effects were assessed by measuring the inhibition region calculation (mm).

Cell viability assay

The cell line of Lung cancer (A549) was collected from Cairo University, Research Park, faculty of agriculture, Cairo University (Egypt). Dulbecco's Modified Eagle's medium (DMEM) with 10% heat-inactivated fetal bovine serum, 100 µg/ml streptomycin, and 100 unit/ml penicillin G potassium, 90% humidified and 5% (V/V) CO_2 at 37ºC is used for cells preserve. The neutral red (NR) assay was evaluated the cytotoxicity of honey solution by Repetto et al. [25]. In 96-well plates, exponentially growing cells were collected and seeded at 2000 cells/well using 0.25% trypsin–EDTA. Honey solutions were applied after incubation (overnight) in concentrations (2, 1 and 0.5 mg/ml). The media was eliminated after 24 h of exposure with honey solutions, and the cells were exposed to a neutral red solution at 37 ºC for 4 h. The destination solution was applied toward strip stained neutral red cells and colour test at 540 nm microplate reader (Biotek, ELX808, United States).

Statistical analysis

Each treatment uses three batches and three replicates, one from each batch. The findings were expressed as means ± SD and subjected to a one-way analysis (ANOVA). Indicated values were distinguished by Duncan's multiple at $p \leq 0.05$ relevant level [26].

Results and discussion

Physical and chemical studies have been conducted to detect any major improvements that have occurred as a result of the use of various dose levels of gamma irradiation. It contains low pH (acidic nature) and reducing sugars at high concentration that makes honey is difficult to support microbial development, because if these parameters changed, honey's shelf life may be impaired because it will boost the conditions of microbial survival and therefore their multiplication and other variables of food spoilage is equally critical. Maintaining the physicochemical parameters to preserve or enhance the shelf life and natural feature of honey is important.

Honey moisture content

The moisture content of honey is a significant feature that contributes to its stability against fermentation and granulation during storage. This is due to the presence of high water content and the yeast cells found in honey will be ready to ferment the sugar. In general, there was a significant decrease induced by γ-rays in the moisture content of honey. The 10.0 kGy dosage level in the analyzed samples proved the lowest moisture content (17.18%) compared with the control (18.38%) treatment (Fig. 1). Thus, gamma rays help maintain the freshness of honey by reducing the moisture content and subsequently lowering the rate of fermentation.

These finding are inconsistent with Bera et al. [27] who revealed that there were no significant variations in the moisture values of honey tests exposed to 10.0 kGy. However, Hussein et al. [15] reported that gamma irradiation (25 kGy) resulted in a significant reduction in the honey moisture content. During storage, elevated moisture content might promote to unwanted honey fermentation caused by the exploitation of osmotolerant yeasts resulting in the formation of ethyl alcohol and carbon dioxide; the alcohol can be additionally oxidized to acetic acid and water resulting in sour flavor [28] and the honey can be protected from microbiological effect for longer time by keeping its moisture content low [2].

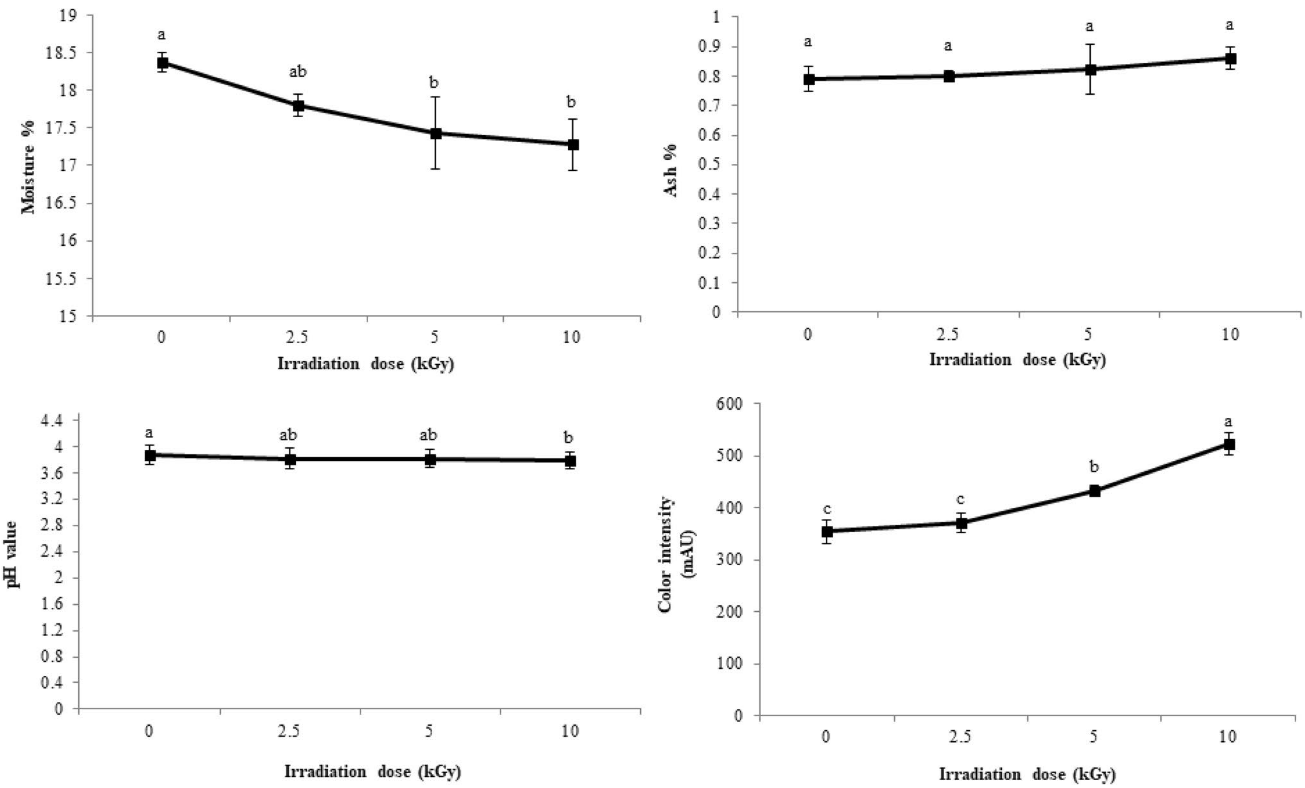

Fig. 1 Different dose levels of γ-rays affect moisture %, ash %, pH value and color intensity (mAU) of honey. Bars ± SD ($n = 3$). Different letters indicate statistically significant differences at $p \leq 0.05$

Ash content

The content of ash is a consistency principle that specifies the honey origin and can also be used to assess the organic and inorganic acids concentrations and the occurrence of postponed solids in honey [19]. The result indicated that the ash content varied from 0.79% to 0.86% (Fig. 1). There was No major difference in ash content found between samples. These findings are agreed with Bera et al. [27].

pH of honey

Honey is a high acidity substance with pH values for various forms of honey ranging from 3.6 to about 6.5 [19]. The low pH also prevents the development of honey microorganisms, thus leading to extend shelf life of honey product's. The pH results ranged from pH 3.79 to pH 3.87 (Fig. 1), the honey samples after treatment with gamma irradiation, there is no significant variation in pH values. This result is in consistent with Bera et al. [27] and Hussein et al. [15] on honey samples reaching pH values in the 3.8 to 4.2 range and the 3.8 ± 1 to 6.0 ± 1 standard Codex range.

Color intensity

A 50% (w/v) honey solution absorbance ranged from 355 mAU to 522.7 mAU (Fig. 1). For all honey treatments, gamma irradiation produced a significant improvement in the color strength. The color strength outcome of this investigation is in accordance with Hussein et al. [15]. The honey color represents in part the quality of antioxidant properties (carotenoids and flavonoids) as reported by Khalil et al. [29]. The honey color also depends on its mineral and phenolic contents. Moreover, Hussein et al. [15] showed that light-colored honeys usually have lower ash content, while dark-colored honeys generally have higher ash content. Irradiated honey showed higher antioxidant phenolic compounds than un-irradiated honey, and this is probably one of the reasons for the increased color intensity in irradiated honey.

Total soluble solids (TSS%) and reducing sugars content

The contents of TSS and reducing sugars are presented in Fig. 2. There were no significant differences between gamma irradiated and control samples, gamma irradiation had a slight effect on the outcomes. The total soluble solids

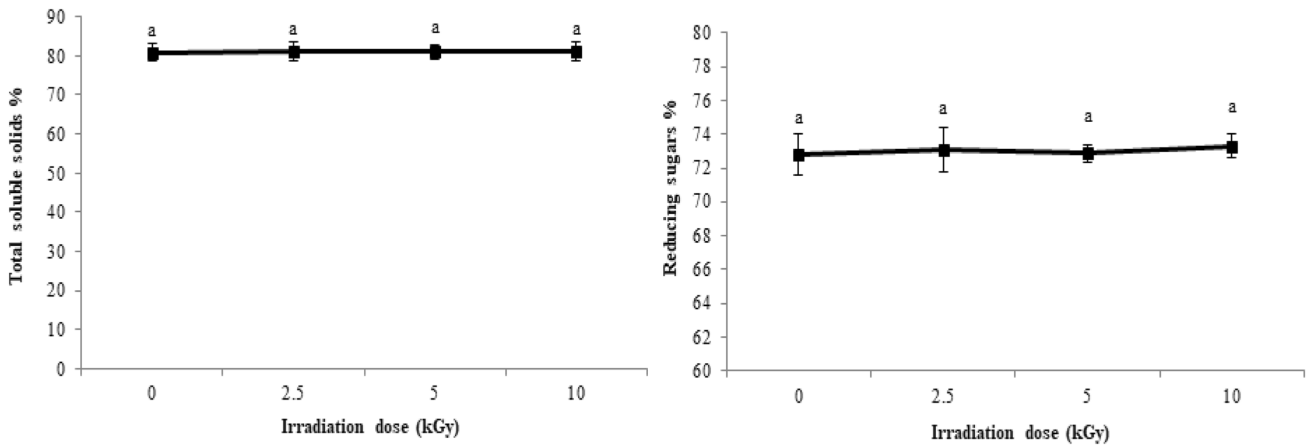

Fig. 2 Different dose levels of γ-rays affect total soluble solids (TSS %) and reducing sugars content (%) of honey. Bars ± SD ($n=3$). Different letters indicate statistically significant differences at $p \leq 0.05$

ranged from 80.80% to 81.20% in honey samples. Sugars are the main compounds of all the forms of honey, glucose, fructose and sucrose are the main constituents of honey. The results showed that in the honey samples tested, γ-rays did not provoke significant differences in the reducing sugars content and Bera et al. [27] and Hussein et al. [15] are in harmony with these findings.

The HPLC sugars profile in honey samples was almost identical (Table 1), suggesting that the majority of sugars such as fructose and glucose are of reducing form. They ranged from 34.164 to 34.475% and 28.185 to 28.422% respectively. In honey samples the sucrose content ranged from 2.707% to 2.819%, which is lower than 5% of the maximum approved frontier as per the Codex norm [30]. No major variation were found between the honey samples in all the sugar compounents (fructose, glucose and sucrose). Irradiation is known to break down starch and other carbohydrates into simpler sugars [31]. Since the majority of sugars in honey are simple sugars in the form of glucose and fructose, no significant effect on the sugar content was observed upon radiation treatment. In addition, these sugars are present in honey in combination with many other macromolecules, which can decrease the radiation sensitivity of sugars to radiolytic attack [32].

The content of phenolic compounds, flavonoids and antioxidant activity

In general polyphenols serve as key antioxidants, offering defense by eliminating free radicals, thus ending oxidative chain reactions [33]. Depending on geographical origin and floral source, honey phenolic outlines differ [34]; it was found that darker types have been found to possess more phenols content than the light colored honey forms [35]. Various irradiated honey samples were tested for the phenolic compounds and the scavenging activity, a s significant higher phenolic content was found relative to the controls as phenolic compounds content increased by increasing irradiation dose level. The highest phenols content (17.48 mg/100 g) was obtained by high dose (10.0 kGy) compared with control (13.06 mg/100 g) which gave the lowest content (Fig. 3). As indicated by Harrison and were [36] that rise in phenols content is refer to the liberate of phenolic ingredients from glycosidic constituents and the degradation of larger phenolic compounds by γ-rays into smaller ones. A broad group of phenolic compounds are flavonoids, and has multiple biological functions, like antioxidants, anticancer, antimicrobials, etc. [37]. The results showed an improvement in the flavonoids content by

Table 1 Different dose levels of γ-rays affect sugars honey profile (%) by HPLC

Sugar	Irradiation dose (kGy)			
	0.0	2.5	5.0	10.0
Fructose	$34.202^{a} \pm 0.987$	$34.164^{a} \pm 1.163$	$34.475^{a} \pm 0.571$	$34.456^{a} \pm 0.471$
Glucose	$28.185^{a} \pm 0.466$	$28.217^{a} \pm 0.556$	$28.164^{a} \pm 0.327$	$28.422^{a} \pm 0.465$
Sucrose	$2.707^{a} \pm 0.195$	$2.732^{a} \pm 0.198$	$2.807^{a} \pm 0.085$	$2.819^{a} \pm 0.080$
Total	65.094	65.113	65.446	65.695

Values are mean ± SD ($n=3$). Different letters in the same row indicate statistically significant differences at $p \leq 0.05$

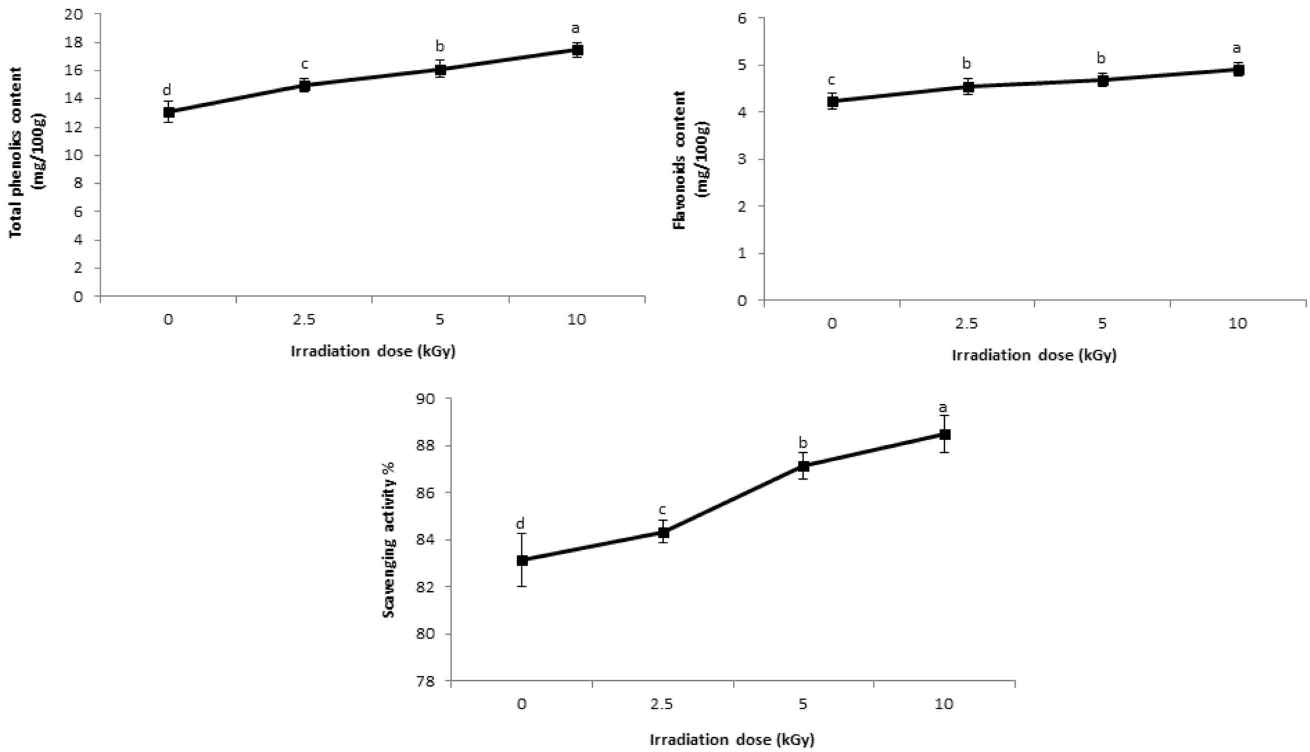

Fig. 3 Different dose level of γ-rays affect phenolic compounds content, flavonoids (mg/100 g) and scavenging activity (%) of honey. Bars ± SD ($n = 3$). Different letters indicate statistically significant differences at $p \leq 0.05$

elevating the level of irradiation dosage to 10.0 kGy which gave the maximum increase (4.91 mg/100 g) compared to control (4.23 mg/100 g) (Fig. 3). Some compounds as well as other antioxidants are present in the honey samples, which might cause a rise within the absorption rates. It can also be assumed that dark honey produces the best level of other antioxidants such as flavonoids, ascorbic acid and carotene, which also lead to the stronger antioxidant assets could found in the 10.0 kGy dose level treatment. Such findings are far greater than those achieved in other researches, the average value obtained by Beretta et al. [18] for the total phenols compounds of *Italian acacia* honey was (55.2 ± 2.8) mg of GAE per kg of honey. Similar results were obtained by Bertoncelj et al. [35] with an average value of 44.8 mg of GAE per kg of honey. Al et al. [38], who measured phenolic content in *Romanian acacia* honey to be in the range of 2.0–39.0 mg of GAE per kg of honey. Antioxidant may be extensively characterized as any substances that hold-ups or reduces the oxidative harm of a target molecule [39]. The power of the free radicals to intercept is the primary characteristic of the antioxidant. Antioxidant substances like polyphenols, phenolic acids, and flavonoids eliminate free radicals and thus reducing the oxidative damage caused by a lot of diseases [40]. Findings in the current study showed that antioxidant activity was improved by increasing gamma rays and the highest antioxidant activity was obtained by highest dose level (10.0 kGy) compared to antioxidant of control (Fig. 3). In addition, the function of antioxidants isn't confined to the free radicals scavenging; it also has better potential for adjusting the trails of signal transduction which affected the free radicals during oxidation stress. Free radicals are impaired and accountable for cellular reactions (e.g. survival, inflammation, proliferation and death) in different infections [41]. Darker coloured honeys have been associated with greater antioxidant like the strong colour comes from various dyes substances as flavonoids, carotenoids and phenolic ingredients, which provide honey substances with antioxidant characteristic [42].

Diastase (amylase) activity (DN)

Diastase content (more correctly known as amylase) has been used in the past as an indicator of honey quality. The reasoning that when honey was heated, would show a decrease in the diastase number (DN), which would suggest that during processing the honey had been exposed to extreme heat. One diastase activity unit is classified as the amount of α-amylase which changes 0.01 g of starch to the identified endpoint at 40 °C in one hour. As shown in Table 2 major variations were evident in all the treatments performed on honey there were clear significant differences in all treatments which was conducted. The diastase

Table 2 Different dose levels of γ-rays affect hydroxy methyl furfural (HMF) and diastase activity (DN) of honey

Parameters	Irradiation dose (kGy)			
	0.0	2.5	5.0	10.0
Diastase activity (DN)	$13.30^{d} \pm 0.352$	$15.60^{c} \pm 0.458$	$19.90^{b} \pm 0.265$	$23.70^{a} \pm 0.173$
HMF (mg/kg)	$6.00^{a} \pm 0.361$	$5.50^{b} \pm 0.173$	$4.10^{c} \pm 0.100$	$1.90^{d} \pm 0.100$

Values are mean ± SD (*n* = *3*). Different letters in the same row indicate statistically significant differences at $p \leq 0.05$

content ranged from 13.30 to 23.70 DN for honey samples. The results revealed that the diastase content improved by increasing gamma rays dose level and the maximum content (23.70 DN) was obtained by 10.0 kGy treatment compared to control treatment (13.30 DN). This finding is not consistence with Baggio et al. [43] who found the activity of the enzyme was decreased by gamma rays.

Hydroxymethylfurfural (HMF) content

The HMF maximum concentration set by the International Honey Commission [44] and Codex Alimentarius Commission (CAC) [30] was 80 mg/kg for honey from tropical regions and 40 mg/kg for honey from non-tropical region. Many variables affect HMF concentrations, like heating rate and heating period, storeroom states, pH and flower resource [45]. As revealed in Table 2 gamma irradiation induced a significant ($p \leq 0.05$) reduction in HMF content relative to the control sample for all honey treatments. Compared to regulation (6.0 mg/kg) the maximum dosage (10.0 kGy) resulted in the highest decrease (1.6 mg/kg). In general, the HMF was lowered by increasing the amount of the irradiation dose level. Hussein et al. [15] confirmed that a significant decrease in HMF explained the substance by gamma irradiation of Malaysian Gelam and Nenas honeys, agreed with the results of the current research. By decreasing the amount of microorganisms that can speed up the development and decrease the freshness of the honey, irradiation may have decreased the formation of the HFM. Irradiation is also a major factor in preserving the quality of honey [16].

Antimicrobial activity

The data provided in Table 3 showed that the inhibition zone around the discs varied in line with irradiation dose levels and therefore the compassion of bacteria. This may refer to variations in bacteria resistance to anti-tested substances refer to cell membrane extracellular fluid changes, hampering the enzymes entry or extractions through altering the chemical structure of constituents or by altering the nature of some components [46]. The dose level 10.0 kGy was more efficient dose for inhibiting the growth of both Gram-positive and Gram-negative bacteria as well as fungal development. An especially rich source of anti-infection agents is the use of natural inventions. Phenolic and flavonoid compounds have antimicrobial activity because they play a task in DNA inhibition [47]. Some big molecules may also break down into tiny molecules that have antibacterial activity because of gamma irradiation [48]. The effect was explained by Taormina et al. [49] described the impact of honey on Gram-negative bacteria, attributing it to the existence of hydrogen peroxide and potent antioxidants in bee honey, as well as to a naturally low pH, which is inappropriate for bacterial development, and to the attendance of phenolic acids, flavonoids and lysozymes. Acidity would potentially add to the honey's antibacterial capacity [50]. It

Table 3 Different dose levels of γ-rays affect antimicrobial activity (inhibition zone diameter in mm) of honey

Microbial strains	Inhibition zone (mm)					
	Irradiation dose (kGy)				Standard	
	0.0	2.5	5.0	10.0	CN	NS
Gram-negative						
E. coli	$15^{c} \pm 0.954$	$16^{b} \pm 0.00$	$17^{a} \pm 0.557$	$17^{a} \pm 0.560$	$11^{d} \pm 0.00$	NT
K. pneumoniae	$18^{d} \pm 0.557$	$20^{c} \pm 1.732$	$22^{b} \pm 0.200$	$25^{a} \pm 0.917$	$10^{e} \pm 0.00$	NT
Gram-positive						
B. subtilis	$13^{c} \pm 0.00$	$14^{c} \pm 0.500$	$16^{b} \pm 0.400$	$20^{a} \pm 1.323$	$13^{c} \pm 0.346$	NT
S. aureus	$13^{d} \pm 0.608$	$14^{cd} \pm 0.00$	$17^{b} \pm 0.889$	$20^{a} \pm 1.758$	$15^{c} \pm 0.964$	NT
Fungal strain						
Candida albicans	$14^{d} \pm 0.208$	$15^{c} \pm 0.265$	$17^{b} \pm 0.900$	$19^{a} \pm 0.100$	NT	$12^{e} \pm 0.625$

Values are mean ± SD (*n* = *3*). Different letters in the same row indicate statistically significant differences at $p \leq 0.05$. CN (Gentamycin, 10 µg), NS (Nystatin, 100 µg), NT (Not tested) has been shown that gamma irradiation of honey destroys all vegetative microbial cells moreover microbial spores without affecting the overall antibacterial activity of honey [32]. The biological properties of honey like antioxidants, antibacterial and antibiotic actions can differ depending on their origin source and contents [51]. Honey's antibacterial activity has been broadly investigated and various other antibacterial honey substances are detected. Two of those substances, glucose oxidase (GOX) and bee defensin-1 that arbitrate release of hydrogen peroxide (H_2O_2) are regular ingredients in honey. Such substances contribute greatly honey's total antibacterial effect in opposite to both Gram-negative and Gram-positive bacteria [52].

Cell viability assay

Cancer consider of the mainly reasons of passing away and poses a huge health load [53]. It is remain a challenge to avoid and handle this pervasive condition. In spite of substantial research input, conventional cancer treatment approaches have significant side effects that include cooking for innovative and little hazardous treatments. Around 90–95% of cancer holders are believed to be linked to an individual's climate and lifestyle of a personal [54], emphasizing the possible role of nutrition in carcinogenesis [55]. Treated Lung cancer cells varied from those of untreated cells, the lung cancer cell line (A549), was examined to explore cell changes after 24 h of treatment with honey. Throughout the experimental phase, the density of untreated cells (positive and negative control) remained convergent. Treated cells displayed a decline in cell viability and decreased cell density after treatment with irradiated honey at different dosage levels of 2.5, 5.0 and 10.0 kGy. Irradiated honey with a dose level 10.0 kGy gave the lowest IC_{50} according to the findings obtained in (Table 4), and this indicates that this dose prevents cell proliferation or reduction within the viability of lung cancer cell line (A549). In general, honey includes substances that can grant their anticancer assets, except with its composition efficacy is likely differing.

Table 4 Cell viability% age of irradiated honey against lung cancer cell line (A549)

Concentration (mg/ml)	Viability (%) and IC_{50} for lung cancer cell line			
	Irradiation dose (kGy)			
	0.0	2.5	5.0	10.0
2	93.8	90.4	84	80.4
1	94	92	87.9	87
0.5	95.7	93	93.4	91.7
IC_{50}	41.10 mg/ml	25.55 mg/ml	7.65 mg/ml	6.08 mg/ml

*Incubation time was 24 h

Few investigations have been done on the biocompatibility of honey phenols, conversely addition with 1.5 g/kg buckwheat honey (containing ~ 1.171 mg of phenolic antioxidants/g) and stated for inducing substantial raises in plasma phenolics two hours after addition, with points staying elevated towards six hours [56]. There has been a growing interest in looking for chemo-preventive and anti-cancer drugs extracted from dietary or organic ingredients in recent years. Compared to traditional cancer treatment, the relative security of meals constitutes makes that an attractive option. Honey has been utilized for decades as a foodstuff resource and medication, also a study indicates it can be helpful cancer therapy support. In the process of carcinogenesis, inflammation and reactive oxygen species (ROS) participates significance task [57] and associated with its phenolic components [58]. These embrace phenolic acids as ellagic acid, caffeic acid, gallic acid, ferulic acid, chlorogenic acid, *p*-coumaric acid, syringic acid, and therefore the flavonoids appreciate chrysin, catechin, galangin, quercetin, kaempferol, luteolin, pinocembrin, pinobankskin and myricetin [59].

Conclusion

Several physicochemical properties were obtained for the Egyptian clover honey, before and after gamma irradiation. Gamma irradiation treatment at 10.0 kGy induced no significant changes in some parameters such as ash content, pH values, total soluble solids (TSS%) and the sugars. A significant decrease in moisture content was observed after gamma irradiation while color intensity, phenolic content, flavonoids and antioxidant activity were signifcantly increased. Otherwise, compared with their respective control, HMF values of irradiated samples were lower in contrast to the diastase activity which increased indicating the positive behavior of honey samples when exposed to gamma rays. Gamma radiation doses proven effective in inhibiting the growth of bacteria and fungi. Thus, the use of gamma rays can help honey maintain its properties and increase its resistance to the microbial activity, which may provide an opportunity to store it for longer periods, without affecting its quality properties. The dose level of 10.0 kGy dose of gamma rays was more effective in reducing the vitality and density of lung cancer cells, which has become a common disease, and therefore the possibility of using this irradiated honey in the treatment of lung diseases may later contribute to the treatment or protection of the lung from any lung diseases and this is what must be studied next. This study considers a good evidence for studying the impact of gamma irradiation on the physiochemical and quality properties of Egyptian clover honey.

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Declarations

Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this paper.

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