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Effect of Gamma Irradiated Chitosan and Green Tea on Pod Quality of Fresh-Cut Green Bean under Cold Storage Conditions

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Abstract: This investigation was conducted for two successive seasons (2016 and 2017) to evaluate the influence of irradiated chitosan and green tea with 0, 5 and 10 K Gy of gamma irradiation on the quality of fresh-cut green beans under cold storage conditions at 5°C and 90-95% relative humidity for 20 days. Our results indicated that weight loss, decay, polyphenol oxidase activity, microbial load and yellowness (b* value) were increased by increasing storage duration, while general appearance, lightness (L*) and greenness (a* values) were decreased. Chitosan and green tea irradiated with 10K Gy of gamma irradiation showed the most effective treatments for maintaining physical characters, lightness and greenness of fresh-cut green beans, reducing the activity of polyphenol oxidase activity, minimizing the microbial load and yellowness of fresh-cut green beans during the first five days of storage period.

Key words: Fresh-cut green beans · Chitosan · Green tea · Gamma irradiation

INTRODUCTION

The consumption of fresh-cut vegetables and fruits increases during the last decate due to their convenience, ease of consumption, freshness and provision of processing time [1]. Fresh cut products involve exfoliation, cutting and ripping before packing and storing [2]. The International Fresh-cut Produce Association (IFPA) defines fresh-cut products as fruits or vegetables that have been trimmed and/or peeled and/or cut into 100% usable product that is bagged or pre-packaged to offer consumers high nutrition, convenience and flavor while still maintaining its freshness [3]. Cutting of green beans accelerates respiration rate more than in intact beans. Therefore, the quality of fresh-cut green beans decreases rapidly due to exposure of inner flesh to environment, thus they have much shorter shelf life 3days at 5°C [4]. It is necessary to use some post-harvest treatments (irradiation, chitosan and green tea) in conjuction with low temperature to extend shelf life of fresh-cut green beans.

In the last period, consumers have become more worried of the insertion of artificial chemical additives in food industry such as artificial preservatives, color, flavor and nutritional value [5].

Green tea (*Camellia sinensis* L.) is one of the most popular drinks in the world and plays an important role in resisting many diseases such as chemopreventive, anticarcinogenic, antiatherogenic, antioxidant and antimicrobial activities [6-9]. Some researchers reported that some varieties of tea or tea extract like Oolong, jasmine and black had characteristics of food-borne pathogen resistance e.g. *Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus* and *Campylobacter jejuni* [6, 10 -12].

Some researchers found that the lowest concentrations of green tea extract (0.25g $100ml^{-1}$) preserve some chemical constituents such as ascorbic acid and carotenoids of fresh-cut lettuce [13].

The important role of green tea as an antimicrobial, is refer to catechins, Hydroxyl moieties at 3, 4 and 5 on the B ring in the catechin molecules [14, 15].

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Another important role played by green tea as an antioxidant is affined to its content of polyphenolic compounds such as epicatechin gallate , epicatechin and catechin [16]. The main role of green tea as an antioxidant is the inhibition of lipid peroxidation through the interaction with free radicals, also scavenging superoxide radical , singlet oxygen , hydroxyl , nitric oxide , nitrogen dioxide and peroxynitrite [17-20].

Shrimps, crabs, insects and invertebrates are the main source of chitin which is transformed to chitosan through deacetylation in concentrated alkali solution [21]. Chitosan is an useful and safe polymer which can be used in food preservation as a result of its antimicrobial and antioxidant properties [22-25]. The mechanism of chitosan as an antimicrobial agent is concluded in three points:(1) the attraction between chitosan molecule positive charge and negative charge of microbial cell membrane [26, 27]. (2) Inhibition of toxins production and microbial development and (3) Stopping mRNA synthesis [28]. A lot of researchers have been shown that chitosan enhance the visual characters and increase the shelf life of different fruits like papaya, strawberry and mango [29-31]. As it was observed also that chitosan retard fruit ripening through change in carbon biological processes, holding the sugars levels. Also retarding fruit maturation [32]. Increase the shelf life of fresh cut mushroom by delaying the enzymes activity and decreasing the microbial load during storage [33].

Gamma irradiation plays an important role in changing both physical and chemical properties of some polymeric materials such as highly molecular weight chitosan [34], which helps in enhancing the solubility of chitosan via decreasing its molecular weight through breaking the polymeric chain which enhance its effectiveness against food borne pathogens and antioxidant properties [35-38].

The penetration of gamma radiation in biological cell increases the formation of free radicals whereas a reaction carried out between gamma rays and water molecule known as hydrolysis reaction [39, 40]. The main factor that affects this reaction is that the irradiated material contains a proper amount of water. These free radicals react with several components in irradiated food such as, enzymes, vitamins and antioxidant compounds [41, 42]. The World Health Organization in 1994 confirmed that dry food like wheat flour , dehydrated vegetables and nuts have significant amount of water almost (13%, 10% and 5%) respectively.

Thus, the aim of this study was to evaluate the effect of gamma irradiation on chitosan and green tea properties to extend the shelf life of fresh-cut green bean under cold storage conditions.

MATERIALS AND METHODS

Raw Material: Two experiments were carried out on green bean (*Phaseolus vulgaris* L.) cv. Paulista. Pods were obtained, from private farm at El Sharkia Governorate during the two successive seasons of 2016-2017 and 2017-2018 respectively. Green bean pods were harvested at commercial maturity (i.e., bright green, tender fleshy and with small green immature seeds) at the 15th and 17th of December in the first and second seasons, respectively. The precooling process was carried out and then green pods were transferred to the laboratory in Horticulture Research Institute, Giza governorate under cooling. Pods without any visual defects were washed with tap water to eliminate soil residues, prepared according to safety statements and recommendations [43, 44] and then air dried .All cutting utensils used (Knife and cutting board) were washed with soap and water and rinsed with 0.1% H_2O_2 solution prior to use .Pod were cut from both ends, using a sharp stainless steel knife. The fresh-cut green beans were washed in tap water, dipped in chlorinated water (20 mL l^{-1}) for 1 min and washed with distilled water, then air-dried for 10 min at room temperature.

Chitosan Coating, Green Tea Solution Preparation and Packaging Procedure: High molecular weight chitosan 360KDa and 90% deacetylation was obtained from Sigma-Aldrich and green tea leaves were purchased from local market. Chitosan and green tea were exposed to gamma irradiation at 5 and 10 KGy. Gamma irradiation process was carried out at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt, using Indian gamma cell which contains cobalt 60 as a gamma radiation source.

Both 1% (w/v) irradiated and un-irradiated chitosan solution was dissolved in aqueous solution of 0.5% v/v acetic acid and stirred at 45°C and pH of 5.2 according to Petriccione *et al*. [45]. 20% Green tea water extract solution was prepared by mixing irradiated or un-irradiated ground tea powder in distilled water 200 g of green tea in 1 liter controlled at 90°C in an Erlenmeyer flask and stirred in a water bath shaking incubator at 100 oscillation /min for 10 min. The water extract of green tea was filtered through filter paper No.1 according to Siripatrawan and Noipha [46].

Similar green pods in size were dipped through the cutting end in distilled water as control treatment, 1%, 20 % of irradiated and non-irradiated chitosan (chi.) and green tea (G.T.) respectively then dried. Each treatment consists of 200 g of fresh- cut green beans packed in foam plat wrapped with stretch film and stored at 5°C, 90-95%

relative humidity and stored for 20 days .Measurements were examined immediately after treatment and at 5 days intervals for the following properties.

Weight Loss Percentage: It was estimated according to the following equation: Weight loss% = [(Initial weight - weight of fruits at sampling date)/Initial weight of fruits] x 100.

Decay: It was determined as score system of 1= none, 2= slight, 3= moderate, 4= moderately severe, 5= severe.

General Appearance: General appearance was determined as score system of excellent> 9, good> 7 to 8.9, fair> 5 to 6.9, poor> 3 to 4.9 and unassailable> 2.9. The scale depends on morphological defects such as shriveling, fresh appearance, color change of pods and decay. Pods rating (5) or below are considered unmarketable [47, 48].

Polyphenol Oxidase (PPO): PPO was extracted by homogenizing fruit samples with 5 fold of their weight sodium phosphate buffer (0.1 m, pH 6.5) containing 30 mM sodium ascorbate and 0.4 M sucrose at 25°C. The fruit homogenate was centrifuged at 10000 g for 15 min. Supernatant was collected and stored at 4°C. Catechol was dissolved in the phosphate buffer (10 mM) then a volume of 3 ml was mixed with 1.0 enzyme extract. The increment of absorption at 495 nm was spectrophotometrically recorded. The increase in absorbance of 0.01 per minute at 495 nm at the specified condition was defined as one unit of PPO activity. The results were expressed aspercentage of the activity of the respective zero experiment [49].

Microbiological Load: Each sample was prepared by homogenizing 10 gm of sliced green bean with 100 ml sterile 0.1 peptone water for 2 min. Dilution by 0.1 pepton water was made as needed in Plate Count Agar (PCA) and incubated for 24 hr at 37 °C for the determination of mesophilic aerobic microorganisms. Viable counts were determined by counting the number of colonies and reported as colony forming units per gram, CFU / g [50].

Color Measurement: Color measurements (L*, a* and b* values) were performed using a Chroma meter CR-400 (Konica Minolta Inc. Osaka, Japan) with illuminant D65 with 8 mm aperture. The instrument was calibrated with a white reference tile (L*=97.52, a*=–5.06, b*=3.57) prior to measurements. The L* (0=black, 100=white), a* (+red, –green) and b* (+yellow, –blue) color coordinates were determined according to the CIELAB coordinate color space system.

Statistical Analysis: This experiment was arranged in a completely randomized design (CRD), all data were subjected to statistical analysis according to the procedures reported by Snedecor and Cochran [51] and means were compared by Duncan's multiple range tests ($P \leq 0.05$) [52].

RESULTS AND DISCUSSION

Weight Loss Percent: Data presented in Table (1) show that there was significant differences among different treatments concerning weight loss percentage; It was observed that the lowest weight loss percent was obtained from fresh-cut green beans dipped in 1% irradiated chitosan with 10 K Gy followed by 20% of irradiated green tea with the same dose during the two successive seasons. This result is in agreement with that obtained by Inkha *et al*. [53] who stated that dipping fresh cut strawberries in 10 % chitosan reduces the weight loss percentage.

As for the effect of storage period and its impact on weight loss percent, a remarkable increase in weight loss was obtained by increasing storage period in both seasons. The main reason which led to the increase in fresh-cut green beans weight loss during storage period is the transpiration and respiration which increase fresh-cut green beans water loss [54].

Respecting the interaction between different post-harvest treatments, storage period and their effect on weight loss percentage, results showed that weight loss percentage increased in untreated fresh-cut green beans during storage period and reach its maximum lost at 20 days after storage. Less weight loss was recorded from fresh-cut green beans dipped in 1% irradiated chitosan with 10 K Gy of gamma irradiation followed by 20% green tea irradiated with 10 K Gy and stored for 5 days than other treatments in both seasons. Chitosan treatment plays an important role for controlling weight loss by forming a thin wrapper around the fruit or cutting part which regulates gas exchange and water loss through transpiration [55]. The effectiveness of chitosan for decreasing fruit weight loss was observed in some crops such as papaya and strawberry [56, 30].

Decay: As shown in Table (1) the lowest decay was marked in fresh-cut green beans dipped in 1% irradiated chitosan with 10 K Gy. Respecting storage period and its influence on decay, it was observed that the increase in storage period till 20 days was linked by a gradual increase in decay.

Concerning the influence of different post-harvest treatments and their effect on decay of fresh-cut green beans through storage periods, the results indicate that fresh-cut green beans dipped in 1% irradiated chitosan with 10 K Gy and stored for 15 and 20 days scored the lowest decay, followed by dipping fresh-cut green beans in the solution of 20% irradiated green tea with 10 K Gy and stored for 10 days. The effect of chitosan on delaying decay score might be attributed to its role as an obstacle against water and oxygen losing which inhibit the physiological and biochemical changes of fruit during storage, in addition diminish fruit damage through inhibiting the growth of pathogenic fungi as a result of its antimicrobial effect [57].

On the other hand, green tea ability in decreasing fresh-cut green beans decay might be attributed to its important role as an antimicrobial agent as a result of its content of catechins, which affect food borne pathogens [58].

General Appearance: As for the effect of different treatments and their effect on general appearance, data in Table (1) revealed that fresh-cut green beans dipped in 1% irradiated chitosan with 10 K Gy, 5 K Gy and 20% irradiated green tea with 10 K Gy in the first season and with 1% irradiated chitosan with 10 KGy in the second season scored the highest general appearance comparing with other treatments.

Concerning storage period and its impact on general appearance, data show that there was a relative decrease in general appearance of fresh-cut green beans accompanied by the increase in storage period and reaches its maximum depression at 20 days after storage. Similar results were reported by Kasimi and Kasimi [4] on fresh-cut snap beans.

Regarding the interaction between storage period and different treatments, it was observed that all treatments during the first five days of storage maintain the general appearance of fresh-cut green beans in both seasons. On the other hand, it was found that 20 % green tea irradiated with 5 K Gy and 1% un-irradiated chitosan gave higher general appearance than other treatments till 10 days of storage. On the other side, it was remarked that both 20% irradiated green tea with 10 K Gy and 1% irradiated chitosan with 5 K Gy preserve the general appearance of fresh-cut green beans till 15 days of storage period. In the second season, fresh-cut green beans dipped in 20 % irradiated green tea with10 K Gy or in 1% irradiated chitosan with 5and 10 K Gy gave the highest general appearance during 10 days of storage period. Finally, it was found that dipping fresh-cut green beans in 1 % irradiated chitosan with 10 K Gy scored the highest general appearance during 15 days of storage period compared with other treatments during the same storage period. The effect of chitosan on maintaining the general appearance might be referred to its ability on decreasing fruit water loss [55], reducing the expansion of food borne pathogens during storage period [57]. As for green tea, the presence of chatechin has an important role in diminishing the microbial load during storage, also in decreasing the browning of fruit through its ability in decreasing the activity of polyphenol oxidase [14]. All these previous factors had a positive effect on maintaining fruit general appearance during cold storage.

Polyphenol Oxidase Activity: Data presented in Table (2) show the inhibitory effect of non-irradiated, irradiated chitosan, green tea and their impact on polyphenol oxidase activity of fresh cut green bean pods under cold storage. It could be concluded that dipping fresh-cut green beans in 1% irradiated chitosan with 10 K Gy followed by 20% irradiated green tea with the same dose of gamma rays inhibit the activity of polyphenol oxidase. On the other hand, the increase in storage period led to an increase in the activity of polyphenol oxidase. From the other side, it was observed that 1% irradiated chitosan with 10 K Gy or 20% irradiated green tea with the same dose respectively inhibit the activity of polyphenol oxidase during storage period especially during the first five days. Although there was an increase in polyphenol oxidase percentage through the increase of storage period with all treatments, but both irradiated chitosan and green tea scored the lowest percentage during the first season. In the second one, the same trend was observed but there was no significant difference between both 1% irradiated chitosan and 20 % irradiated green tea with 5 or 10K Gy. The inhibitory effect of chitosan on polyphenol oxidase activity might be related to its role in the extraction of metal ions at enzyme active sites through chelating mechanism which makes polyphenol oxidase inactive enzyme [59]. As for the effect of green tea as an inhibitor of polyphenol oxidase activity , this result might be attributed to catechins, the principal polyphenol compounds in green tea, which scavenging free radicals, overcome hydroxyl radicals (OH^-) or super oxide anion radicals (O^-_2) [60-62].

Microbial Load: As shown in Table (2), results indicate that the most effective antimicrobial treatment which diminish the microbial load was dipping fresh-cut green beans in 1% irradiated chitosan with 10 K Gy followed by dipping fresh-cut green beans in the solution of 20 %

Table 1: Effect of irradiated chitosan and green tea on weight loss %, decay and general appearance of fresh-cut green beans under cold storage conditions

		First season						Second season					
		Storage period (Days)											
Characters	Treatments	0	5	10	15	20	Mean	0	5	10	15	20	Mean
Weight loss %	Control	-	2.73s	5.10m	8.16d	11.54a	6.89A	-	2.95r	5.44m	8.52d	11.83a	7.19A
	20%G.T 0 KGY	-	1.93u	3.73o	6.53h	8.91b	5.28B	-	2.25t	3.93o	6.83h	9.15b	5.54B
	20%G.T.5KGy	-	1.53w	3.12q	6.03j	8.14d	4.70D	-	1.84v	3.45p	6.35j	8.45d	5.02D
	20%G.T.10KGy	-	0.94y	2.41t	5.22l	7.23f	3.95F	-	1.23x	2.73s	5.53l	7.62f	4.28F
	1% Chi.0KGy	-	1.83v	3.51p	6.33i	8.54c	5.05C	-	2.16u	3.85o	6.62i	8.81c	5.36C
	1% Chi.5KGy	-	1.14x	2.84r	5.66k	7.71e	4.34E	-	1.47w	3.17q	5.96k	8.02e	4.66E
	1%Chi.10KGy	-	0.63z	1.83v	4.43n	6.73g	3.40G	-	1.03y	2.13u	4.83n	7.05g	3.76G
	Mean	-	1.53D	3.22C	6.05B	8.40A	----	-	1.85D	3.53C	6.38B	8.70A	----
Decay	Control	1.00g	2.00d-g	3.00bd	3.67ab	4.33a	2.80A	1.00f	1.67d-f	3.00b	4.00a	4.67a	2.87A
	20%G.T 0 KGY	1.00g	1.67e-g	2.67b-e	3.00b-d	3.67ab	2.40AB	1.00f	1.33e-f	2.33b-d	3.00b	4.00a	2.33B
	20%G.T.5KGy	1.00g	1.33fg	2.00d-g	2.33c-f	3.00b-d	1.93C	1.00f	1.00f	2.00c-e	2.33b-d	3.00b	1.87CD
	20%G.T.10KGy	1.00g	1.00g	1.67e-g	2.00d-g	2.67b-e	1.67CD	1.00f	1.00f	1.67d-f	2.00c-e	2.67bc	1.67D
	1% Chi.0KGy	1.00g	1.33fg	2.00d-g	2.67b-e	3.33a-c	2.07BC	1.00f	1.00f	2.00c-e	3.00b	3.00b	2.00C
	1% Chi.5KGy	1.00g	1.00g	1.67e-g	2.00d-g	2.67b-e	1.67CD	1.00f	1.00f	2.00c-e	2.00c-e	2.67bc	1.73CD
	1%Chi.10KGy	1.00g	1.00g	1.33fg	1.67e-g	2.00d-g	1.40D	1.00f	1.00f	1.00f	1.33e-f	2.00c-e	1.27E
	Mean	1.00E	1.33D	2.05C	2.48B	3.10A	----	1.00D	1.14D	2.00C	2.52B	3.14A	----
General appearance	Control	9.00a	7.00a-d	5.00d-f	3.67fg	2.33g	5.40D	9.00a	7.67a-c	5.00e	3.00f	1.67f	5.27D
	20%G.T 0 KGY	9.00a	7.67a-c	6.33b-e	5.00d-f	3.67fg	6.33C	9.00a	8.33a	6.33c-e	5.00e	3.00f	6.33C
	20%G.T.5KGy	9.00a	8.33ab	7.00a-d	6.33b-e	5.00d-f	7.13BC	9.00a	9.00a	7.00b-d	6.33c-e	5.00e	7.27B
	20%G.T.10KGy	9.00a	9.00a	7.67a-c	7.00a-d	5.67c-f	7.67AB	9.00a	9.00a	7.67a-c	7.00b-d	5.67de	7.67B
	1% Chi.0KGy	9.00a	8.33ab	7.00a-d	5.67c-f	4.33e-g	6.87BC	9.00a	9.00a	7.00b-d	5.00e	5.00e	7.00BC
	1% Chi.5KGy	9.00a	9.00a	7.67a-c	7.00a-d	5.67c-f	7.67AB	9.00a	9.00a	7.67a-c	7.00b-d	5.67de	7.67B
	1%Chi.10KGy	9.00a	9.00a	8.33ab	7.67a-c	7.00a-d	8.20A	9.00a	9.00a	9.00a	8.33ab	7.00b-d	8.47A
	Mean	9.00A	8.33B	7.00C	6.05D	4.81E	----	9.00A	8.71A	7.10B	5.95C	4.71D	----

Means followed by different letters are significantly different at $P \leq 0.05$ level; Duncan´s multiple range test

irradiated green tea with the same dose . Similar result was obtained by Ghasemnezhad *et al.* [63] who found that treated arils of pomegranate with 0.25 and 1 % of chitosan decrease the total bacterial counts and Matan *et al.* [64] who concluded that green tea play an important role in controlling bacterial growth in stored fresh cut dragon fruit.

On the other hand, there was a linear relation between the microbial load and storage period, where it was found that the increase in storage period was accompanied by an increase in microbial load.

Concerning the interaction among different treatments and storage period, there was a significant difference between them, where it was noticed that all treatments affected the microbial load and decrease it until the first 5 days compared with control specially fresh-cut green beans dipped in the solution of 1% irradiated chitosan with 10 K Gy. Then after this storage period a remarkable increase in microbial load was observed with the increase in storage period in both seasons. Although there were a lot of conflicts around the influential role of chitosan as an anti-microbial, but it is well known that it has the ability to destroy the cell wall, causing disintegration and alteration of membrane qualities which elevate the permeability of cells and led to the infiltration of intracellular components which eventually leads to cell death [65-66]. Also it might be referred to the low content of oxygen level formed by a fence film of chitosan, low inner oxygen levels is one of the factors which affect fungal development and biological processes [67]. On the other hand, another factor which reduces disease incidence of fruit treated with chitosan is the reduction of phenylalanine ammonia-lyase, chitinase and β-1, 3-glucanase activities [68]. As for green tea effect on decreasing microbial load, it is considered one of the most important sources of polyphenolic compounds such as catechin which inhibit the activity of some pathogens [69].

L* value: Data presented in Table (3) show the effect of different treatments on fresh-cut green beans lightness (L* value), it was observed that both 1% irradiated chitosan with 10 K Gy followed by 20% green tea irradiated with the same dose preserve the L* value (lightness) of fresh-cut green beans. This result might be attributed to the effect of chitosan in delaying fruit senescence through its effect on metabolic activities and inhibit chlorophyll degradation through the chlorophyllase enzyme [70].

Table 2: Effect of irradiated chitosan and green tea on polyphenol oxidase activity % and microbial load CFU/g of fresh-cut green beans under cold storage conditions

		First season						Second season					
		Storage period (Days)											
Characters	Treatments	0	5	10	15	20	Mean	0	5	10	15	20	Mean
	Control	72.21y	75.83r	80.14l	86.43f	93.23a	81.57A	73.56f	76.35d-f	81.14c	84.91b	95.12a	82.22A
	20%G.T 0 KGY	72.21y	75.34s	79.73m	86.03g	92.83b	81.23B	73.56f	75.92ef	80.84c	87.52b	94.66a	82.50A
PPO	20%G.T.5KGy	72.21y	74.84u	79.14n	85.41h	92.22c	80.76D	73.56f	75.13f	80.02c	86.66b	93.92a	81.86AB
	20%G.T.10KGy	72.21y	74.05w	78.03p	84.42j	91.34e	80.01F	73.56f	74.33f	79.22cd	85.52b	93.05a	81.13BC
	1% Chi.0KGy	72.21y	75.11t	79.54m	85.82g	92.62b	81.06C	73.56f	75.52f	80.44c	86.48b	94.33a	82.07AB
	1% Chi.5KGy	72.21y	74.53v	78.62o	85.07i	91.83d	80.45E	73.56f	74.81f	79.63c	86.12b	93.62a	81.55A-C
	1%Chi.10KGy	72.21y	73.62x	77.35q	83.80k	91.83d	79.76G	73.56f	73.92f	78.73c-e	84.90b	92.41a	80.70C
	Mean	72.21E	74.76D	78.94C	85.28B	92.27A	----	73.56E	75.14D	80.00C	86.02B	93.87A	----
	Control	0.42z	3.73q	5.21l	7.52c	9.95a	5.36A	0.49z	3.96p	5.44k	7.72d	9.93a	5.51A
	20%G.T 0 KGY	0.42z	2.64u	4.03o	5.92i	7.62b	4.13B	0.49z	2.80t	4.34n	6.13h	8.05b	4.36B
Microbial load	20%G.T.5KGy	0.42z	2.23w	3.62r	5.44k	7.13e	3.77D	0.49z	2.27v	3.85q	5.72j	7.32e	3.93D
	20%G.T.10KGy	0.42z	2.05x	3.04t	4.97m	6.44g	3.38F	0.49z	1.86x	3.23s	5.03l	6.66g	3.45F
	1% Chi.0KGy	0.42z	2.55v	3.87p	5.73j	7.42d	4.00C	0.49z	2.67u	4.17o	6.03i	7.93c	4.26C
	1% Chi.5KGy	0.42z	2.04x	3.34s	5.21l	6.81f	3.56F	0.49z	2.07w	3.63r	5.42k	7.05f	3.73E
	1%Chi.10KGy	0.42z	1.80y	2.65u	4.44n	6.04h	3.07G	0.49z	1.51y	2.92t	4.64m	6.14h	3.14G
	Mean	0.42E	2.44D	3.68C	5.60D	7.34A	----	0.49E	2.45D	3.94C	5.81B	7.58A	----

Means followed by different letters are significantly different at P ≤ 0.05 level; Duncan ´s multiple range test

As for the effect of storage period on L* value, data show that L* value of fresh- cut green bean decreased gradually with increasing storage period and reach its maximum depression at the end of storage period. This decrease in L* value might be due to the increase in polyphenol oxidase activity which leads to enzymatic browning appearance [13].

On the other side, fresh-cut green beans treated with 1% irradiated chitosan with 10 K Gy followed by 20% irradiated green tea with 10 K Gy and stored for 5 days had higher L* value than other treatments during the same storage period, then a slow depression was observed with the increase in storage period, taking into consideration that the same both treatments were the most effective till the end of storage period. The effect of chitosan on maintaining fresh-cut green beans peel color might be attributed to its role as an edible coating which modifies the internal atmosphere of fruit and delay of chlorophyll degradation [67]. As for green tea and its role in preserving fresh-cut green beans color, this result might be due to its effect as an antioxidant contains catechins, the principal polyphenol compounds in green tea that makes polyphenol oxidase inactive enzyme and delay peel color degradation [59].

a* Value: As for fresh-cut green beans greenness (a* value), data presented in Table (3) show that fresh-cut green beans dipped in 1% irradiated chitosan with 10KGy followed by 20% irradiated green tea with the same dose scored the highest a* value in both seasons.

Regarding the effect of storage period and its effect on a* value, data show that the increment in storage period was accompanied by a linear decrease in a* value and a degradation in green color especially at the end of storage period (20 days). This result agrees with that obtained by Martín-Diana *et al*. [13] who found that the increase in storage period led to a decrease in a* value of fresh-cut lettuce. This decrease might be due to the oxidation with oxygen radical of the chlorophyll molecule which led to chlorophyll breakdown [71].

On the other side, the interaction between storage period and different treatments show that 1% irradiated chitosan with 10 KGy followed by 1 % irradiated chitosan with 5 KGy and 20% irradiated green tea with 10 K Gy gave higher a* value of fresh-cut green beans during the first five days of storage than other treatments then the same trend was observed till the end of storage period during the two successive seasons. Same results were obtained by Hong *et al.* [32] who declared that treated guava fruit with chitosan had higher chlorophyll content than untreated fruit, also Xing *et al*. [72] who found that chitosan play an important role in chlorophyll content of sweet pepper. As for the effect of green tea, it might be related to its role as an antioxidant agent [14].

Table 3: Effect of gamma irradiated chitosan and green tea on lightness (L*), greenness (a*) and yellowness (b*) values of fresh-cut green beans under cold storage conditions

		First season						Second season					
		Storage period (Days)											
Characters	Treatments	0	5	10	15	20	Mean	0	5	10	15	20	Mean
L*value	Control	69.19a	67.14jk	66.21pq	65.73r	64.33t	66.52G	68.05a	65.74k	64.34s	63.21v	62.22w	64.71G
	20%G.T 0 KGY	69.19a	67.53hi	66.80lm	66.02q	65.06s	66.92F	68.05a	66.24i	65.44m	64.32s	63.53u	65.52F
	20%G.T.5KGy	69.19a	68.04de	67.33ij	66.71mn	66.03q	67.46D	68.05a	66.83f	66.04j	65.11o	64.64r	66.13D
	20%G.T.10KGy	69.19a	68.57bc	67.92d-f	67.21jk	66.48no	67.87B	68.05a	67.31c	66.81f	66.03j	65.22n	66.69B
	1% Chi.0KGy	69.19a	67.81e-g	67.03kl	66.33op	65.75r	67.33E	68.05a	66.64g	65.72k	64.73q	64.03t	65.83E
	1% Chi.5KGy	69.19a	68.40c	67.71f-h	67.03kl	66.42op	67.75C	68.05a	67.13d	66.51h	65.62l	64.92p	66.45C
	1%Chi.10KGy	69.19a	68.71b	68.12d	67.62gh	67.05k	68.14A	68.05a	67.51b	67.05e	66.44h	65.71k	66.95A
	Mean	69.19A	68.03B	67.30C	66.66D	65.87E	----	68.05A	66.77B	65.99C	65.07D	64.33E	----
a*value	Control	-17.03a	-15.63f	-13.51s	-12.80v	-11.91x	-14.18G	-16.82a	-14.72k	-13.05t	-12.51u	-11.41v	-13.70G
	20%G.T 0 KGY	-17.03a	-16.05e	-13.93o	-13.23u	-12.61w	-14.57F	-16.82a	-15.64g	-13.93p	-13.24s	-12.53u	-14.43F
	20%G.T.5KGy	-17.03a	-16.53d	-14.54k	-13.94o	-13.62r	-15.13D	-16.82a	-15.04i	-14.62l	-14.02o	-13.44r	-14.79D
	20%G.T.10KGy	-17.03a	-16.71c	-15.04f	-14.64h	-14.03k	-15.49B	-16.82a	-16.43c	-15.82f	-15.15h	-14.73k	-15.79B
	1% Chi.0KGy	-17.03a	-16.10e	-14.13m	-13.73q	-13.35t	-14.87E	-16.82a	-15.82f	-14.22m	-13.83q	-13.02t	-14.74E
	1% Chi.5KGy	-17.03a	-16.63c	-14.92i	-14.22l	-13.84p	-15.33C	-16.83a	-16.23d	-15.64g	-14.92j	-14.11n	-15.55C
	1%Chi.10KGy	-17.03a	-16.71b	-15.23g	-14.92i	-14.53k	-15.68A	-16.82a	-16.53b	-16.04e	-15.65g	-14.94j	-16.00A
	Mean	-17.03A	-16.34B	-14.47C	-13.93D	-13.41E	----	-16.82A	-15.77B	-14.76C	-14.19D	-13.46E	----
b*value	Control	12.61z	13.52v	15.26O	18.62h	21.62a	16.33A	10.53z	11.93v	14.07o	16.82h	19.63a	14.60A
	20%G.T 0 KGY	12.61z	13.38vw	15.11p	18.21i	21.16b	16.10B	10.53z	11.63wx	13.81p	16.54i	19.33b	14.37B
	20%G.T.5KGy	12.61z	13.22w	14.62r	17.64k	20.61d	15.74D	10.53z	11.04y	13.23r	16.04k	18.46d	13.86D
	20%G.T.10KGy	12.61z	12.87x	14.24t	17.03m	19.83f	15.32F	10.53z	10.81yz	12.61t	15.21m	18.02f	13.44F
	1% Chi.0KGy	12.61z	13.33vw	15.02q	17.93j	20.85c	15.95C	10.53z	11.42xy	13.61q	16.33j	18.95c	14.17C
	1% Chi.5KGy	12.61z	13.04wx	14.44s	17.31l	20.23e	15.53E	10.53z	10.93yz	12.92s	15.73l	18.24e	13.67E
	1%Chi.10KGy	12.61z	12.72y	13.81u	16.62n	19.32g	15.02G	10.53z	10.62z	12.10u	14.84n	17.62g	13.14G
	Mean	12.61E	13.15D	14.65C	17.62B	20.52A	----	10.53E	11.20D	13.19C	15.93B	18.61A	----

Means followed by different letters are significantly different at P ≤ 0.05 level; Duncan´s multiple range test

b* Value: As shown in Table (3), fresh-cut green beans yellowness (b* value) of all treatments increase gradually during cold storage, the maximum decrease was remarked at the end of storage period after 20 days of storage. The increase of b* value through the increase of storage period may be related to the breakdown of chlorophyll during storage period [73].

Concerning the effect of different treatments and its impact on b* value, results show that either fresh-cut green beans dipped in 1% of irradiated chitosan with 10 K Gy or in 20% irradiated green tea with the same dose led to a slightly reduction in b * value.

Finally, it was remarked that dipping fresh-cut green beans in 1% irradiated chitosan with 10 K Gy followed by 20% green tea irradiated with the same dose led to a decrease in b* value specially after 5 days of storage period compared with other treatments especially with increasing storage period. This result might be related to the inhibitory effect of both chitosan and green tea on polyphenol oxidase enzyme [59] that retards the appearance of yellow color which increases relatively with the increase of polyphenol oxidase activity.

CONCLUSION

The irradiation of chitosan and green tea with 10 K Gy of gamma irradiation improves their characters which is reflected on maintaining the physical characters of fresh-cut green beans colors and reducing the unfavorable effect of polyphenol oxidase enzyme and microbial load under cold storage conditions specially during the first five days of storage.

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