Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

Comparing the effects of gamma irradiation and thermal processing on unavoidable toxic substances in Egyptian honey

Islam M. Tork [a], Sayed Rashad [a], Manal A. Atwa [a] and Serag A Farag [b]
[a]*Regional Centre for Food and Feed (RCFF), Agricultural Research Center (ARC), EGYPT*
[b]*Food Irradiation Dept., National Centre for Radiation Research &Technology, EGYPT*

Abstract

Honey is usually subjected to heating before commercialization which poses risks for Maillard reactions to take place. The formed Maillard reaction products (MRPs) in honey may generate a small amount of harmful substances such as 5- hydroxymethyl furfural (HMF), Acrylamide (AA) and furan. Therefore, in the present study we tried to use one low heating temperature (80^0C) for two different periods (30 min and 60 min) and three doses of γ-irradiation (5.0,10.0 and 15.0kGy) in an attempt for keeping quality of honey with mitigation of MRPs toxic materials. Honey samples were subjected to different analysis including: microbiological analysis; AA, furan and HMF; elements and heavy metals and antioxidant properties. The results of the microbiological analysis showed that raw Egyptian honey samples had low load of microbes. The total bacteria count (TBC) was found to be $1x10^4$ ±0.4. Whereas Total Spores (TS) were present at $4x10^2$ CFU/g and samples were fungi free. The γ-irradiation eliminated microbes partially at 5.0 key and eliminated all microbes completely at a dose of 10 kGy. Concerning, the chemical analysis, three toxic substances were determined as HMF, AA and furan by GC-MS in raw honey, the amounts of these compounds were changed after applying the different experimental treatments (γ-irradiation or thermal process). HMF, AA and furan were decreased by irradiation and thermal process. The advantages for γ-irradiation was its ability to enhance the total phenols ,antioxidants and flavonoids of honey to high concentrations which adds good quality and more health benefits of irradiated honey (especially at 5.0 and 10 kGy) .The results also proved the presence of 10 trace elements Copper, Chromium, Boron, Cadmium, Cobalt, Lead, Nickel, Zinc, Manganese, Thallium, Iron, Magnesium, Phosphorus, Sodium, Potassium, Calcium, Aluminum and Barium. Though their concentrations were within the permissible limits of WHO. From the results obtained during the present work, it could be recommended that to use low doses of γ- irradiation (5-kGy or 10-kGy) as a safe alternative to harmful thermal processing. This could be a good solution to reduce the toxic substances in honey and enhancing its microbiological and antioxidant quality.

Keywords: Gamma irradiation; microbiology; acrylamide; furan; antioxidant activity, trace elements; thermal processing.

1. Introduction

Honey is naturally made from flower nectar by honey bees (*Apis mellifera*). Depending on the plant source, honey can have an extremely complicated chemical makeup. Since ancient times, it has been used as food and medicine [1]. To guarantee a safe, enjoyable, and uniform appearance of the finished product, a number of processing activities have been established. The honey must typically be heated throughout these processing methods to minimize its viscosity and prevent its crystallization or fermentation [2]. Because of this heating process, Maillard reaction products may be formed. Also, Honey is usually contaminated with numerous microorganisms, depending on the origin of plants and their surrounding area. Mainly, the presence of the spores of *Clostridium botulinum* has been reported by many workers [3]. Bacteria also cause a problem if the honey is intended to be applied for a therapeutic purpose. For instance, honey may provoke an alimentary infection or contaminate wounds. Osmophlic yeasts, in turn, may initiate a fermentation of the honey [4]. Also, honey is easily to be contaminated from many sources especially microbes besides chemicals as herbicides and other elements depending on origin and location [3, 5].

5-hydroxymethyl furfural (HMF) is a substance that is produced when the reducing sugars in honey and other processed foods are heated via the Maillard reaction in an acidic environment. HMF has harmful and it is converted into the non-excretable, genotoxic compound 5-sulfoxymethylfurfural. HMF

*Corresponding author e-mail: Islamtork2008@gmail.com.; (Islam M. Tork).
Receive Date: 02 January 2023, Revise Date: 23 March 2023, Accept Date: 26 March 2023, First Publish Date: 26 March 2023
DOI: 10.21608/ejchem.2023.178393.7260

is therefore a contaminant that causes neoplasms and is extensively researched by scientists [6]. Acrylamide (AA) and Furan, two potentially cancer-causing substances that are frequently produced in processed foods as a result of heat temperature. Maillard reaction is thought to be highly damaging in honey recently because of thermal processing; these compounds are thought to be possibly or probably carcinogenic to humans has sparked an extended discussion on the healthfulness of even basic foods. Acrylamde (AA), furan, and hydroxymethyl furfural (HMF) are substances that are considered to be reasonably expected human carcinogens [7].

Honey has many beneficial health, therapeutic, and preventative qualities. Honey has the potential to serve as an essential source of antioxidants in human nutrition and can have positive benefits on human health, such as anticancer and cardiovascular protection, because of its natural antioxidants and their synergistic interaction with other components [8]. Honey contains flavonoids, phenolic acids, ascorbic acid, catalase, peroxidase, carotenoids, and MRPs. Due to its natural antioxidants and their synergic interaction with other components, honey has the potential capacity to serve as an important source of antioxidants in human nutrition and can exhibit beneficial effects on human health, as antitumor and cardiovascular protection [8]. Honey antioxidant capacity is strongly correlated with the concentration of its phenolic contents [9,10]. The phenolic content of honey comes from the plant that the bees feed on [11]. Thus, botanical origin plays a significant role in determining the constituents and antioxidant activity of honey [12].

Honey also contains a variety of macro- and micro minerals that are the minor constituents of honey present in the range 0.02–2.03%. Trace elements are inorganic compounds which have to exist in the human body for vital activities, such as Se, Cu, Mn, Fe, Ni, and Zn are essential for normal metabolism [13] but above tolerance limits they are hazardous for human health. Trace elements such as Pb, Cd, and Al are considered as toxic and should damage the human metabolism [14]. Recently, γ-irradiation has been established as one of the best approaches for microbial decontamination of food without altering the quality of the ingredients [15]. The utilization of γ-irradiation for microbial decontamination of whole-packaged food, called cold sterilization, is legally permitted in more than 50 countries worldwide. Irradiation of honey (15kGy) results in high quality sterilization and does not affect the antibacterial action of honey [15]. The success of irradiation is widely reported, especially at 10–15 kGy for vegetative microorganisms [5] and at 25 kGy onward for bacterial spores [16, 17]. The present work introduces a comparison between the effects of low γ-irradiation and low thermal treatment on honey's quality, microbiological, and bioactive properties as well as the biochemical characteristics of honey aiming at mitigating the toxic substances in honey that may be formed during its processing. Organization of the manuscript.

2. Experimental

2.1 Sampling and Irradiation process

Egyptian honey samples were obtained from "Honey bee Division" at the Faculty of Agriculture - Ain Shams University. No additional treatment was done. The fresh samples were then divided into 6 treatments (with four replicates each). Three treatment groups for irradiation at different doses as 5.0, 10.0, 15.0 kGy and two for thermal treatments (at 80 °C for 30 min. and 60 min.) + one control group without any treatment were done. The irradiation process was done by using Cobalt-60 source (Indian cell) at the laboratory of food irradiation department, National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. All samples were kept refrigerated until analysis.

2.2. Microbiological analysis

The microbial count of the samples was determined according to the procedure given by [18]. A 25-g portion of each sample was blended under sterile conditions with 225 ml of sterile saline solution (0.85% NaCl) for 1 minute. Next, the mixture was diluted ten-fold with saline solution. Total bacteria count (TBC) was determined on a plate count agar medium via the pour plate technique. Total fungi count (TFC) (CFU/ml) of samples were measured as previously described by [18]. The total samples' spore count (TSC) (CFU/ml) was calculated as previously stated by [19].

2.3. Chemical analysis

2.3.1. Acrylamide (AA) analysis

Samples were allowed to swell adding water in an amount normally corresponding to 3 times the weight of the sample (more for exceptionally dry samples). Preparation, 10 g of the homogenate was weighed into a 100 ml centrifuge glass with a screw cap and thoroughly mixed with 40 ml of 1-propanol.all analysis steps were done according [10] using GC-MS.

2.3.2. Furan analysis

(HS-GC/MS furan analysis): Samples were prepared and analyzed by gas chromatography-mass spectrometry (GC/MS) with head space; sampling was used to detect furan in selected-ion monitoring mode (SIM) using ions: m/z 39 and 68. The reagents involved (in the determination are furan, purified water and ethanol) Furan standard minimum purity 99% (Fluka) and stored in (-20°C) freezer. Also, water purified by water purification system (Milli-Q) and Methanol (HPLC grade). Furan was

determined by HS GC–MS. Individual stock standard solutions of furan were prepared by transferring each pure analyze via a micro syringe through the septum of 20 mL headspace vials containing methanol. GC/MS/MS (Agilent 7890N GC with Agilent 7000 MSD with Dynamic headspace), auto-sampler, GC column: HP-5, 15 m, 0.32 mm I.D., 20 μm films were used [10, 20].

2.3.3. HMF.

5-Hydroxymethyl-2-furfural (5-HMF) was determined according to [21]. Chromatography analyses were carried out with an Agilent 1100 HPLC device. Separations were carried out in an ACE C18 column, 250 x 4.6 mm x 5 μm particle sized. The mobile phase used was methanol: water (90: 10, v/v).

2.3.4. Determination of total antioxidant capacity (TAC)

The total antioxidant capacity (TAC) of the samples was done spectophotometricaly at 595 nm using the "phosphomolebdenum assay" using ascorbic acid as a standard antioxidant [22].

2.3.5. Determination of total phenolic content (TPC)

Total phenolic content (TPC) of the samples was determined by using the Folin–Ciocalteu method [23] using a spectrophotometer set at 760nm and Gallic acid (GA) was used as a standard.

2.3.6. Determination of total flavonoids (TF):

Total flavonoid content (TF) of the samples was determined by using the aluminium chloride method with using a spectrophotometer set at 415nm and Querectin as standard reference [24].

2.3.7. Determination of DPPH radical scavenging assay

The free radical 1, 1diphenyl2 picrylhydrazyl (DPPH) scavenging activity of the samples was also evaluated as earlier reported by [25].

2.3.8. Determination of macro and micro elements in honey

Samples were digested using Microwave digestion technique by analytikjena instrument. Analytical performance of minerals determination (Cu, Cr, B, Cd, Pb, Ni, Zn, Mn, Tl, Fe, Mg, P, Na, K, Ca, Al, and Ba) has been done by using the inductively coupled plasma- mass spectrometry (ICP-MS/MS Agilent 8800) according to the [26] method and to the instrumental manual. The standard ICP-MS helium mode was used to provide lower detection limits and higher sensitivities for elements [27].

3.4. Statistical analysis

Four replicates were performed for each analysis and the values were expressed as mean ±S.D. One-way analysis of variance (ANOVA) described by [28] with significant differences between means determined at $p<0.05$. A Linear regression analysis was applied using Excel program; a Microsoft computer software to get value of R2. All values were average of the replicates with standard deviation.

3. Results and discussion

3.1. Effect of different treatments on microbial load of honey

Honey is a semi-liquid substance with water percentage (15–18%) and pH (3.8) [6]. A complex variety of carbohydrates, mostly glucose and fructose, are found in honey as traces, depending on the floral source. All these may activates microorganism's development in honey. As shown in Table 1, the obtained microbiological results showed a low load of microbes in the raw Egyptian honey samples. The initial total bacteria count (TBC) in raw honey was very low ($1x10^4$ ±0.4), whereas total spores (TSC) were present at $4x10^2$ CFU/g and samples were fungi free. The irradiation eliminated microbes partially at 5.0 kGy and completely eliminated all microbes at 10 kGy. Same doses are recommended for honey at EU standards [18, 29]. Thus, the results revealed that γ-irradiation treatment was explored as a possible mean to achieve microbial decontamination of honey, and it completely sterilized all samples, especially at high doses of 10 and 15kGy. It has been established that γ-irradiation is a cold and safe process, that in general does not affect the intrinsic physicochemical properties of treated products [29, 30, 31].

The success of irradiation is widely reported, especially at 10–15 kGy for vegetative microorganisms [5]. Irradiation is considered as a cold pasteurization. Emergent processing technologies may constitute a promising alternative to ensure honey microbiological safety with preserved fresh like quality [32]. By comparing the effect of thermal treatments and γ- irradiation on microbial load as shown in Table (1), either at short or long-time treatments at 80 °C eliminated all microbes in honey. But according to previous reports, the heat treatment affects honey quality especially viscosity and may cause the production of Maillard reaction products [33].

3.2. Effect of treatments on toxic compounds (HMF, AA and furan)

Most of honey producers use heating at mild temperatures below 100 °C chiefly in order to prevent post-bottling crystallization. During, this process, the so-called Maillard Reaction or non-enzymatic browning occurs. These processes involve

reactions of amino acids, peptides and proteins with reducing sugars and other carbonyl compounds. Furan , Acrylamide ,5-HMF and many of its derivatives are one of the compound classes as toxic ,can be found at very low levels in many foods and drinks as a result of thermal treatment. Some of them showed, its formation of AA and HMF was highly negative healthy effects, correlated and with high temperature and time [34].

Table (1): Effect of γ-irradiation and thermal treatments on the microbial load of Egyptian honey

Treatments	TBC	TSC	TFC
Control	$1x10^4$ ±0.4	$4x10^2$± 0.3	$1x10^2$± 0.5
Irradiation doses ((kGy)			
5.0	$3x10^3$± 0.3	$2x10^2$± 0.4	N.D
10.0	$1x10^3$± 0.2	N.D	N.D
15.0	N.D	N.D	N.D
Thermal treatments (80°C)			
Short time 30 min.	N.D	N.D	N.D
Long time 60 min.	N.D	N.D	N.D

ND = not detected. total bacteria count (TBC), total spores (TSC) and total fungi counts (TFC)

3.2.1. Effect of treatments on 5-HMF

As shown in Table (2), control samples of honey were free of HMF. Concerning, irradiation effects, as shown in Table 2, Fig.1.irradiation increased HMF, at 5.0 kGy, from zero to (9.09±1.5ppm) with calculated rate (%) near 9 % then increased gradually with increasing irradiation doses. Then paralleled with doses linearly, a linear equation resulted as ($y=32.6\ X-3.7, R^2=0.9$) as recorded in Table (3). This rate was faster by thermal process as 14.29%, 19.2% by short and long time at 80˚C respectively. Whereas, irradiated samples recorded the rates as 9.1, 10.5,13.02 % for 5.0,10.0 and 15.0 kGy respectively. In all cases all samples either irradiated or not showed HMF concentrations lower than the permissible levels by WHO/FAO, EU and Egyptian standard [29]. Most workers mentioned that the irradiation of honey did not show any significant effect. Therefore, the results introduce using 5.0 kGy as recommended dose not more for sterilizing honey with safe, nearest HMF value to Egyptian Standard (HMF limit in honey of 40 ppm ,80 mg/kg) [29, 35].

The highest values of HMF were produced during thermal process as shown in Table (2), Fig. (1). The thermal treatments increased HMF from zero in raw honey, to at 80^0C as (14.29±3.9) for 30min and (19.29±5.3) for 60 min. HMF is formed upon thermal processing and/or long-term storage as an intermediate product of MRPs [6, 35]. It is important to notice that HMF in honey is extensively studied as an indicator of honey's quality and freshness. Some workers showed that the honey processed at the 75°C/ 24 h presented an increase in HMF from 173.4 to 226.35 mg/kg) [36].

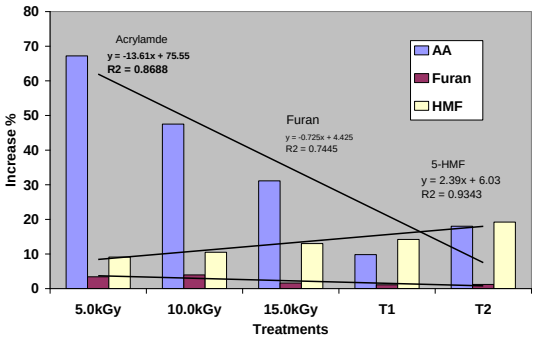

Fig (1): Concentration as (%) for toxic substances under different treatments. (T1=thermal treatment at80 °C /30 min), (T2= thermal treatment at80 °C /60 min).

Generally, it is recommended using irradiation dose 5.0kGy as alternative for thermal processing, as it produces low levels of HMF (9.1ppm) than thermal processing. These levels allow export to EU countries or other countries.

3.2.2. Effect of treatments on Acrylamide (AA):

The raw honey samples recorded presence of AA (6.1 ppb) at zero time before any treatment.

Table (2): Effect of γ-irradiation and thermal treatments on toxic substances of honey.

Treatment	HMF (ppm)	Increase (%)*	AA (ppb)	Increase (%)	Furan (ppb)	Increase (%)
Control	N.D	---------	6.1 ±1.1	---------	3.8±2.1	--------
Irra. dose(kGy)						
5.0	9.09±1.5	*9.1*	10.2±2.0	*67.2*	14.0±2.3	*268.4*
10.0	10.58±2.4	*10.5*	9.0±1.21	*47.5*	10.2±1.6	*168.4*
15.0	13.02±2.9	*13.02*	8.0±3.11	*31.1*	6.57±2.6	*72.9*
T1	14.29±3.9	*14.29*	6.7±2.5	*9.8*	4.91±2.1	*29.21*
T2	19.29±5.3	*19.29*	7.2±3.2	*18.0*	5.23±1.1	*37.9*

* Increase (%) =control-samples /controlX100.

*Thermal treatments (800C) =T1 short time (30 min) and T2= Long time (60 min), 5-Hydroxymethyl-2-furfural (HMF) and Acrylamide (AA)

The control values increased from (6.1 ppb) to (10.2 ppb) at 5.0 kGy then decreased to (9.0 ppb) at (10.0kGy) and to (8.0ppb) at (15.0 kGy) respectively. By calculating the increasing rate as (%), the results were as follow 67 %, 47% and 31% for 5.0, 10.0 and 15.0 kGy respectively. As shown in Fig. (1), the reduction of AA by treatments was linear ($y=-13.6x+75.6$, $R^2=0.87$). The results suggested that the formation of AA and HMF was highly correlated with tested treatments either γ-irradiation, thermal treatments and time.

AA and 5-hydroxymethyl-2-furfural (5-HMF) are resulting as MRPs during food processing by either thermal or irradiation treatments in presence of organic acid, phenols, free amino acids, sugars mainly glucose or fructose. Some pathways of their formation have been proven to share the first step of the Maillard reaction, which makes acrylamide and 5-HMF have a widespread occurrence in daily consumed foods [37].

3.2.3. Effect of treatments on furan:

Results in both Table (2) and Fig (1) , show that, the tested treatments either γ-irradiation or thermal treatments increased furan content γ-irradiation has a descending increase in furan concentration from (3.8±2.1ppb) control to 14.0,10,6.5 ppb when using 5.0 ,10, and 15 kGy respectively. The calculated rates were 268.42%, 168.42% and 72.9 for same doses, respectively. While the proportional increase of furan concentration with the increase of thermal exposure period was 29.21and 37.61, respectively.

It is generally recommended by [38] that exposures for such compound should be as low as reasonably achievable. For ,furan some levels are recommended (0.29–1.17 ppb for adults, 0.27–1.01ppb for infants 3–12 months 0.60–2.22ppb for adults (95th) 1.14–1.34 ppb for infants 6–9 months (95th) Denmark 0.95–1.02ppb for adults 0.08 ppb for children 4–6 year [39].

3.4. Effect of tested treatments on antioxidant, phenols, flavonoid and DPPH scavenging activity:

Four methods were carried out to study the effect of tested treatments on total phenols, flavonoids and antioxidant capacity of honey samples, as shown in Table (3).

The results of the present study showed that Egyptian honey exhibit high antioxidant activity naturally, along with its high phenolic contents. Interestingly, we also noted that irradiated honey has higher antioxidant activities, total phenolic content and total flavonoid contents compared to non-irradiated honeys. In conclusion, irradiation of honey caused an improvement in its antioxidant capacity and its phenolic and flavonoid content. The thermal treatment at short time increased phenolic content of honey but by long thermal treatments this content decreased. Similar trend was obtained by [9].

As shown in Fig, 4 the enhancement of TP by γ-irradiation recorded high values clearly. The enhancement effects by irradiation was clearly, compared with control as % showed that irradiation at 5.0, 10.0 kGy resulted in an increase in TP by 172% and 201%, respectively. Whereas, irradiation by 15 kGy increased 74.%TP. Also, thermal treatment for short time increased TP 61% but thermal at long time decreased TP.

Thermal results raised the injuries of heat process which usually use by factories for destroying the phenols content [33] The reduction of antioxidant activity during thermal processing was showed by [40]

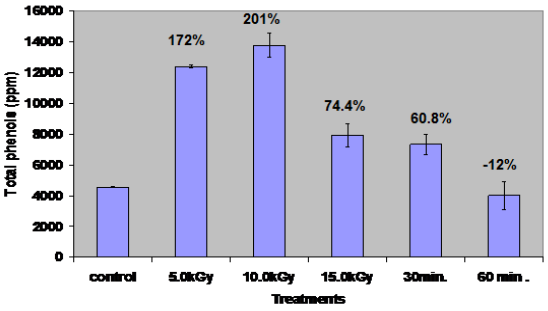

Fig.(4) Comparing effects of γ-irradiation and low thermal temperature on Total phenols (ppm)(Gallic acid equivalent)(T1=short time=30 min,T2= long time 60 min / 80 ^{0}C).

Table (3) Biochemical analysis of treated honey with low irradiation doses and thermal Treatments

Treatments	TP	TAC	TF	DPPH
Control	4,564.4±32	10,739.7±800	8.06±.20	36.65±0.3
Irradiation(kGy) 5.0	12,412.09±119	21,991.9±290	174.86±5.0	27.68±1.0
10.0	13,769.31±783	7,612.04±120	63.33±3	35.76±1.0
15.0	7,953.2±761	15,207.2±140	7.91±.32	39.0±0.12
Thermal treatments(80°C) -Short time 30min. - Long time 60min	7,340.1±678.6 4,012.87±922	15,338.9±120 9851.9±397	19.71±0.8 81.0±2.1	42.8±.6 44.03±0.1

TP=Total phenols ppm (Ascorbic acid equivalent), - TF= Total flavonoid ppm (Quercetin equivalent) , DPPH= (IC_{50} (ppm) (Gallic acid equivalent), TAS= Total antioxidant ppm.

Concerning, TAC (ppm) as (Ascorbic acid equivalent),concerning , TAC as shown in Table (3) and fig (5) it is clear that some doses as 5.0 ,15 .0kGy enhance TA clearly ,like short time - thermal process ,but 10.0 kGy and long thermal caused reduction as in fig,(5). Same trend for enhancing by irradiation of honey was observed with Malyasian honeys [9].

Also, it is observed that methods of DPPH values were not raised obviously the differences between treatments. Whereas ,TAC or TP showed that differences between treatments with high significance values (Table 3).

Same results were observed by some workers [9, 40]. But, thermal even at low temperature more decreasing quality and phenols and flavonoids of honey as reported [33].

Elements and heavy metals concentrations

The quality and value of honey, as natural bio-product, depends on its sort and origin. The quantitative and qualitative relations of chemical elements are characteristic to each blossom of the plant from each region of the country, so general quantity of mineral materials depends on the location. Eighteen elements were detected by ICP-MS-MS in samples of Egyptian honey, everyone has concentration less than 1.0 (ppm)with except of lead as shown in Table (4). Whereas, the elements more than 1.0 ppm are tabulated in Table (4). Revise table no All detected values less recommended by [39]. No markedly differences were observed for tested treatments either of irradiation or thermal treatments.

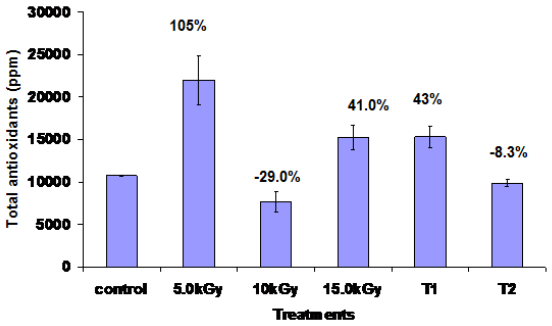

Fig.(5) Comparing effects of **γ-irradiation** and low thermal temperature on(TA) total antioxidant (Ascorbic acid equivalent as ppm), (T1=short time=30 min,T2= long time 60 min / 80 ^{0}C).

Most of these levels were less than 1.0 ppm with exception zinc, whereas water, soils for irrigation the plants are the main source. Generally, irradiation doses increased the levels whereas, thermal reduces these metals may be due to water content which evaporated by thermal only ,but gamma irradiation caused dialysis to produce free radicals to activate reaction of all biological molecules .Same trends was observed in Table (5),but most these metals more than 1.0 ppm.

Higher Pb concentrations (up to 80.37 mg/kg) were detected in honey samples collected from contaminated areas in Italy [41]. Finally, tested honey were free from harmful metals and less [39].

Table (4) Heavy metals content (ppm) in Egyptian honey under treatments (more than 1.0 ppm).

Treatments	Cu	Cr	B	Cd	Co	Pb	Ni	Zinc	Mn	TI
Control	0.769	0.207	0.616	0.243	0.338	0.314	0.249	1.130	0.325	0.467
Irradiation										
-5	0.561	0.373	1.084	0.431	0.637	0.575	0.502	1.104	0.368	0.875
-10	0.212	0.235	0.734	0.264	0.364	0.354	0.290	1.098	0.408	0.558
-15	0.488	0.376	1.179	0.242	0.339	0.354	0.261	1.167	0308	0.518
Thermal										
-Short time	0.071	0.169	0.540	0.192	0.254	0.259	0.194	0.977	0.192	0.409
-Long time	0.030	0.256	0.653	0.236	0.304	0.318	0.239	1.914	0.236	0.505
*FAO/WHO	73.3	--------	--------	0.2	-------	0.3	67.9	99.4	------	------

*FAO/WHO-maximum permissible values. (mg/kg)=ppm.

Table (5) Heavy metals content (ppm) in Egyptian honey under treatments

Treatments	Fe.	Mg	P	Na	K	Ca	Al	Ba
Control	5.35	22.323	36.935	64.879	221.81	25.37	1.08	4.36
Irradiation								
-5	5.89	25.660	37.296	67.973	234.46	11.39	0.93	5.58
-10	5.32	24.475	37.588	65.888	227.17	13.53	1.08	3.86
-15	8.05	27.912	37.165	74.216	214.17	18.84	1.24	2. 902
Thermal								
-Short time	4.88	24.811	36.702	66.993	217.79	14.92	1.09	1.619
-Long time .	6.79	30.624	37.309	79.684	220.87	22.34	3.32	1.329
FAO/WHO*	425.5	--------	-------	-------	---------	---------	--------	--------

*FAO/WHO-maximum permissible values.(mg/kg).

4. Conclusion

Honey is a natural food with a considerable economic worth worldwide. One of the biggest safety issues with regard to its use is its propensity to become contaminated with bacterial and fungus spores. The use of thermal processing increases the risk of formation of potentially harmful or cancer-causing chemicals such as AA, furan, and 5-HMF as well as inferior quality and undesirable color. Some suggestions are subjected to mitigation procedures, some of which call for using low radiation doses. It is advised to use the doses (5.0, 10.0 kGy) as an alternative to thermal processing, even at low temperatures, to introduce safe honey that is of good quality, free of microbes, and contains few harmful compounds.

5. REFERENCES

1. Eteraf-Oskouei, T., & Najafi, M. (2013). Traditional and Modern Uses of Natural Honey in Human Diseases: A Review. Iranian Journal of Basic Medical Sciences, 16(6), 731.
2. Blidi, S., Panagiota, G., Sofia, L., Spyros, G., & Antony, C. C. (2017). Effect of Thermal Treatment on the Quality of Honey Samples from Crete. Advances in Food Science and Engineering, 1(1).
3. Musa, M.Y., Elfaki E., A. and Mohammed Seif Eldin. A. (2013) 'Microbiological Characterization and Physicochemical Properties of Sudanese Honeys', British Microbiology Research Journal, 4(6), pp. 715–722. Available at: https://doi.org/10.9734/bmrj/2014/5330.
4. Migdal, W., Owczarczyk, H.B., Kedzia, B., Holderna-Kedzia, E., Madajczyk, D., (2000). Microbiological decontamination of natural honey by irradiation. Radiation Physics and Chemistry 57, 285–288.
5. Saxena, S., Gautam, S., Sharma, A. (2010) Microbial Decontamination of Honey of Indian Origin Using Gamma Radiation and Its Biochemical and Organoleptic Properties. J Food Sci. 75(1), 19-27.
6. Shapla, U. M., Solayman, M., Alam, N., Khalil, M. I., & Gan, S. H. (2018). 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: effects on bees and human health. Chemistry Central Journal, 12(1), 35.
7. Perera,D. N. Hewavitharana G. G., Navaratne, S. B., (2021)."Comprehensive Study on the Acrylamide Content of High Thermally Processed Foods", BioMed Res Internatl. 13, 6258508.
8. Olas, B. (2020). Honey and its phenolic compounds as an effective natural medicine for cardiovascular diseases in humans? Nutrients,12(2), 1–14.
9. Hussein, S. Z., Yusoff, K. M., Makpol, S., & Yusof, Y. A. (2011). Antioxidant capacities and total phenolic contents increase with gamma irradiation in two types of Malaysian honey. Molecules (Basel, Switzerland), 16(8), 6378–6395.
10. Chua, L.S.; Rahaman, N.L.A.; Adnan, N.A.; Tan, T.T.E. (2013). Antioxidant Activity of Three Honey Samples in relation with Their Biochemical Components. J. Anal. Methods Chem. 2013, 1–8.
11. Kek, S.P.; Chin, N.L.; Yusof, Y.A.; Tan, S.W.; Chua, L.S. (2014). Total Phenolic Contents and Colour Intensity of Malaysian Honeys from the Apis spp. and Trigona spp. Bees. Agric. Agric. Sci. Procedia 2, 150–155.
12. Tomczyk, M.; Tarapatskyy, M.; Dżugan, M. (2019). The influence of geographical origin on honey composition studied by Polish and Slovak honeys. Czech J. Food Sci., 37, 232–238.
13. Kılıç Altun, S., Dinç, H., Paksoy, N., Temamoğulları, F. K., & Savrunlu, M. (2017). Analyses of Mineral Content and Heavy Metal of Honey Samples from South and East Region of Turkey by Using ICP-MS. International Journal of Analytical Chemistry, 2017, 6391454.
14. Fiseha, D., Dessalegn, E., & Nigussie, G. (2019). Determination of Some Essential Minerals in Honey Samples Collected from Chena District, Ethiopia. Chemistry and Materials Research, 11, 1–5.
15. Balakrishnan, N., Yusop, S. M., Rahman, I. A., Dauqan, E., & Abdullah, A. (2021). Efficacy of Gamma Irradiation in Improving the Microbial and Physical Quality Properties of Dried Chillies (Capsicum annuum L.): A Review. Foods (Basel, Switzerland), 11(1).
16. Molan, P. C. and Allen, K. L. (1996). The effect of gamma-irradiation on the antibacterial activity of honey. Journal of Pharmacy and Pharmacology, 48(11), 1206–1209.
17. Postmes, T., van den Bogaard, A. E., & Hazen, M. (1995). The sterilization of honey with cobalt 60 gamma radiation:Astudy of honey spiked with spores of Clostridium botulinum and Bacillus subtilis. Experientia, 51(9–10), 986–989.
18. Ichinoe Zirnstein G.W. and Rehberger T.G. (1983) Microbiol. Rev. in central Montana. Interna-tional Symposium on Biological Control. 51, 221- 227.
19. Nicholson, W.L., Setlow, P. 1990. Sporulation, germinationand outgrowth .In C.R Harwood. & S.M. Cutting (Eds),Molecular Biolgical Methods for Bacillus (pp.391-450).
20. Morehouse K. M., Nyman P. J., McNeal T. P., Dinovi M. J. and Perfetti G.A.(2008) Survey of furan in heat processed foods by headspace gas chromatography/mass spectrometry and estimated adult exposure. Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment 25(3): 259-64.

21. Songül Ü. (2018). Determination of 5-hydroxymethylfurfural (5-HMF) in Expired Pharmaceutical Syrups by Using HPLC-DAD Method Ünüvar S. JOTCSA. 2018; 5(3): 1431-1440.
22. Prieto, P., Pineda, M. & Aguilar, M., (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical biochemistry, 269(2), 337–341.
23. Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M. (1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299, 152-178.
24. Mohdaly, A.; Sarhan, M.; Smetanska, I. and Mahmoud, A. (2010). Antioxidant properties of various solvent extracts of potato peel, sugar beet pulp and sesame cake. J Sci Food Agr., 90: 218–226.
25. Aliyu A. B.; Ibrahim, M. A.; Musa, A. M. and Musa, A.O. (2013). Free radical scavenging and total antioxidant capacity of root extracts of Anchomanes difformis Engl. (Araceae). Acta Pol Pharm., 70: 115-121.
26. AOAC (2019) Official Methods of Analysis of the Association of Official Analytical Chemists: Official Methods of Analysis of AOAC International. 21st Edition, AOAC, Washington DC.
27. Arı, A., Ertürk Arı, P., Ermişer, D., Cındık, B., Yalçın, E. and Gaga, O., E. (2022). Multi-elemental Characterization of Semolina Samples by Inductively Coupled Plasma-Tandem Mass Spectrometry (ICP-MS/MS).Biological Trace Element Research 200, pp.3462–3473.
28. Snedecor GW, Cochran WG (1987) Statistical methods, 7th edn. The Iowa State University Press Ames, I. A., USA. 221–230
29. Codex Alimentarius Commission. (2001) Co-dex Standards for Honey. Codex Stan 12-1981, Rev. 1 (1987), Rev. 2 (2001);Codex Alimen-tarius Commission: Rome, Italy, 2001
30. Jo, C., Kim, J. K., Kang, H. J., Lee, E. Y., Byun, M. W. (2005). Irradiation Effects on the Decontamination of Microorganisms in Honey International Symposium “New Frontier of Irradiated food and Non-Food Products ” 2005, KMUTT, Bangkok, Thailand. 22-23.
31. Cheorun Jo, Jae Kyung Kim, Ho Jin Kang, Eun Young Lee, and Myung Woo Byun*,2005. Irradiation Effects on the Decontamination of Microorganisms in Honey International Symposium “New Frontier of Irradiated food and Non-Food Products” 22-23 September 2005, KMUTT, Bangkok, Thailand
32. Scepankova, H.; Pinto, C.A.; Paula, V.; Estevinho, L.M.; Saraiva, J.A. Conventional and Emergent Technologies for Honey Processing: A Perspective on Microbiological Safety, Bioactivity, and Quality. Comp. Rev. Food Sci. Food Safe. 2021, 20, 5393–5420.
33. Jaya F., Radiati L. E., Estiasih T., Rosyidi D., Junus A. L. M. , Batoro J , Erwan E., Lamerkabel J. S. A., Masyithoh D., , Ustadi U. and Pinandita E. P. (2022). Honey moisture reduction using several thermal methods and their effects on its quality .E3S Web of Conferences 335, 00026 (2022) 202 The 2nd ICESAI.
34. Miao Y, Zhang H, Zhang L, Wu S, Sun Y, Shan Y, Yuan Y. Acrylamide and 5-hydroxymethylfurfural formation in reconstituted potato chips during frying. J Food Sci Technol. 2014 Dec; 51(12):4005-11. doi: 10.1007/s13197-013-0951-9. Epub 2013 Feb 15. PMID: 25477673; PMCID: PMC4252457.
35. Husoy T., Haugen M., Murkovic M., Jobstl D., Stolen L. H., Bjellaas T., Ronningborg C., Glatt H. & Alexander J. (2008). Dietary exposure to 5-hydroxymethylfurfural from Norwegian food and correlations with urine metabolites of short-term exposure. Food and Chemical Toxicology, 46(12), 3697–3702.
36. Karabournioti S, Zervalaki P. (2001). The effects of heating on HMF and invertase in honeys. Apiacta, 36(4): 178- 181.
37. Alizadeh, M.; Khodaei, H.; Abbasi, M.M.; Saleh-Ghadimi, S. Assessing the effect of 5-hydroxymethylfurfuralon on selected components of immune responses in mice immunized with ovalbumin: E_ect of 5-HMF on immune response. J. Sci. Food Agric. 2017, 97, 3979–3984.
38. FAO/WHO.2007. Joint FAO/WHO Food Standards Programme CODEX Committee on Contaminants in Foods. Proposed draft code of practice for the reduction of acrylamide in food Retrieved from.
39. WHO (2015). World Health Orgnaisation expert committee on food additives. Summary and conclusions in: 74th meeting, Rome, 18 – 23.
40. Zarei, M., Fazlara, A., & Tulabifard, N. (2019). Effect of thermal treatment on physicochemical and antioxidant properties of honey. Heliyon, 5, e01894.
41. Dambrosio M. and Marchesihi A. (2002). Research on contamination by heavy metals in honey sample. Attidella Societa Italiana di Scienze Naturali, 123: 342 – 348.