

Impact of γ -Irradiation on Aroma Flavour, Bio-Active Constituents and Quality Attributes of Water Melon Juice

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Abstract

γ -Irradiation is highly effective in inactivating microorganisms and enzymes activity in various foods and it offers a safe alternative method of food decontamination and maintaining quality. Water melon juice was exposed to γ -irradiation of 1, 3 and 5 kGy at room temperature ($25 \pm 1^\circ\text{C}$). With regard to colorimetric parameters the fresh water melon juice was lower than the irradiated samples. There was an improvement in the Hunter color value in irradiated water melon juice compared with fresh samples. The ascorbic acid, other antioxidants measured (DPPH, β -carotene assays, total phenols, flavonoids and antioxidant capacity) showed enhancement on exposure to γ -irradiation. Microbial studies showed reduction in total bacterial counts in irradiated juice at 5 kGy. γ -irradiation improves microbial decontamination and antioxidant activity as well as colour of the water melon juice (WMJ) without any adverse change in bioactive and volatile compounds qualities. These results support the application of γ -irradiation as a measure of food preservation technique for water melon juice that can be explored commercially to benefit both the producers and consumers.

Keywords: Water melon; Juice, γ -irradiation; Bio-active; Aroma; Antioxidant; Colour; Microbiological

Introduction

Consumers are becoming very health conscious, and this attitude is also supported by governments which invest high levels of resources in promoting the consumption of fresh fruits and vegetables. Water melon (*Citrullus lanatus cv.*) is largely consumed as refreshing summer fruit throughout the Mediterranean region especially in Egypt, where its consumption is seasonal and occurs mainly between May and September. Watermelon provides a wide variety of dietary antioxidants such as carotenoids (lycopene and β -carotene), phenols, vitamins (A, B, C and E) and specific amino acids [1], which are thought to exert a protective role in reducing the risk of certain types of cancers, cardiovascular diseases and age-related degenerative pathologies [2]. The health benefits of eating watermelon, as well as its low caloric value, make it a very attractive fruit. Identification and quantification of bioactive compounds and antioxidant properties of many fruits and vegetables are well defined; however, studies on the characterization and quantification of the bioactive compounds and antioxidant activity properties of watermelon are very limited. It has been reported that the levels of the health promoting bioactive compounds and the antioxidant activity of fruits and vegetables are strongly influenced by genotype differences and external factors such as agro-technical processes, environmental conditions, ripening stage, harvest and postharvest manipulations [3].

Several studies have investigated the characteristics of specific compounds thought to be among the most important flavour components of water melon. The flavour of watermelon has been the subject of much debate. The root of the problem is due to the fact that water melon aroma is formed via a dynamic enzyme system, thus it is constantly changing. It is hypothesized that water melon fruits have no aroma until they are cut open. The presence of other enzymes further alters the volatiles initially formed. Two of the most important parameters that determine the quality and consumer preferences of the fruits are their sweetness and aroma properties. The aroma of fruits is ordinarily composed of complex mixtures of volatile compounds

present in the headspace [4,5]. In the fruit juice industry, juice is typically pasteurized by high temperature short time (HTST) pasteurization. Although this method is effective at inactivating microorganisms and enzymes, it can cause detrimental effects on the quality of the juice, which may cause colour change, pigments degrade, separation of particles, and a change in flavor and/or smell of fruit juices [6].

Ma et al., [7] reported that, the flesh of melon is heat sensitive, the sensitive nutrients, color and aromatic profile will be spoiled greatly or off-odour when it was produced with high temperature treatment. It was reported that the total esters decreased 20%, and 6-carbon, 9-carbon alcohol and aldehyde contents decreased significantly after thermal treatment. Meanwhile, new aroma compounds, such as dimethyl disulfide (0.56%), dimethyltrisulfide (0.09%), 2-methylpiperazine (0.04%) and N-ethyl-methylthiamine (0.21%) were produced. The flavor of high temperature-treated melon juice had a cooked off-odor and no green flavor from the sensory [8]. So it is important to find an innovative food process to inactivate the enzyme, microorganism, protect the nutrient and unique flavor. Traditionally, γ -irradiation is known to be effective in reducing microorganisms in foods. Ahn et al. [9] suggested that irradiation of food natural extracts improved their colour without adverse changes in physiological activities. Rajeev et al. [10] reported that radiation could be employed for enhancing selected antioxidant compounds along with reduction in the microbial load in star fruit juices.

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Song et al. [11] concluded that irradiation treatments of carrot and kale juice improve the microbiological safety with maintaining or even enhancing the antioxidative activity. Tiwari et al. [12] revealed that most of the reported applications of irradiation are limited to solid foods and there is scarcity of information regarding treatment of fruit juices. As for the colourimetric parameters a^* and b^* values it has been obtained that both decreased with the irradiation time, indicating with it that the effect that produces the irradiation is contrary to browning process [13].

The purpose of this study was to evaluate the effectiveness of γ -irradiation on the bioactive compounds, aroma volatiles and on the microbiological quality of water melon juice. It is envisaged that the baseline information generated will be useful for the successful implementation of γ -irradiation technology, to benefit health conscious consumers as well as being useful in implementing the hazard analysis and critical control point (HACCP) approach for preservation and shelf life improvement of water melon juice. Also, the objective of the present investigation was to impact the effects of γ -irradiation with different doses on bioactive and volatile compounds, colour, microbiological and quality of fresh WMJ for industrial applications. These watermelon juices are successfully commercialized and currently available in Egypt.

Material and Methods

Materials

Diethyl ether, HPLC-grade ethanol, hexane, chloroform, sodium sulphate anhydrous, linoleic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, Folin-Ciocalteu reagent, catechin, β -carotene, sodium nitrite, sodium carbonate, sodium hydroxide, aluminium chloride, Tween-40 (polyoxyethylene sorbitan monopalmitate), n-alkanes (C7-C21), tert-butyl hydroquinone (TBHQ), butylated hydroxyl toluene (BHT), 2,6-dichloroindophenol Na salt, metaphosphoric acid. Also, linoleic acid and chloroform (HPLC) were purchased from Sigma-Aldrich Chemical Co (St. Louis, MO, USA).

Extraction and processing of watermelon juice

Fresh watermelon (*Citrullus lanatus* cv.) at a commercial maturity purchased from a local supermarket with Agriculture Ministry (Cairo, Egypt). The WMJ was made by blended and filtered on a double layer cheese cloth to remove pulp and stored at 4°C prior to processing. The fruits were ground and then filtered through a double layer cheese cloth to remove pulp, obtain the juice and stored at 4°C prior to processing.

γ -Irradiation process

WMJ samples were packaged in a sanitized brown glass capped bottles (1L) and irradiated in Nuclear Research Centre, Cairo, Egypt by γ - cell, cobalt-60 γ -irradiator at dose rate 1.29744 KGY/hours. The applied doses in this study were 0,1,3 and 5 kGy. The actual doses were within 75.4% of the target dose [14]. The irradiation room temperature was 18°C. The non-irradiated control was placed outside the irradiation chamber to have the same environmental temperature effect with the irradiating sample. The irradiated WMJ samples were transferred to a refrigerator (4°C) directly for analysis.

Physico-chemical analyses

The pH of WMJ samples was measured using a digital pH-meter (HANNA, HI 902 meter, Germany). The percent of Total Soluble Solids (TSS), expressed as oBrix (0-32), was determined with a Hand refractometer (ATAGO, Japan). Titratable acidity and clarity or

turbidity of WMJ was determined according to the method reported by Tung-Sun et al. [15].

Extraction of volatile aroma compounds from water melon

Water melon volatile samples were prepared from 10-12 cubes of tissue. Tissue was rapidly juiced (1 minute) into slurries with a mixer (Moulynex, France), and then the volatiles were isolated by using a dynamic headspace system (DHS). The samples were purged for 1 h with nitrogen gas (grade of N2<99.99) at a flow rate 100 ml/min. The headspace volatiles were swept into cold traps containing diethyl ether and held at -10°C. The volatile extracts were dried over anhydrous sodium sulfate for 1 h., and then reduced to 1 ml by using rotary evaporator (Heidolph, Germany).

Identification of volatiles compounds

Gas chromatography (GC) analysis: GC analysis was performed by using Hewlett-Packard model 5890 equipped with a flame ionization detector (FID). A fused silica capillary column DB-5 (60 m \times 0.32 mm. id.) was used. The oven temperature was maintained initially at 50°C for 5 min., and then programmed from 50 to 250°C at a rate of 4°C/min. Helium was used as the carrier gas, at flow rate of 1 ml/min. The injector and detector temperatures were 220 and 250°C, respectively. Linear retention indices (LRIs) of the separated volatile components were calculated using n-alkanes (C7-C21) as references [16].

Gas chromatographic-mass spectrometric analysis (GC/ MS): The analysis was carried out by using a coupled gas chromatography Varian model. The ionization voltage was 70 eV mass range m/z 39-400 a.m.u. The GC condition was carried out as mentioned above. The isolated peaks were identified by matching with data from the library of mass spectra (National Institute of Standard and Technology) and compared with those of authentic compounds and published data. The quantitative determination was carried out based on peak area integration. Identification of the GC components also was confirmed with NIST mass spectra library data. As well as on comparison of their retention indices either with those of authentic compounds [16].

Determination Total phenolic and flavonoid (TP and TF) contents: Total phenolic and flavonoid compound contents were determined by the Folin-Ciocalteu and Colorimetric aluminium chloride methods respectively [17]. TPC of WMJ at 0, 1, 3 and 5 kGy samples were determined using gallic acid as a standard (0 to 75 mg/ml). WMJ (0.5 ml) was mixed with 0.5 ml distilled water in a test tube, followed by the addition of 4 ml of Folin-Ciocalteu reagent, and allowed to stand for 3 min. Then 2 ml of 7% sodium carbonate solution was added. Each sample was allowed to stand for 60 min at room temperature and measured at 760 nm against the blank on a Shimadzu, Japan spectrophotometer. To correct for any possible interference of ascorbic acid in the total phenolic assay, ascorbic acid content in WMJ samples was determined with the 2,6-dichloroindophenol titrimetric method 967.21 [18].

0.5ml aliquots of the methanolic extract were used for flavonoid determination. Samples were diluted with distilled water to a final volume of 1 mL, and 0.3 mL of 5% NaNO₂ was added. After 5 min, 0.3 mL of 10% AlCl₃ was added and finally 1 mL of 1 M NaOH was added after 6 min. The absorbance was read at 510 nm, using a Shimadzu, Japan spectrophotometer, and flavonoid content was expressed as mg of catechin equivalents per kg of fw (mg CAT/kg fw). All measurements were carried in triplicate.

Lycopene content determination: Lycopene content was measured spectrophotometrically following the method described

by Davis et al., [19] and Oliu et al., [20]. Watermelon juice samples of 0.6 g were weighed and added to 5 mL of 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone, 5 mL of 95% USP-grade ethanol and 10 mL of hexane. The homogenate was centrifuged at 320 g for 15 min at 4°C. After shaking, 3 mL of distilled water was added. The vials were then agitated for 5 min and left at room temperature to allow phase separation. The absorbance of the upper, hexane layer was measured in a 1 cm path length quartz cuvette at 503 nm using a spectrophotometer. Hexane was used as blank.

The lycopene content of each sample was estimated according to the following equation:

$$\text{Lycopene (mg / kg tissue)} = A_{503} \times 31.2 / \text{mass of tissue (g)} \quad (1)$$

where A_{503} is the absorbance of upper hexane layer

Lycopene retention was expressed as the percentage of lycopene compared to that of the untreated samples. All measurements were carried in triplicate.

Vitamin C content determination: Vitamin C was analyzed using the AOAC method 967.21 [18]. The titrant was prepared with 50 mg of 2, 6-dichloroindophenol Na salt and 42 mg of sodium bicarbonate in 50 mL of water. The solution was diluted to 200 mL with distilled water. The extracting solution was prepared with 15 g of metaphosphoric acid and 40 mL of acetic acid and then diluted to 500 mL with distilled water. Solutions were stored in amber bottles at 4°C. A 100 mL aliquot of watermelon juice was added to 100 mL of the extracting solution and then filtered using a No.1 filter paper (Whatman, Maidstone, England). The solution was then titrated with the titrant until the solution turned bright pink for at least 5 s. A standard curve was created using pure ascorbic acid (Sigma Aldrich, St. Louis, MO). Vitamin C retention was calculated using equation (2).

$$\text{Retention (\%)} = \frac{\text{mg ascorbic aci / 100mL juice after treatment}}{\text{mg ascorbic acid / 100mL juice before treatment}} \times 100 \quad (2)$$

Polyphenoloxidase enzyme activity determination: Polyphenoloxidase extraction: untreated and irradiated WMJ samples were homogenised in 100 mL 0.2 M sodium phosphate buffer pH 7 in a laboratory blender. The homogenates were centrifuged at 5000 r.p.m. at 4°C for 10 min. The supernatant was stored at 25°C until assay. Polyphenoloxidase was determined spectrophotometrically at 420 nm using 2 mL catechol 0.1 M as substrate. The reaction was initiated by adding 1 mL of the PPO extract at 25°C. Abs. per 30 s was measured at 420 nm for 3 min in the linear range of a 4054 UV-Visible spectrophotometer, LKB-Biochrom [21]. Polyphenoloxidase units: One unit of PPO enzyme activity was defined as an increase in absorbance unit per minute at 420 nm [21].

Non-enzymatic browning determination: Non-enzymatic browning was measured spectrophotometrically by 4054 - UV/Visible spectrophotometer, (LKB-Biochrom Comp., London, England), as absorbance at 420nm using ethanol as blank according to the method of Birk et al., [22].

Colour determination of watermelon juice: Colour of Egyptian irradiated non-irradiated WMJ was measured using spectro-colourimeter (Tristimulus Colour Machine) with the CIE lab colour scale (International Commission on Illumination) as mentioned by Hunter [23] and Sapers and Douglas [24]. Colour of irradiated watermelon juice samples was measured using a HunterLab colourimeter. Hunter a^* , b^* and L^* parameters were measured with a colour difference meter using a spectro-colourimeter (Tristimulus Colour Machine) with the CIE lab colour scale (Hunter, Lab Scan

XE - Reston VA, USA) in the reflection mode. The instrument was standardized each time with white tile of Hunter Lab Colour Standard (LX No.16379): $X = 72.26$, $Y = 81.94$ and $Z = 88.14$ ($L^* = 92.46$; $a^* = -0.86$; $b^* = -0.16$). The instrument ($65^\circ/0^\circ$ geometry, D25 optical sensor, 10° observer) was calibrated using white and black reference tiles. The colour values were expressed as L^* (lightness or brightness/ darkness), a^* (redness/greenness) and b^* (yellowness/blueness). The Hue (H^*), Chroma (C^*) and Browning Index (BI) was calculated according to the method of Palou et al. [25] as follows:

$$H^* = \tan^{-1}[b^*/a^*] \quad (3)$$

$$C^* = \text{square root of}[a_2^* + b_2^*] \quad (4)$$

$$BI = [100(x - 0.31)]10.72 \quad (5)$$

Where: $X = (a^* + 1.75L^*) / (5.645L^* + a^* - 3.012b^*)$

Total colour difference (TCD) was determined using Eq. (6) which indicates the magnitude of the colour change after treatment. Colour measurements were taken in triplicate.

$$TCD = \sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2} \quad (6)$$

where L_0 is initial value of L^* , a_0 is initial value of a^* , and b_0 is initial value of b^* . L^* , a^* and b^* values were recorded as the mean of triplicate readings.

Antioxidant activity determination: Since many authors recommend evaluating the antioxidant activity of fruit and vegetable by a number of different methods rather than a single method [26], in the present study, the measurement of the WMJ samples at 0, 1, 3 and 5 kGy were performed using two different methods, DPPH and β -carotene assays.

DPPH radical scavenging activity assay: Antioxidant activity was determined by DPPH assay according to [27]. Each extract of different concentrations was used. Four milliliter of 0.1 mM methanol solution of DPPH was added to these tubes and shaken vigorously. The tubes were allowed to stand at room temperature for 30 min. The control was prepared as the same without any extract and MeOH. The changes in the absorbance of the prepared samples were measured at 517 nm. Radical scavenging activity was estimated as the inhibition percentage and was calculated using the following formula,

$$\% \text{Inhibition} = [(AB - AA) / AB] \times 100 \quad (7)$$

Where: AB: absorption of blank sample ($t=0$ min), AA: absorption of sample solution ($t=30$ min). IC50 value is the concentration of the sample required to scavenge 50% of the DPPH free radical. Also, anti-scavenging activity (ASA) equal $1/EC_{50}$.

β -Carotene-linoleate scavenging assay (BCBA): The antioxidant activity of WMJ at 0, 1, 3 and 5 kGy was performed using β -carotene bleaching assay (BCBA) according to Gülçin [28]. β -carotene (0.1 mg) in 0.2 ml of chloroform, 10 mg of linoleic acid and 100 mg of Tween-40 were mixed. The solvent was removed at 40°C under vacuum and the resulting mixture was diluted with 10 ml of water and was mixed well. To this mixture, 20 ml of oxygenated water was added. Four milliliter aliquots mixtures were pipetted into different test tubes containing 500 μ L of each WMJ samples (20, 40, 80 and 100 μ g/ml) and the same concentrations in TBHQ (20, 40, 80 and 100 μ g/ml) in ethanol. TBHQ was used for comparative purposes. All determinations were carried out in triplicate. The antioxidant activity (AA) WMJ at 0, 1, 3 and 5 kGy were evaluated in terms of bleaching of the β -carotene using the following formula,

$$\% \text{Inhibition} = [(AB - AA) / AB] \times 100 \quad (8)$$

Where: AB: absorption of blank sample (t=0 min) and AA: absorption of sample solution (t=60 min). The results were expressed in % basis in preventing bleaching of β -carotene. The EC50 value is the concentration of the sample required to scavenge 50% of the β -carotene free radical. Also, anti-scavenging activity (ASA) equal 1/ EC50.

Microbiological evaluation

Watermelon juice was determined in triplicate for total aerobic bacteria and yeast & moulds according to BAM [29]. Untreated and irradiated samples were serially diluted with 0.1% peptone (DIFCO Labs, Detroit, MI) and pour-plated in duplicate. Total aerobic bacteria counts: one mL aliquot of each sample was plated using a plate count agar medium (Merck KGaA, Darmstadt, Germany), and incubated at 35-37°C for 48 h to counting. Yeast and moulds (Y & M) were determined using malt extract agar (Merck KGaA, Darmstadt, Germany) after incubation at 25°C for 3 days. The number of colonies (total aerobic bacteria or yeast and moulds) that appeared on the plates was counted and expressed as log Colony Forming Unit per gram or log (CFU/ g).

Statistical analysis

Mean values from the three separate experiments or replicate analysis were reported. The obtained results were analyzed statistically using Standard Deviations (n=3) and average as described by Richard and Gouri [30].

Results and Discussion

Effect of γ - irradiation on aroma volatile compounds in fresh and irradiated water melon juice

volatile compounds were identified by comparing their linear retention indices (LRI) and MS fragmented patterns with those of standard compounds and published data, as well as by comparing their mass spectra with the MS library of NIST08 (National Institute of Standard Technology). LRI was calculated using a mixture of n-alkanes, C7–C21, as standards according to the method of Vandendool and Kratz [31]. Compounds without reference volatiles were considered to be tentatively identified. Table 1 showed the areas (as %) obtained from WMJ after exposure to gamma-rays. Twenty-nine aroma compounds were identified in both fresh and irradiated WMJ at 1, 3 and 5 kGy.

Aliphatic esters were the predominant aroma volatiles in both WMJ at 0, 1, 3 and 5 kGy especially, ethyl-2-methylbutanoate in WMJ at 0, 1, 3 and 5 kGy with 60.7, 58.7, 50.96 and 49.01 %, respectively. While, irradiated WMJ which have a sweetly odor was increased by increasing the radiation dose till 3 kGy and dramatically decreased at 5 kGy with 9.7%. Schumacher et al., [32] reported ethyl-2-methylbutanoate and methyl propionate responsible for the fruity character in a number of fruits and are typically found in relatively large quantities in water melon and cantaloupe.

No.	Identified compound	LRI a	Relative area %				IDf
			γ -irradiation dose				
			Control	1kGy	31kGy	51kGy	
1	2-Methylpropanal	610	5.5	4.6	4	3.87	MS, RI
2	Methyl propionate	618	6.67	20.2	20.28	18.7	MS, RI
3	1-Penten-3-ol	678	1.48	ND	ND	3.88	MS, RI
4	Pentanal	695	1.98	0.82	0.45	1.84	MS, RI
5	2-Methyl- 1-butanol	729	1.75	ND	ND	ND	MS, RI
6	(E)-2-pentenal	740	1.38	ND	ND	ND	RI
7	Ethyl- 2-methylpropanoate	746	0.66	ND	ND	ND	RI
8	(E)-2-pentenal	753	1.82	ND	ND	ND	RI
9	2-Methylpropyl acetate	775	3.87	ND	ND	ND	RI
10	Ethyl butanoate	798	0.43	1.39	2.91	1.31	RI
11	Ethyl-2-methylbutanoate	855	60.7	58.7	56.96	40.01	MS, RI
12	Alkyl acetate	973	0.19	ND	ND	0.46	MS, RI
13	Octanal	1000	ND	0.31	ND	6	MS, RI
14	(E)-3-Hexenyl acetate	1017	6	2.6	2	1.82	MS, RI
15	Benzyl alcohol	1027	5.4	4.2	ND	ND	MS, RI
16	2-Ethylhexan-1-ol	1038	ND	1.61	ND	2.6	MS, RI
17	(2E,6Z)-nona-2,6-dienal	1114	ND	1.05	1.6	2.08	MS, RI
18	(E)-2-Octenal	1052	ND	0.6	0.9	3.9	MS, RI
19	Decanal	1209	ND	ND	0.44	1.67	MS, RI
20	2-(4-methylcyclohex-3-en-1-yl) propan-2-ol	1212	ND	ND	0.99	0.34	MS, RI
21	(5E)-6,10-dimethylundeca-5,9-dien-2-one	1365	0.2	ND	0.54	ND	MS, RI
22	butyl octanoate	1389	ND	0.16	0.28	0.34	MS, RI
23	ethyldecanoate	1397	ND	0.13	0.43	ND	RI
24	Octyl acetate	1478	ND	ND	0.23	3	MS, RI
25	2-(Methylthio)ethyl acetate	1491	0.14	0.37	0.42	1.3	MS, RI
26	(Z)-5-Octen-1-yl acetate	1521	0.73	ND	4.37	ND	RI
27	Nonyl acetate	1570	ND	ND	0.14	2.13	MS, RI
28	(Z)-6-dodecene- α -lactone	1670	ND	0.12	ND	ND	MS, RI
29	Pentadecanal	1713	ND	0.92	1	1.6	MS, RI
Total volatiles			98.9	97.78	97.94	96.85	

LRI a: linear retention indices on DB5 column; MS, mass spectra; RI, retention index; IDf: identification methods; and ND: not identified

Table 1: aroma volatile components of WMJ at different γ -irradiation dose (1, 3 and 5 kGy).

	pH	TSS	Total Acidity	TSS/Acidity Ratio	Clarity (660nm)
Control	3.78 ± 0.014	4.2 ± 0.1	0.25 ± 0.01	16.80	2.28 ± 0.046
1KGY	3.71 ± 0.01	4.5 ± 0.0	0.27 ± 0.005	16.67	2.29 ± 0.076
3KGY	3.81 ± 0.024	4.0 ± 0.1	0.25 ± 0.01	16.00	2.35 ± 0.054
5KGY	3.86 ± 0.018	3.8 ± 0.05	0.23 ± 0.01	16.52	2.34 ± 0.061

*Total acidity expressed as citric acid (mg/100g)

Table 2: Effect of γ - irradiation on the physico-chemical attributes of sweet melon juice.

	PPO (unit/ml)	% inhibition
Control	0.203 ± 0.16	0.00
1KGY	0.018 ± 0.24	91.18
3KGY	0.024 ± 0.18	88.18
5KGY	0.025 ± 0.09	87.93

Table 3: Effect of γ - irradiation on polyphenoloxidase (PPO) activity and % inhibition of WMJ.

Our results found the percentages of contributor active compounds which responsible for fruity aroma in WMJ samples such as ethyl-2-methylbutanoate, (E)-3-hexenyl acetate and 2-methylpropanal were decreased with irradiation dose increased and other aroma compounds as 2-Methyl- 1-butanol, ethyl 2-methylpropanoate, (E)-2-pentenal, 2-methylpropyl acetate, benzyl alcohol and 2-methyl-1-butanol were disappeared. The irradiation process after 1Kgy leads to off-flavour for irradiated WMJ, which was likely produced by long chain aldehydes and esters such as octanal, (2E,6Z)-nona-2,6-dienal, (E)-2-octenal, decanal, octyl acetate, pentadecanal and nonyl acetate. The same data were obtained by Zhengfu et al., [33] who stated, Sensory evaluation of cantaloupe juice indicated that the juice had a slight irradiation off-odor after treatment at 1 kGy, and had strong off-odor at 2 kGy and above. Also, Ma [8] indicated that, after irradiation, the new the alcohols (nonyl alcohol) and carbonyl compound (nonylaldehyde, nonanone) with C6 - C9 carbon produced had great changes in their relative contents and varieties, which were probably responsible for the formation of off-flavour in the melon juice.

Alcohols, ketones, aldehydes and esters with C5-C9 carbons, such as 2-Ethylhexan-1-ol, (2E,6Z)-nona-2,6-dienal, octanal, (E)-2-octanol, decanal, 2-(4-methylcyclohex-3-en-1-yl) propan-2-ol, (5E)-6,10-dimethylundeca-5,9-dien-2-one, 2-(Methylthio)ethyl acetate, (Z)-5-Octen-1-yl acetate octyl acetate, nonyl alcohol, pentadecanal were revealed with high concentration in irradiated WMJ at 3 and 5 Kgy. Those alcohols, aldehydes, and ketones with C5-C9 carbons made a relatively great contribution to the formation of irradiation off-flavor in the WMJ according to Guadagni fragrance theory. Wang et al. [34] reported the irradiation reaction influences on the formation of off-odor compounds in melon juice because their fragrance thresholds are lower. However, their degree of contribution to the off-odor is still unknown.

Effect of γ - irradiation on the physico-chemical characteristics of fresh and irradiated water melon juice

The irradiated treated watermelon juice at 1, 3 and 5 KGY had slightly higher pH values than those of un-irradiated-control watermelon juice samples, but the total soluble solids (TSS) and total acidity slightly lower than those of un-irradiated-control watermelon juice samples (Table 2). The pH of all samples was ranged between 3.71-3.86, except untreated sample which was 3.78. These results in agreement the results of Kim et al., [35]. The °Brix values for irradiated treatments ranged from 3.8-4.5. The % titratable acidity in untreated and irradiated of watermelon juices ranged from 0.23 to 0.27 (Table 2).

The TSS / acidity ratio is the major analytical measurement for quality in juice. The larger ratio leads to the better the flavor of the juice [14], it was higher in fresh juice (16.80), 1KGY treated juice (16.67) and 5KGY treated juice (16.52) compared to in 3KGY treated juice (16.00). This indicates that irradiated watermelon juice may be advisable for fresh use. However, it might be processed into acceptable quality juice. Fellers et al. [36] reported that grapefruit juice with TSS/ acidity ratios 7.0 had lower consumer preference scores than juice with TSS / acidity ratios above 11.0. The percent of transmission (%T) or clarity was high in 5KGY treated juice (2.34%) versus 2.29 % in 1KGY treated juice. Which means that the untreated and 1KGY treated watermelon juice was clearer than the 5KGY treated juice. However, the percent of transmission (%T) was very low in control and 1KGY irradiated watermelon juice (2.28 and 2.29) (Table 2).

Effect of γ - irradiation on polyphenoloxidase enzyme activity of fresh and irradiated water melon juice

Enzymatic browning causes colour change of fruits and vegetables, which result from the action of a group of enzymes called polyphenoloxidase (PPO). The PPO activities between irradiated and the non-irradiated samples were determined (Table 3). The PPO activity of the non-irradiated WMJ increased and then decreased for irradiated samples, which resulted from natural senescence of fresh WMJ (Table 3). In this work, irradiation with 1, 3 and 5kGy was effective in inhibiting the PPO activities, thus retarding juice browning and maintaining the fresh quality. From the same (Table 3), it could be seen that the activity of PPO in FWMJ was 0.203 unit/ml but the activity of PPO in 5KGY treated juice (0.025 unit/ml) was slightly higher than the activity of PPO in 1KGY treated juice (0.018 Unit/ml). However, the efficacy of inhibition to PPO activity increased as the irradiation dose from 1 Kgy with 91%.

Effect of γ - irradiation on bioactive compounds (ascorbic acid, flavonoids, Lycopene and total phenols) in fresh water melon juice

The contents of the main bioactive compounds including ascorbic acid, lycopene, flavonoids and polyphenolics in watermelon juices were measured to examine the effects of irradiation. The ascorbic acid content of fresh WMJ was 2.7 mg% after manufacturing, which was not different from the sample irradiated at 1 kGy (Table 4). However, the content of ascorbic acid was reduced when 3 and 5kGy of irradiation were applied. WMJ originally had higher content of ascorbic acid, but irradiation decreased the content of ascorbic acid to 64.81% of the original amount at 5 KGY (Table 4). However, irradiation with 1kGy was able to control microbial, while irradiation with 3kGy caused tissue damage, which also led to more loss of vitamin C. Therefore, irradiation with 1 kGy was the optimal irradiated treatment for maintaining vitamin C of fresh WMJ.

On the other hand, effect of quarantine doses of γ - irradiation on the content of total phenolics and flavonoids of fresh and irradiated WMJ, expressed as mg equivalents of gallic acid and catechin/100 g sample

	Vitamin C (mg/100 ml)	Total Lycopene (mg/100 ml)	Total phenolics content (mg GA/100 g fw)	flavonoid content (mg CAT/100 g fw)
Control	2.70 ± 0.12	10.0 ± 0.1	236.5 ± 3.24	192 ± 2.89
1KGY	2.70 ± 0.15	7.67 ± 0.05	251.3 ± 3.54	205.5 ± 2.94
3KGY	2.08 ± 0.18	8.82 ± 0.05	272.9 ± 2.98	221.4 ± 3.1
5KGY	1.75 ± 0.26	9.91 ± 0.1	295.5 ± 3.84	240.7 ± 3.62

Table 4: Effect of γ - irradiation on bioactive compounds (ascorbic acid, Total lycopene, Total phenolics and flavonoids contents) in WMJ.

Irradiation dose (kGy)	DPPH (%)				BCBA (EC50, $\mu\text{g/ml}$)
	20 $\mu\text{g/ml}$	40 $\mu\text{g/ml}$	80 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$	
Control	5.3 \pm 0.9	10 \pm 1.02	19.4 \pm 2.6	34.4 \pm 3.4	164.36 \pm 2.8
1KGY	7.2 \pm 1.35	12.9 \pm 2.35	24.1 \pm 2.8	31.4 \pm 3.1	158.88 \pm 2.95
3 KGY	12.6 \pm 2.98	17.5 \pm 2.9	28.3 \pm 3.9	40.2 \pm 3.8	150.86 \pm 3.2
5 KGY	14.8 \pm 2.87	25.6 \pm 3.21	32.1 \pm 3.5	42.7 \pm 2.9	140.2 \pm 3.24

Table 5: effect of γ -irradiation on antioxidant activity of WMJ using DPPH and β -carotene assays.

respectively, (Table 4). Both total phenols and flavonoids contents were higher in irradiated samples than in non-irradiated samples. As the irradiation dose increased, higher total phenolic contents (TPC) were found, being this only in 3 and 5 kGy irradiated samples (Table 4). As shown in Table 4, it was found that the WMJ at 5 kGy demonstrated much stronger TPC and TFC with 295.5 mg GAE/ 100g fw and 240 mg CAT/ 100g fw, respectively. While the non-irradiated WMJ revealed the lowest TPC and TFC with 236.5 mg GAE/ 100g fw and 192.2 mg GAE/ 100g fw, respectively. After irradiation, increase in TPC and TFC may be due to the release of phenolic compounds from glycosidic components and the degradation of larger phenolic compounds into smaller ones by γ - irradiation as suggested by Harrison and Were [37] and Fan and Mastovska [38], which correlates with the present study. Previous results showed that increasing in biological activity upon irradiation of biomaterials have been reported by Kim et al., [39]; Jo et al., [40,41]. Topuz and Ozdemir [42] reported an increase in capsaicinoid content in irradiated paprika. Also, Harrison and Were [37] reported enhancement in total phenolic content of irradiated almond skin extracts above 4 kGy and 12.7 kGy. While, Schindler et al. [43] found that the gamma-radiation treatment (2, 4, and 6 kGy) markedly reduced the concentration of the phenolic compounds (p-hydroxybenzaldehyde, p-coumaric acid, ferulic acid, rutin and naringenin) in tomato juice. Irradiation exerts its effects as direct and indirect mechanisms, in case of indirect mechanism, radiolysis of water results in the production of radicals such as hydrated electrons, hydroxyl radicals, and hydrogen atoms [38].

These radicals may break the glycosidic bonds of procyanidin trimer, tetramer, pentamer, hexamer that are present in watermelon juice, leading to the formation of procyanidin monomers which increase the total polyphenolic content in IWMJ. Topuz and Ozdemir [42] reported an increase in capsaicinoid content in irradiated paprika. Soybean samples treated with γ - irradiation at levels ranging from 0.5 to 5 kGy had increased free phenolic content [44]. Also, Harrison and Were [37] reported enhancement in total phenolic content of irradiated almond skin extracts above 4 kGy and 12.7 kGy. However, our results revealed that the total phenol and flavonoid content were increased in irradiated water melon samples. It could be attributed to the release of phenolic compounds, degradation of larger phenolic compounds into smaller ones by γ - irradiation, with a consequent improvement in the extraction yield of the phenolic compounds because of the change in tissue structure by γ - irradiation [45].

The lycopene content of fresh watermelon juice was 10 mg/ 100 ml which is folder to the value reported by Rawson et al., [46]. Lycopene retention in processed irradiated watermelon juice ranged from 77% to 99% compared with untreated samples (Table 4). A decrease of lycopene was observed in samples processed at low dose 1 kGy compared to both untreated samples and other irradiated samples at dose 3 and 5kGy. Similar results were reported by Akanbi and Oludemi [47] and Kaur et al., [48]. This may be due to lycopene destruction by heat and oxidation resulting in fragment products like acetone, methyl-heptenone, laevulinic aldehyde and glyoxal [49,50]. However at lower dose 1 kGy a reduction was observed in lycopene content.

The observed changes in lycopene may be caused by cavitation, which governs various physical, chemical and biological reactions, such as accelerating chemical reactions, increasing diffusion rates, dispersing aggregates or inactivating enzymes and microorganisms [51,52]. In general, sensory evaluation showed that WMJs displayed no significant differences (data not shown). Irradiation may produce off-flavor and off-colour problems [53]. However, WMJ inherently has a very strong flavor; thus, there was no distinctive irradiation-induced off-flavor reported in the sensory analysis when irradiation was applied up to 5kGy.

Effect of γ - irradiation on antioxidant activity content in fresh water melon juice

The effect of γ - irradiation on the antioxidant activity in WMJ at 0, 1, 3 and 5 kGy was performed by DPPH and BCBA assays as shown in Table 5. The perusal of the data revealed that antioxidant activity increased in irradiated WMJ with increasing of irradiation dose. For DPPH radical of antioxidant activity assay, which is based on the transfer of electrons from a donor molecule to the corresponding radical, enhancement of inhibition % (42.7%) in WMJ at 5 k Gy. Ahn et al. [9] found that, immediately after irradiation, the scavenging ability of Chinese cabbage was reduced by 2 kGy of irradiation. Irradiation also resulted in a significant tendency to decreasing DPPH radical scavenging activity of black pepper methanolic extracts [54]. In some case, no significant changes of the radical scavenging abilities were observed in un-irradiated and 5, 10 and 20 kGy-irradiated Chungkookjang and Doenjang [55].

By contract, Jo et al. [56] was found irradiation doses between 10 and 20 kGy of ethanol extract from green tea leaves gave rise to a significant increase in DPPH radical scavenging ability immediately after treatment. Other studies reported that phytic acid exposed to high dose of γ - irradiation (20 kGy) showed significantly higher DPPH radical scavenging capacity than ascorbic acid, while the scavenging effect was not observed in non-irradiated phytic acid [57].

β -Carotene is an important compound and shows strong biological activity. If β -carotene is decomposed before its intake, its biological functions in the body would not be observed. However, its 11 pairs of double bonds are extremely sensitive to free radical mediated oxidation and it is discoloured easily with oxidation of linoleic acid. The data of the present study indicated significant ($p \leq 0.05$) inhibition in the free radical induced β -carotene bleaching mediated through oxidation of linoleic acid, which due to the increasing of γ - irradiation dose.

Irradiation at 1, 3 and 5 kGy of WMJ lead to increase of the inhibition of β -carotene bleaching with EC50, 158.88, 150.86 and 140.2 $\mu\text{g/ml}$, respectively, when compared with un-irradiated WMJ. Our results revealed γ -irradiation did not affect the antioxidant activity of WMJ samples. Variyar et al. [44] reported that γ -irradiation is capable of breaking the glycosidic bonds of polyphenols, thereby releasing soluble phenols of low molecular weight, leading to an increase of antioxidant rich phenolics responsible for higher antioxidant activities.

	L*	a*	b*	E	C*	H*	BI	TCD	420 nm	NEB 420 nm
Control	87.37	-6.5	-2.75	6.9	7.06	22.93	-15.27	-	79.09	0.013 ± 0.005
1 KGY	87.38	-6.56	-2.87	7.09	7.16	23.63	-15.59	0.47	79.03	0.004 ± 0.01
3 KGY	87.35	-7.35	-3.05	7.78	7.96	22.54	-17.14	1.08	79.39	0.006 ± 0.01
5 KGY	90.46	-0.87	-0.19	0.09	0.89	12.32	-1.62	3.35	81.25	0.005 ± 0.005

Table 6: Effect of γ -irradiation on colour characteristics of sweet melon juice.

Sample	Viable cell counts (CFU/mL)	
	Total aerobic bacteria	Yeast & Molds
Control	460 ± 0.13 ^a	ND ^b
1KGY	280 ± 0.09	ND
3KGY	150 ± 0.15	ND
5KGY	45 ± 0.11	ND

a; Means ± standard deviation (n = 3) and b ;Viable colony was not detected above the detection limit of 102 CFU/mL.

Table 7: Effect of γ - irradiation on the microbial population (total aerobic bacteria, and yeast and moulds) of WMJ.

Effect of γ - irradiation on colour characteristics and non-enzymatic browning (A420 nm) in fresh water melon juice

The surface colour of watermelon juice was measured with a colour difference meter, using the Hunter Lab colour scale. Instrumental colour was monitored and modeled as it is a key quality index influencing consumer acceptance of water melon juice products. Colour values for fresh untreated water melon juice were 87.37, -6.5 – 2.75 for L*, a*, b*, respectively. Under all irradiated tested conditions, 1KGY showed much higher efficient values based on a* values than A420 measurements, whereas the other doses behaved an opposite trend. The Hunter colour L*-value (brightness), a*-value (redness) and b*-value (yellowness) were found to be closely not changes by irradiation in both fresh and 1KGY irradiated WMJ, but increased by irradiation in 5 Kgy irradiated WMJ which indicate WMJ revealed a bright coloration (Table 6).

For all irradiated tested samples, the increase in the doses to 3KGY of irradiated juices revealed an increase in inhibition efficient of browning. Such a trend is in agreement with previous studies of Janovitz-Klapp et al. [58] and Ozoglu and Bayindirh [59]. The colour of the Curcuma aromatic an extract was improved by γ - irradiation [39]. Our study is in coherence with the previous findings by Ju et al., [60] and Jo et al., [56], which showed a significant improvement in the colour in fresh and irradiated tamarind juice and green tea.

Table 6 shows the inhibitory effect of various doses irradiated juice based on measurements at their maximum doses for water melon juice irradiated in the following order: 1 > 3 > 5 KGY. It is obvious that irradiated treatments of juices was very slightly increased the development of red colour a* value as non-enzymatic browning. In addition, the Hunter colour value of 1KGY treatment in juice was lower than that of 3 and 5KGY irradiated samples. These results indicated that browning (redness) increased in untreated samples than in irradiated samples for juice. However, PPO enzyme activity was higher in untreated samples than in irradiated samples, as seen in Table 3. According to our results, the main colour change in untreated and irradiated juice was because of increase in the BI and a* value, which were in high correlation to browning measurement.

These colour changes may be due to independent or interaction effects of the extrinsic control variables of irradiation doses KGY level. Differences in visual colour can be classified based on total colour difference (TCD). Choi et al. [61] reported that TCD values correspond to noticeable differences in the visual perception of products. In the present study TCD was observed to be very distinct for the maximum treatment conditions investigated. It should be noted that changes

in colour values may be regarded as a negative sensory impact of processing. A correlation between different parameters investigated is shown in Table 6. However, the results from Table 6 showed that the TCD increased by increasing irradiation dose from 0.47 at 1 KGY, 1.08 at 3KGY and 3.35 at 5KGY of irradiated WMJ. Other colour parameters, such as hue angle (H*) and chroma (C*), also indicated that heat caused a slight colour change. The samples of untreated juices had a BI higher than in the case of irradiated juice samples. But, BI values in 1KGY samples were lower than in case of 3 and 5kGy samples, as seen in Table 6. These results are in good agreement with those of Hayta [62] and Ozoglu and Bayindirh [59].

It was generally found that irradiated process improved the colour of WMJ (Table 6). However, untreated samples had higher increase in colour as optical density (A420nm) and non-enzymatic browning (420nm) compared with the irradiated juice samples. The decrease in colour (browning as A420nm) and non-enzymatic browning (420nm) could be attributed to the reaction that occurred between amino groups and active carbonyl groups (Maillard reaction) after irradiated process. However, 1KGY has been shown to be effective in preventing browning by combining with carbonyl groups, as seen in Table 6. From the above mentioned results, it could be concluded that irradiated juice have the best colour values (a* and BI) and lower non-enzymatic browning as compared with untreated samples, as seen in Table 6. The most effective irradiated process for the inhibition of oxidative enzymes (PPO), good colour characteristics and lower non-enzymatic browning in WMJ was 1KGY.

Effect of γ - irradiation on microbiological evaluation in fresh water melon juice

Water melon is the most widely used raw materials in the fresh juice industry. The total aerobic bacteria (TAB) number in fresh WMJ was 460 CFU/mL (Table 7), which was already higher than the legal standard (less than 105 CFU/mL) [63]. During the processing are used to decontaminate the microorganisms, but if they are poorly managed, the original contamination can remain very high even in freshly made juice [64]. From the results, it was thought that sterilization process is necessary to ensure minimal adverse changes in quality. Table 7 shows the total counts of aerobic bacteria, yeast and mould, of non-irradiated and γ - irradiated WMJ samples. The microbiological tests were carried out immediately after the irradiation. The viable counts of the microorganisms in the non-irradiated samples were high (Table 7).

Irradiation effects on watermelon juice were observed in the populations of the total aerobic, yeast and mold (Table 7). Differences between fresh and irradiated samples were observed, with the

exception of the 5 kGy samples which values decreased (45 CFU/ml). The initial populations of the total aerobic bacteria in watermelon juice were reduced by increasing γ - irradiation dose at 1 kGy (280 CFU/ml), 3KGY (150 CFU/ml) and 5KGY (45 CFU/ml) or above. Approximately 100–200 CFU/ml were reduced by irradiation, while the non-irradiated control showed the highest population (460 CFU/ml) in the samples. In fresh watermelon juice, yeast and mold were not detected (<100 CFU/ml) in all samples. No detectable yeast and molds cells were found at untreated and irradiated WMJ.

The same microbiological result was also reported in fruit juices [11,60]. γ - Irradiation was shown to be efficient in the reduction of microbial contamination in WMJ, even at a low dose of 1 kGy. However, there could be different microbiological populations in WMJ from the different processing conditions such as the cutting tools, temperature and the qualities of raw materials, these many irradiation doses were employed in this study. It was well known that various environmental factors affected the sensitivity of microorganisms to radiation. At a higher availability of water (aw), the microorganisms are more sensitive to radiation because of the more free radicals generated by the irradiation. It has been reported that the water contents of WMJ were higher than 0.80 g water/1 g juice [65]. Therefore, the radio sensitivity of microorganisms in the WMJ was high.

Farkas et al. [66] reported that ionizing radiation at 1kGy reduced loads of bacteria, improved microbiological shelf life, and extended sensory quality of pre-cut peppers and carrots. Improvement in microbiological quality by radiation processing was evident by the dose-dependent reduction in total viable count, yeast, and mold. The same microbiological result was also reported in irradiated tamarind juices [60]. Chervin and Boisseau [67] reported that the growth of aerobic and lactic microflora on shredded carrots was inhibited by irradiation at 2 kGy and chlorination, and the sensory analysis panelists preferred the irradiated vegetables. Prakash et al. [68] also reported that irradiation at 0.5 kGy can reduce the microbial counts of diced tomatoes substantially to improve the microbial shelf life without any adverse effects on the sensory qualities.

Conclusion

This study confirms that irradiation was an effective method for sterilizing fresh watermelon juice without compromising sensory properties, which cannot be subjected to heat pasteurization due to changes in the bioactivities of the products. Microbiological assay of the watermelon juice showed better quality after γ - irradiation. γ - irradiation of watermelon juice at 1, 3 and 5kGy also resulted in increase or maintenance of the antioxidants which is essential to preserve them for transportation. The colour in both the fresh and irradiated watermelon juice changed to bright colour, which gives the possibility to use in food industrial application. Irradiated watermelon juice was found to maintain its bio active and volatile compounds until 3 kGy irradiation dose. It can be found that the best preservation effect appeared to be 1 kGy irradiation, in which the microbial population could be lowered and nutrition quality of WMJ was maintained well after irradiation processing. Furthermore, from a technological point of view, it would be conceivable to use this irradiation in processed WMJ provided that their safety is assessed and their commercial feasibility is demonstrated. These results support the application of γ - irradiation as a measure of food preservation technique for water melon juice that can be explored commercially to benefit both the producers and consumers.

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