Microbiological, Nutritional and Sensorial Changes in Fresh Carrot Juice Preserved by Irradiation

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Abstract

Fresh carrot juice has perishable nature and very limited shelf life, and may pose a microbiological hazard. Gamma irradiation (1.5, 3.0 and 4.0 kGy) as non-thermal processing was performed to improve microbial quality, ensure safety and extending the refrigerated shelf life of fresh carrot juice. Irradiation dose of 3.0 kGy greatly reduced total aerobic bacterial counts, lactic acid bacteria and total molds and yeasts. While, it completely eliminated coliform bacteria, *Escherishia coli* and *Enterococcus faecalis*. Irradiation dose of 1.5 kGy had no significant effect on ascorbic acid content of the juice, while irradiation doses of 3.0 and 4.0 kGy significantly decreased ascorbic acid content. However, there was no significant difference in total carotene content between all irradiated samples and non-irradiated control. It was found that immediately after irradiation the sensory scores of irradiated (1.5 and 3.0 kGy) and non-irradiated samples were not significantly different. Irradiation dose of 4.0 kGy significantly reduced the sensory quality attributes of the juice. Thus, irradiation dose of 3.0 kGy can be successfully used to improve the microbial quality and extend the refrigerated shelf-life of fresh carrot juice, where it extended the shelf-life to 8 days against only 2 day for non-irradiated control.

Key words: *Fresh carrot juice, Gamma irradiation, Microbial quality, Nutritional quality.*

Introduction:

Carrot is one of the most commonly used and well known vegetables in the every day kitchen that is rich in functional food components such as vitamins (A, D, B, C and K) and minerals (calcium, potassium, phosphorus, sodium and iron). The carotenoids and other antioxidants present in carrot play an important role in the inhibition and / or interruption of oxidation processes, as well as in counterbalancing free radical activities. Therefore, carrots and their fresh produce (shredded carrots, sliced carrots and carrot juice) may protect humans against certain types of cancer and cardiovascular diseases**(Krinsky & Johnson, 2005)**.

The consumption of fresh (unpasteurized) fruit and vegetable juices has increased in recent years due to their characteristics of freshness, high vitamins content, low calorie contribution and an active promotion of fruits and vegetable of healthy diet **(Allende *et al.* 2006).** Fresh carrot juice as natural extract from carrot crop enjoys high consumer acceptance as account of its taste and nutritional benefits. It is preferred as fresh squeezed without pasteurization. However, fresh carrot juice have highly perishable nature and very limited shelf-life rarely exceed one day even under a cold chains system **(Song *et al.* 2007).** This may be due to the high microbial contamination in particular acid-loving or acid-tolerant bacteria and fungi (yeasts and molds). Bacteria in fresh-carrot juice especially pathogens that come from raw carrots; soil, water or the hands of workers affect not only the public health, but also the product quality. There are a number of reports that raw vegetables may harbor the potential food borne pathogens **(Abadias *et al.* 2008 & Gutierrez *et al.* 2009).** Therefore, prolonging shelf-life and elimination of pathogenic bacteria while maintaining juice sensorial and nutritional qualities is of a great importance for industries and for consumers.

Conventional thermal processing of fruit and vegetables juices remains the most widely adapted technology for shelf-life extension and preservation of these juices. However, consumer demand for fresh and nutritious juices has led to interest in a non-thermal technology to apply in the processing of fresh vegetable juices to avoid the deleterious effects that heat has on the flavor, color and nutrients **(Tiwari *et al.* 2009).** Ionizing radiation (gamma radiation, X-rays and electron beam) have approved now as a physical non-thermal technology for food preservation. It is highly effective in controlling both spoilage and pathogenic microorganisms contaminating food and has offered a safe alternative as a decontamination method of food and public health products **(Kader, 1986; Niemira *et al.* 2001 & Hammad *et al.* 2010).** Several researchers have shown that ionizing radiation is a suitable and effective non-thermal method to control microorganisms of fresh fruits and vegetables and their minimally processed produce **(Chervin & Biosseav, 1994; Buchanan *et al.* 1998).**

The aim of the present investigation is to use gamma radiation as a non-thermal process to improve the microbiological quality of fresh carrot juice. The effect of different gamma irradiation doses on the nutritional, physiochemical and sensorial characteristics of fresh juice were investigated.

Materials and methods:

Preparation of juice:

Commercially ripe fresh carrots (Daucus carrota) were obtained from a local vegetable market at Cairo. The green part of the carrot and the wounded area were removed and the sound samples were soaked in tap water over night. The juice was prepared by direct squeezing using a commercial juice (National Juicer Blender, Model M5-130 N). The juice samples were packaged in aluminum foil bags (each 100 ml).

Gamma irradiation:

Irradiation of the packaged juice samples was carried out using cobalt-60 irradiator (Gamma Chamber 4000, India), located at National Center for Radiation Research and Technology, Naser City, Cairo, Egypt. The carrot juice samples were divided into four groups, the first group was left without irradiation and served as control, while the second, third and fourth groups were subjected to gamma radiation at dose levels of 1.5, 3.0 and 4.0 kGy. The dose rate of the irradiation source at the time of the experiment was 3.903 kGy/h. Dosimetry was performed using 5mm diameter alanine dosimeters traceable to National Physical Laboratory (NPL), UK.

Storage:

Immediately after irradiation, both irradiated and unirradiated juice samples were stored at 4°C±1 until rejection. Samples were withdrawn at 0, 3, 5 and 10 days for microbiological, nutritional, physicochemical and sensory analysis. The analyses were performed in triplicate for each treatment at each time point.

Microbiological analysis:

Total aerobic bacterial count (CFU.ml^{-1}) was determined by using Plate Count Agar (Difco-Labs, Detroit, Michigan, USA) after incubation at 30°C for 72h. Lactic acid bacteria was counted on Man Rogosa, and Sharp (MRS) (Difco-Labs, Detroit, Michigan, USA) after incubation at 30°C for 72 h. Total mold and yeast counts were enumerated with Malt Extract Agar (MEA) medium (Difco-Labs, Detroit, Michigan, USA) after incubation at 25°C for 72h. Total coliforms and *Escherishia coli* were determined on MacConkey Broth (Oxid Comp., Basingstoke, Hant, UK) using Most Probable Number (MPB) technique according to **WHO (1993).** *E. coli* was confirmed on Eosine methylene blue agar medium. *Enterococcus faecalis* was enumerated on Kanamycin Aecolin Azide Agar (KAAA) medium (Oxide Comp, Basingstoke, Hants, UK) after incubation at 37°C for 24h according to **Mosselc (1978).**

Nutritional analysis:

Vitamin C (ascorbic acid) content was determined using 2,6-dichlorophenol indophenol reagent (Fluka, Deisehofen, Germany) according to the method described by **AOAC (2000).**

Total carotenoids were determined spectrophotometrically at 450 nm using ATI unicam 5600 series UV/VIS spectrophotometer, Model V-200-RS). Total carotenoids content was calculated with reference to a standard curve based on beta carotene (El-nasr Comp., Cairo, Egypt) according to **Sharon-Raber & Khan, 1983).**

Physicochemical analysis:

Viscosity of the juice was measured using a Brookfield digital Rheometer (Model DV-II, Brookfield Engineering Laboratories, Inc. Stongton, M.A). Viscosity measurement was made by using 8-16ml of carrot juice in a small sample adapter. The temperature was adjusted at 20°C using water bath circulator and coolers. Each sample measured against non-irradiated control.

pH values were determined by using a bench pH meter (pH 211 Microprocessor pH meter, Hanna instruments) and pH electrode (EPP-1) at room temperature according to **AOAC (2000).**

Sensory evaluation:

Unirradiated and irradiated carrot juice samples were given to the panelists immediately after irradiation and during refrigerated storage. The procedure carried out for evaluation was similar to that described by **Min *et al.* (2003).** Ten panelists belonging to the Department of Microbiology at the National Center for Radiation Research and Technology, Egypt, Atomic Energy Authority were participated in the sensory tests. 5ml of each sample were served into 20ml polypropylene containers with polyethylene screw-cap (Delta lab) coded with three digits randomly numbered. A glass containing potable water and a piece of non-salted cracker were provided to panelists for eliminating the residual taste between samples. The panelists were asked to judge the preference of odor, color, taste and overall acceptability in a hedonic scale from 0 to 9, where (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = borderline of acceptability, 3 = dislike moderately, 2 = dislike very much, and 1 dislike extremely). A score of 4 or below was regarded as unacceptable and taken to indicate the end of shelf-life.

Statistical Analysis:

The significance of the data with different factors was evaluated using tow-way analysis of variance ANOVA. All analysis was performed with SAS software package version 6.12 **(SAS, 1997).**

Results and Discussion:

Microbiological quality

The effect of gamma irradiation on the microbiological qualities of fresh carrot juice is shown in Table (1). It is obvious that the initial log viable count of the total aerobic bacteria was 4.98 CFU.ml^{-1}. These counts are within the accepted level for fresh vegetables and their fresh produce (**PHLS, 2000**). The log initial populations of lactic acid bacteria and molds and yeasts were 4.76 and 3.25 CFU.ml^{-1}, respectively. It is well known that acid-loving bacteria or acid-tolerant bacteria (lactic acid bacteria) and fungi (molds and yeasts) are the main causal of the rapid deterioration of fresh vegetable juices. Coliform bacteria and *E. coli* which are considered significant from public health of view were found in fresh carrot juice at levels of 460 and 21 MPN/ml, respectively. Meanwhile *Enterococcus faecalis* found at level of 3.2 x 10^2 CFU.ml^{-1}. All irradiation doses used greatly reduced the initial microbial counts and the reduction was proportion with irradiation dose. Irradiation dose of only 1.5 kGy could eliminate coliforms and *E. coli,* while, irradiation dose of 3.0 kGy could eliminate *Enterococcus faecalis*. Irradiation dose of 4 kGy, could completely eliminate all microorganisms contaminating fresh carrot juice. Similar results have shown by many investigators **(Chervin & Biosseav, 1994, & Song *et al.* 2007).**

During refrigeration storage (4°C±1), the viable counts of aerobic bacteria, lactic acid bacteria and molds and yeasts significantly ($P < 0.05$) increased and reached 7.1, 6.89 and 5.17 log CFU.ml^{-1} after 3 days, where these samples were rejected from the view point of organoleptic tests. The viable counts of these microorganisms in irradiated fresh carrot juice samples also significantly ($P < 0.05$) increased but at lower rate. The log counts of aerobic bacteria, lactic acid bacteria and molds and yeasts in samples receiving 1.5 kGy reached 7.85, 7.78 and 4.52 CFU.ml^{-1} after 10 days of refrigerated storage. The log viable counts of the samples irradiated at 3.0 kGy were almost similar to that of those irradiated at 1.5 kGy after also 10 days. Total molds and yeasts in 4.0 kGy irradiated samples were below the detectable level throughout the refrigerated storage period. In general, all the microbial counts in irradiated samples were significantly lower than those of unirradiated ones at any time of storage. It was considered that the lower values of viable microbial counts was due to the post-irradiation effect where the surviving cells that had been damaged by an irradiation were gradually inactivated, thus no adapting to the surrounding environ-ment during a storage **(Byun *et al.* 2001).** Sublethal damage to cells caused by irradiation is likely to increase their sensitivity to environmental stress factors. In addition, an extension of the log time in the growth of the surviving cells in foods with radiation-related injuries has been reported **(Grant & Patterson, 1992).** On the other hand, irradiated carrot juice samples at 1.5 and 3.0 kGy were free from coliforms and *E. coli* throughout storage period, **Niemira *et al.* (2001)** indicated a 3.5 kGy as the dose required to a chive a 5-log reduction for the most resistant isolate (*Salmonella anatom*) in orange juice.

Influence of irradiation on nutritional quality:

The nutritional quality of irradiated fresh carrot juice was evaluated by determining the contents of ascorbic acid and total carotenoids. Table (2) shows the influence of different irradiation doses on the content of ascorbic acid in carrot juice. It is clear that irradiation dose of 1.5 kGy had no significant effect on ascorbic acid content, while irradiation doses of 3.0 and 4.0 kGy significantly ($P < 0.05$) decreased ascorbic acid content. **Kilcast (1994)** indicated that ascorbic acid (vit. C) was the most sensitive of all water-soluble vitamins to an irradiation. The decrease in ascorbic acid in the present study could be due to the partial oxidation of ascorbic acid to dehydroascorbic acid. Since both compounds have vit.C activity in the body, the reduction in ascorbic acid is not of great importance. **Song *et al.* (2007)** found that irradiation resulted in a dose dependent reduction of the ascorbic acid content in carrot and kale juices, however, the contents of the total ascorbic acid including dehydroascorbic acid were stable up to 3.0 kGy of irradiation. During refrigeration storage of fresh carrot juice ascorbic acid content decreased in both non-irradiated and irradiated samples, but the rate of decrease was higher at 3.0 and 4.0 kGy of irradiation. Similarly **Song *et al.* (2007)** found that 3.0 kGy of irradiation caused a loss by 35.12% in ascorbic acid of carrot juice after 3 days of storage at 10°C.

Carotenoids are among the most important nutrients in food, owing to their diverse functions and actions. It is well known that carrot juice is essential source of β-carotene. Figure (1) indicated that no significant ($P < 0.05$) difference in total carotene content between irradiated (1.5, 3.0 and 4.0 kGy) and non-irradiated carrot juice sample. Similarly it was found that gamma irradiation doses not affect the content of β-carotene or other carotenoids with pro-vitamin A activity **(ICGFT, 1999).** During refrigation storage, the total carotenoids significantly decreased in non-irradiated and irradiated samples.

Physicochemical properties:

The rheological behaviour of fruit and vegetable juices is largely influenced by their quantitative and qualitative composition and therefore, it will depend on the fruit and vegetable types and the treatment at which it is subjected during processing. Figure (2) illustrates the viscosity of carrot juice, it is obvious that all irradiation doses used (1.5, 3.0 and 4.0 kGy) had no significant effect in the viscosity of the juice.

However, a significant increase in the viscosity has observed during refrigeration storage. The increase in the viscosity of the fresh carrot juice upon storage might be due to the increase in the microbial load particularly molds and yeasts, which led to spoilage of the juice and increase its viscosity. Similarly, **Huisint Veld (1996)** reported that spoilage of food may also bring about physical changes such as increase the viscosity, gelation, sedimentation or color change of the food.

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The pH value of fresh carrot juice (non-irradiated) was 6.92 indicating a nutral medium (Fig 3). All irradiation doses used (1.5, 3.0 and 4.0 kGy) had no significant effect on the pH value of the carrot juice. However, during refrigerated storage the pH value of all carrot juice samples significantly decreased, might be due to the increase in the viable counts of lactic acid bacteria. **Wang *et al.* (2006)** attributed the pH reduction of carrot juice to the formation of hydroxyl methyl furfural (HMF) from the reactions involving amino acids and reducing sugars.

Sensory quality attributes:

Sensory quality attributes of non-irradiated and irradiated fresh carrot juice samples were evaluated in the parameters of color, odor, taste and overall acceptability's and the panelist scores were illustrated in Fig (4 a, b, c & d). It was found that the sensory scores of the irradiated (1.5 and 3.0 kGy) and non-irradiated samples were not significantly different immediately after irradiation (zero time of storage). However, the sensory score of the irradiated samples at 4.0 kGy were significantly ($P < 0.05$) lower than those of non-irradiated ones. During refrigerated storage, the sensory quality attributes (odor, taste, overall acceptability) of non-irradiated carrot juice samples significantly decreased where the panelists rejected these samples at the day 3 because of off-odor and very bad taste. Therefore, control samples could not be used for sensory evaluation after that time. The panelists sensory scores of 1.5 kGy-irradiated samples also significantly decreased upon storage reaching the worst at the day 5. Fresh carrot juice samples receiving 3.0 and 4.0 kGy sensorially rejected after 10 days of refrigerated storage. Thus, irradiation dose of 3.0 kGy appeared to be the suitable dose for extending the refrigeration shelf-life of fresh carrot juice without adverse effect on sensory quality attributes. It extended the shelf-life of fresh carrot juice samples to at least 8 days at 4°C±1 and at the same time completely eliminated coliforms, *E. coli* and *Enterococcus faecalis,* thus ensuring its safety.

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Table (1): Effect of γ-irradiation and subsequent storage (4°C±1) on the microbial counts (log/ CFU.ml^{-1}) contaminating carrot juice.

Microorganisms	**Storage (days)**	**Irradiation doses (kGy)**			
		0.0	**1.5**	**3.0**	**4.0**
Total Aerobic Mesophilic Bacteria	0	$4.98^{a}_{a} \pm 0.01$	$3.92^{b}_{a} \pm 0.01$	$2.91^{c}_{a} \pm 0.13$	<10
	3	$7.10^{b}_{b} \pm 0.03$	$4.70^{b}_{b} \pm 0.02$	$3.69^{c}_{b} \pm 0.05$	$2.69^{d}_{b} \pm 0.005$
	5	R	$6.20^{b}_{c} \pm 0.06$	$4.68^{c}_{c} \pm 0.01$	$3.73^{d}_{c} \pm 0.04$
	10	R	$7.85^{b}_{d} \pm 0.05$	$7.03^{c}_{d} \pm 0.03$	$6.05^{d}_{d} \pm 0.05$
Lactic acid Bacteria	0	$4.76^{a}_{a} \pm 0.05$	$2.90^{b}_{a} \pm 0.09$	$2.48^{c}_{a} \pm 0.12$	<10
	3	$6.89^{a}_{b} \pm 0.05$	$4.98^{b}_{b} \pm 0.01$	$2.48^{c}_{b} \pm 0.15$	<10
	5	R	$5.03^{b}_{c} \pm 0.03$	$4.02^{c}_{c} \pm 0.02$	$2.75^{d}_{c} \pm 0.15$
	10	R	$7.78^{b}_{d} \pm 0.01$	$7.34^{c}_{d} \pm 0.04$	$4.02^{d}_{d} \pm 0.02$
Total Molds and yeasts	0	$3.25^{a}_{a} \pm 0.05$	$1.86^{b}_{a} \pm 0.09$	$1.73^{c}_{a} \pm 0.04$	<10
	3	$5.17^{a}_{b} \pm 0.03$	$2.25^{b}_{b} \pm 0.05$	$1.53^{c}_{b} \pm 0.06$	<10
	5	R	$3.66^{b}_{c} \pm 0.02$	$2.85^{c}_{c} \pm 0.01$	<10
	10	R	$4.52^{b}_{d} \pm 0.16$	$3.40^{c}_{d} \pm 0.01$	<10
Coliforms (MPN/ml)	0	460	< 3	< 3	< 3
	3	150	< 3	< 3	< 3
	5	R	< 3	< 3	< 3
	10	R	< 3	< 3	< 3
***Escherichia coli* (MPN/ml)**	0	21	< 3	< 3	< 3
	3	< 3	< 3	< 3	< 3
	5	R	< 3	< 3	< 3
	10	R	< 3	< 3	< 3
***Enterococcus faecalis* (CFU.ml^{-1})**	0	3.5×10^{2}	2.0×10^{2}	< 100	< 100
	3	7.6×10^{3}	< 100	< 100	< 100
	5	< 100	< 100	< 100	< 100
	10	R	< 100	< 100	< 100

R = Samples sensorially rejected. < 10 = below detectable limit (< 10 cfu/ml).
Mean values followed by different superscript (within rows) and different subscripts (within colums) are significantly different (P < 0.05).

Table (2): Ascorbic acid content (mg/100 ml) of carrot juice treated with irradiation during storage at 4°C±1.

Storage (days)	Irradiation dose (kGy)			
	0	1.5	3.0	4.0
0	$8.80^{a}_{a} \pm 1.30$	$8.45^{a}_{a} \pm 0.65$	$6.53^{b}_{a} \pm 0.05$	$3.95^{c}_{a} \pm 0.05$
3	$8.40^{a}_{a} \pm 0.03$	$8.32^{a}_{a} \pm 0.05$	$5.17^{b}_{b} \pm 0.03$	$3.12^{c}_{b} \pm 0.03$
5	R	$8.20^{b}_{a} \pm 1.05$	$3.85^{c}_{c} \pm 0.05$	$2.65^{d}_{c} \pm 0.05$
10	R	$8.05^{b}_{a} \pm 0.20$	$3.82^{c}_{c} \pm 0.02$	$2.46^{d}_{c} \pm 0.04$

R = Samples sensorially rejected.
Mean values followed by different superscript (within rows) and different subscripts (within colums) are significantly different (P < 0.05).

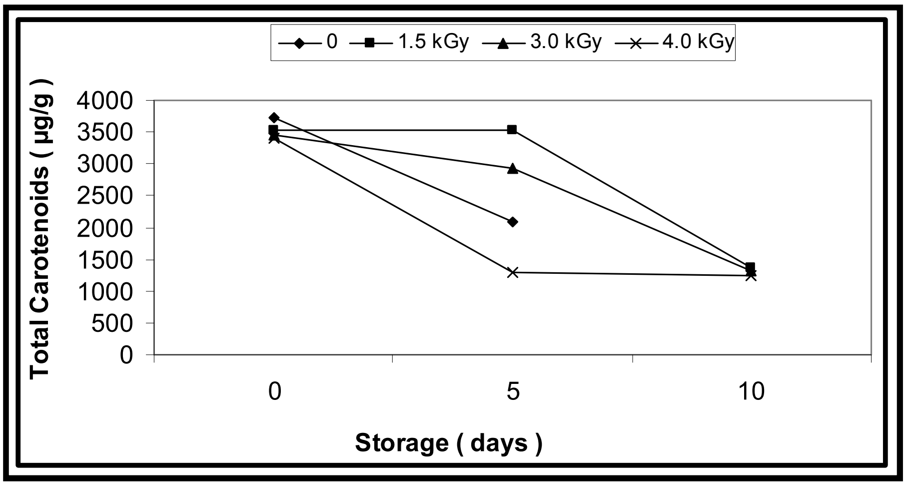

Figure (1): Effect of γ-irradiation and refrigeration storage on the total carotenoids of carrot juice.

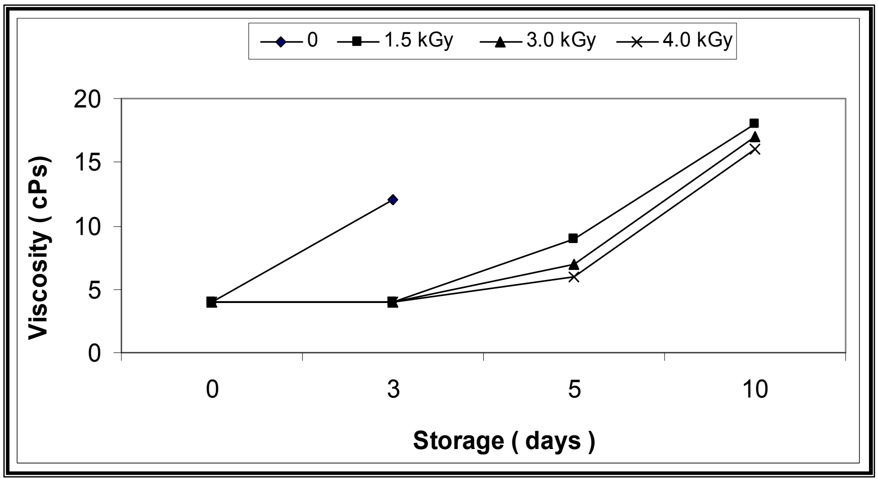

Figure (2): Viscosity (cPs) of carrot juice treated by irradiation during storage at 4°C±1.

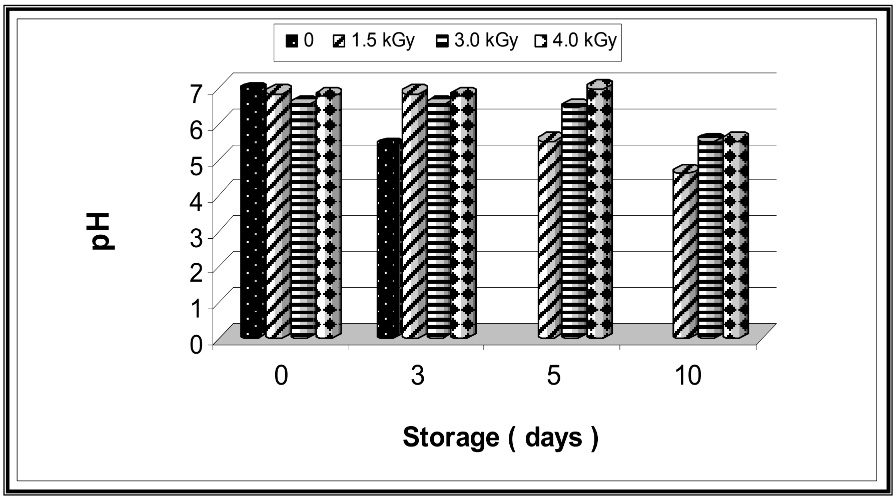

Figure (3): pH value of irradiated and non-irradiated carrot juice during storage at 4°C±1.

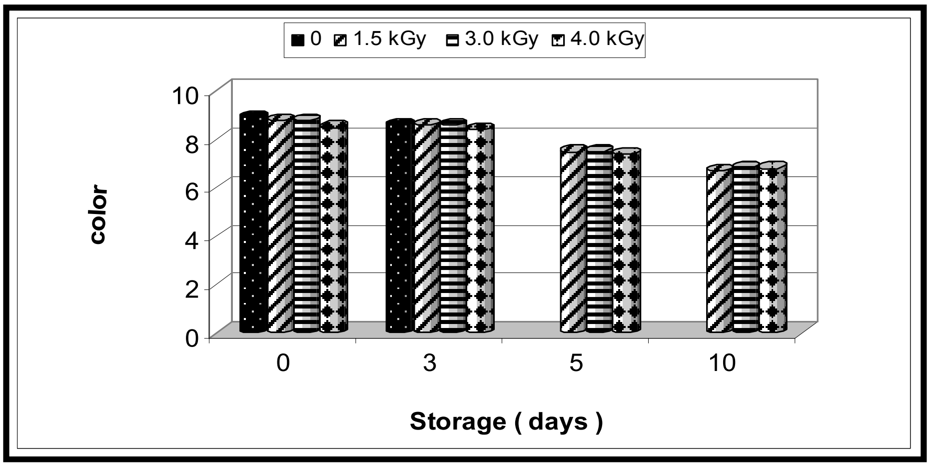

Fig.4 a .Effect of irradiation on the color of carrot juice during storage at 4°C±1.

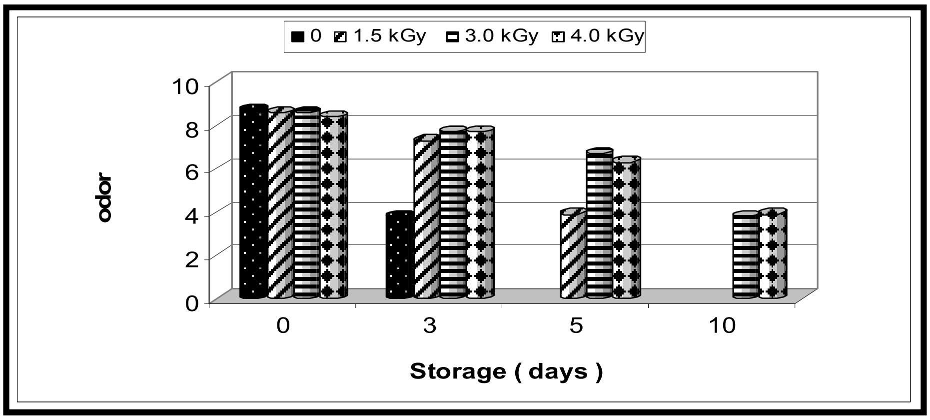

Fig.4 b. Effect of irradiation on the odor of carrot juice during storage at 4°C±1.

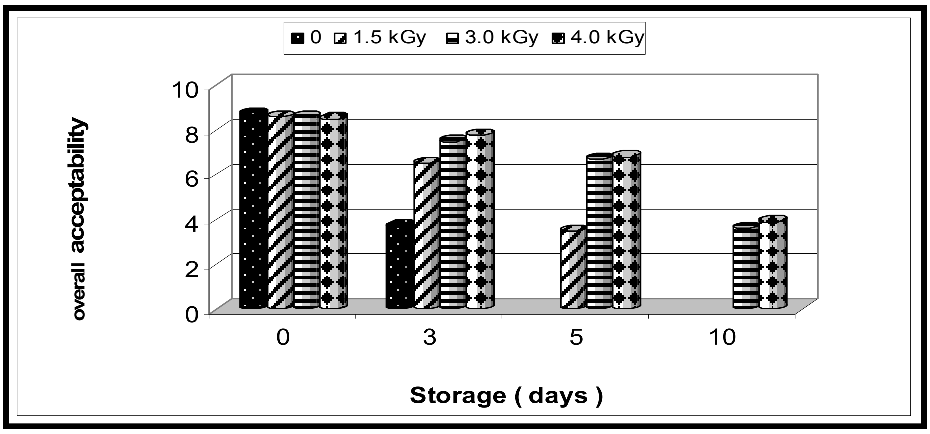

Fig.4 c. Effect of irradiation on the overall acceptability of carrot juice during storage at 4°C±1.

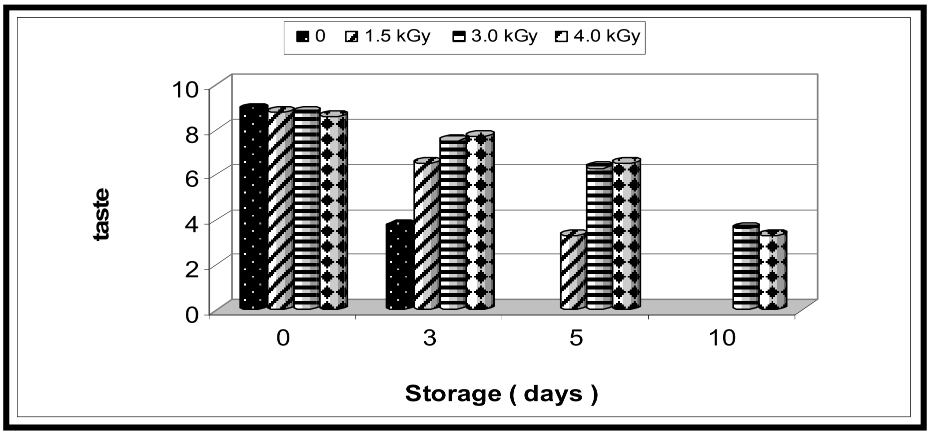

Fig.4 d. Effect of irradiation on the taste of carrot juice during storage at 4°C±1.

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