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Shelf-life Extension and Quality Improvement of Minimally Processed Pear by Combination Treatments with Irradiation

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ABSTRACT

Fresh-cut pears are not yet commercially available in Egypt. Thus, an attempt has been done to produce this minimally processed fruit manually at the laboratory. Fifteen samples of this manually prepared fresh-produce were evaluated for their microbiological quality. Total aerobic bacterial counts (TAPC) ranged from 7.5×10^1 to 3.5×10^4 cfu g^{-1}, lactic acid bacteria (LAB) ranged from < 10 to 3.2×10^3 cfu g^{-1}; total mould and yeast counts (TM & Y) ranged from < 10 to 5.3×10^3 cfu g^{-1} indicating good quality from the view point of microbiological population. Coliform bacteria and *Escherichia coli* were found in only 2 samples at levels of 20 to 43 and > 3 to 9 most probable number per gram (MPN g^{-1}), respectively. On the other hand, *Staphylococcus aureus* was found in also 2 samples at levels of 10^2. *Aeromonas hydrohila*, *Listeria monocytogenes* and *Salmonella* spp., were not found in any of the samples. Fresh-cut pears were dipped in water containing 2% ascorbic acid and 1% calcium lactate. The dipped fresh-cut pears were exposed to 1, 2 and 3 kGy. Dipping fresh-cut pears in 2% ascorbic acid plus 1% calcium lactate prevented browning and enhanced firmness during refrigeration storage. Irradiation dose of 2 kGy was the optimum, since it reduced TAPC by 99.58% and LAB and TM and Y to un-detectable level. These combined treatments extend the shelf life of fresh-cut pears without affecting their chemical, physical and sensorial quality attributes.

Key Words: Irradiation; Quality; Shelf-life; Minimally processed pear; Combination

INTRODUCTION

Minimally processed fruits that are ready-to-eat are more difficult to produce than minimally processed vegetables. Although some minimally processed fruits including apples, pears, peaches, nectarines and strawberries have been marketed for both retail and food service distribution these products are still under study, because of the difficulties in preserving their fresh-like quality during prolonged period (Soliva-Fortuny & Martin-Belloso, 2003). The main problem confronts the production, distribution and marketing of fresh-cut fruits is its short shelf-life (Gorny *et al.,* 1998 a, b). This limited shelf-life may be due to: (1) enzymatic browning for cut surface resulting from peeling, slicing and cutting, which produce an un-desirable color (2) flesh softening or loss of firmness, which cause texture deterioration and (3) microbiological deterioration.

The normal spoilage micro-flora of fruits differs markedly from that of vegetables. Most fruits usually have higher sugars content and more acidic (pH 4.6 or lower). This lower pH and the nature of the organic acids involved usually restrict the micro-flora to acid tolerant microorganisms such as lactic acid bacteria and fungi (Splittstoesser, 1987; Soliva-Fortuny & Martin-Belloso, 2003).

Although extensive work have been conducted on the application of ionizing radiation to extend shelf-life and improve quality and safety of minimally processed vegetables, very few is published about the influence of irradiation on minimally processed fruits (fresh-cut fruits). These published papers only studied the influence of irradiation on the firmness or texture (Gunes *et al*., 2001) and respiration rate of fresh-cut fruits.

The main objectives of this study is to prepare minimally processed fresh produce namely, fresh-cut pear. Study the microbiological quality of this fresh produce. Determine the so-called D_{10}-value for the isolated pathogenic bacteria to identify the so-called 5-log cycle reduction irradiation dose. Investigate the effect of irradiation doses on the hygienic, chemical and sensory quality of fresh-cut pear product during refrigerated storage.

MATERIALS AND METHODS

Preparation and processing of fresh-cut pears. Partially ripened pears (*Pyrus communis* L., variety Leconte) at commercially maturity stage, based on external color and firmness, were purchased (at the same day of harvest) from the farm located in Al Beharra Governorate during the season 2005 and brought to the laboratory. Intact pears fruits of uniform size (130 - 140 g) were manually prepared in the laboratory, where they were rinsed with 0.02% sodium hypochlorite to reduce the surface microbial load. The fruits were washed with tap water and manually peeled and cut

into quarters with sharp stainless steel knife then the cut-pear quarters were cord. A part of pear quarters were dipped in tape water for 3 min (served as control). Another part of pear quarters were also cored but dipped in tap water containing 2% ascorbic acid and 1% calcium lactate for 3 min to control enzymatic browning and loss of firmness. The dipped pear quarters were left to drip-dry for 15 min in a perforated cage. The dried-pear quarters were packaged in foam bags and wrapped with thin film (thickness 10 μm) of polyphenylchloride (non-perforated, permeable, self-clinging). Each package contains 4 quarters (approximately 100 g) from different fruits.

Fifteen samples of manually prepared fresh-cut pears quarters (each sample consists of thee fresh-cut pear packages), were tested for their microbiological quality.

Irradiation experiments. Sixty packages of laboratory prepared fresh-cut pears were also used for irradiation and storage. Fresh-cut pear samples were subjected to 0, 1, 2 and 3 kGy. Irradiation treatments were performed at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The irradiation facility used was Russian CO^{60} Irradiator model ISS LEDOVATED. The dose rate of this source was 4.62 kGy h^{-1}. All irradiated and non-irradiated package samples were stored at 4°C ± 1, for the length of study. All analysis was carried out on three packages (3 samples), each sampling day. Thus, each figure in the results was expressed as the average of the three replicates.

Microbiological analysis. Total aerobic bacterial counts (TAPC) mesophiles, were plated on plate count agar (PCA) medium using pour plate technique according to APHA (1992). Lactic acid bacteria (LAB) were counted on Man, Rogosa and Sharp (MRS) agar medium according to APHA (1992). Total mould and yeast counts (TM & Y) were counted on Czapek′s-Dox yeast extract agar medium according to Koburger and Marth (1984) using pour plate technique. Total coliform were counted on MacConkey broth by Most Probable Number (MPN) technique using three test tubes with Durham′s tubes according to WHO (1993). The inoculated tubes were incubated at 37°C for 24 - 48 h. The presence of acid (yellow color) and gas (in Durham's tubes) indicates positive tubes. Positive test tubes were transferred to another tubes containing MacConkey broth and incubated at 44.4°C for 24 - 48 h. The presence of acid (yellow color) and gas from lactose indicates presence of *Escherichia coli* according to APHA (1992). *Enterococcus faecalis* was enumerated on Kanamycin aesculine azid agar medium using surface spreading technique according to Mossel (1978). *Staphylococcus aureus* was counted on Baird-Parker medium using surface spreading technique according to ICMSF (1978). *Aeromonas hydrophila* was counted on starch ampicillin agar medium using plate surface spreading technique according to Palumbo *et al.* (1985). *Listeria monocytogenes* was plated on *Listeria* selective agar medium (Oxford formulation) using plate surface spreading technique. *Salmonella* was detected according to ISO 6579: 1993 (Harrigan, 1998).

Determination of D_{10}-value. The radiation resistance (D_{10}-value) of *E. coli* and *Staph. aureus,* which were isolated and identified from the product, was determined. For preparation of inoculum the stock cultures of the tested pathogenic bacteria were separately activated by growing each in 100 mL tryptic soy broth (TSB) at 37°C for 24 h. The cultures broth were diluted with sterilized 1% peptone water to obtain suspension of approx. 10^7 - 10^8 cfu mL^{-1} and used for inoculation of the samples (work cultures suspension), For inoculation, aseptic techniques were used throughout the inoculation procedure. Five hundred grams of each product in sealed polyethylene bags were exposed to 20 kGy of gamma radiation for sterilization. Each product was placed in a sterile flask containing 1000 mL of each pathogen work culture suspension and gently shacked for 3 min. The liquid was withdrawn and the samples were drained and kept at 30°C for at least 8 h for equilibrium. After inoculation, 25 g of each inoculated product samples were packed in sterilized polyethylene bags and sealed. They were exposed to different irradiation doses (from 0.25 to 3.0 kGy). Three replicates of each pathogen were used in each dose. After irradiation, survival tested microorganisms were counted on the above tryptic soy agar (TSA) medium after incubation for 24 h at 37°C. The decimal reduction dose (D_{10}-value) of each tested pathogen was calculated.

Chemical analysis. Amino acid determination was performed according to the method of Baxter (1996). The system used for the analysis was High Performance Amino acid Analyzer, Biochom 20, Pharmacia Biotech at the NCRRT. Total carotenoids were determined spectophotometrically by absorbance at 450 nm using ATI Unicam, 5600 series UV/VIS Spectrophotometer and the amounts were calculated with reference to a standard curve based on beta carotene (Sharon-Raber & Kahn, 1983). Ascorbic acid (Vitamin C) was determined using 2, 6 dichlorophenol indophenol reagent according to the method described by AOAC (2000). Polyphenol oxidase (PPO) activity was assayed as described by Benjamin and Montgomery (1973). One unit of PPO activity was defined as an increase of 0.01 absorbance per min mL^{-1} enzymatic extract immediately after extract addition. This means that the increase of 0.01 absorbance at 405 nm = one PPO unit.

Physicochemical analysis. Firmness measurement was carried out on 1 cm^3 sample of fresh-cut pear pulp using a Model 1140 Instron Universal Testing Machine (Instron, High Wycombe, UK). The weight on the load cell was taken as the force (kg cm^{-3}) and converted to pressure (Anina & Oladunjoye, 1993). The pH of the pear pulp slurry was determined using a Hanna Instruments HI 931401 Microprocessor pH Meter according to AOAC (2000). Weight losses were estimated at regular intervals during storage. The model package containing product samples were accurately weighed and weight losses were calculated as g 100^{-1} g of fresh cut products. Weighing was made with

accuracy of ± 1 x 10^4 g.

Sensory evaluation. Sensory quality attributes i.e., color (with special reference to cut surface browning), odor, texture and taste of the un-irradiated and irradiated stored samples were evaluated by ten un-trained panelists (members of the National Center for Radiation Research and Technology, NCRRT). The product samples were coded and presented to a single sensory judge in a clean and odor-free glass plate at room temperature under normal light condition. The sensory quality attributes were scored on a scale of 1 to 9, where: 1 = very poor, 5 = fair and 9 = excellent (Barry-Ryan & O′Beirne, 1998). Recently purchased samples of each product on the same time of sampling were used as reference control every sampling day. A score of 4 or below was regarded as un-acceptable and taken to indicate the end of shelf-life.

Statistical analysis. For statistical evaluation of the results, means ± standard deviation were compared to the corresponding values of control and irradiated samples. Data were analyzed using statistical package for social science (SPSS, Chicago, IL) software (Version 12) by Two Way Analysis of Variance, where the variables were the irradiation dose and storage period according to Scheffé test (Freund & Wilson, 1997). Significance of differences was represented as $P < 0.05$.

RESULTS AND DISCUSSION

Microbiological quality of fresh-cut pears samples. Table I shows that TAPC of manually fresh-cut pear samples ranged from 7.5 × 10^1 to 3.7 × 10^4 cfu g^{-1}. According to French legislation (Nguyen-the & Carline, 1994) and Japanese local prefectural government (Kaneko *et al.,* 1999), TAPC of all tested samples were less than the maximum accepted level (10^5 cfu g^{-1}) for foods. These results are in accordance with those found by other investigators. Corbo *et al.* (2004) found that the log count of mesophilic bacteria in lightly processed cactus pear fruit was 4.17 cfu g^{-1}. Anese *et al.* (1997) found that the log total aerobic bacterial count of minimally processed apple slices packed in air was 3.0 cfu g^{-1}.

From the same table, it is obvious that the count of LAB in the fifteen samples ranged from lesser than 10 to 3.2 × 10^3 cfu g^{-1}. Total mould and yeast count ranged from less than 10 to 5.3 × 10^3 cfu g^{-1}. Soliva-Fortuny and Martin-Belloso (2003) reported that lactic acid bacteria and fungi are the predominant micro-flora in fruits due to low pH value and nature of the organic acid involved.

The results in Table I also show that only two samples contained coliforms with level of 20 - 43 MPN g^{-1} and *E. coli* at level of 9 cfu g^{-1}. Nevertheless, and according to published guidelines (PHLS, 2000) all fresh-cut pear samples considered satisfactory from the view point of *E. coli*. *S.. aureus* was found in two samples at levels of 2.2 × 10^2 and 5.0 × 10^2 cfu g^{-1}. These two samples are considered un-satisfactory, because they contain *S.. aureus* more than 100 cfu g^{-1}. On the other hand, all fresh-cut pear samples were free from of *Salmonella* spp., L. *monocytogenes, A. hydrophila and Ent. faecalis.*

Radiation decimal reduction dose (D_{10}-value). The D_{10}-values of *S.. aureu* and *E. coli,* which were found in fresh-cut pear were determined. Fig. 1 and 2 show that gamma radiation greatly reduced the population of viable counts in the studied pathogenic bacteria and this reduction was proportional with irradiation dose. It is clear that survival curves obtained for viable counts of the tested bacteria were of exponential response (linear regression slopes). Their D_{10}-values on fresh-cut pears were 0.49 and 0.27, respectively. It is obvious that the radiation resistance of *S.. aureus* was higher than that of *E. coli*.

Effect of combined treatment with irradiation on the microbial load of fresh-cut pears. Table II shows that control fresh-cut pear samples, which immersed in only tap water had mean TAPC of 6.4 × 10^3 cfu g^{-1}, while those immersed in ascorbic acid and calcium lactate contained 6.0 × 10^3 cfu g^{-1} indicating that immersion in enzymatic browning inhibitor (ascorbic acid) and firmness agent (calcium lactate) slightly reduced TAPC. Meanwhile, irradiation at all doses used (1, 2 & 3 kGy) caused great reduction in TAPC. The lowest irradiation dose used i.e., 1 kGy reduced TAPC by more than one log cycle (95.3%) from the initial counts of immersed control samples, while higher irradiation dose used (2 & 3 kGy) reduced TAPC by more than two log cycles (99.6 & 99.8%, respectively).

During refrigeration storage, there was an increase in TAPC in un-irradiated samples either immersed in only tap water or in tap water containing chemicals. This increase could be due to the proliferation and growth of psychrotrophic bacteria. TAPC in samples exposed to 1 kGy reached 1.5 × 10^4 cfu g^{-1} after 14 days of refrigeration storage. TAPC in samples exposed to 2 and 3 kGy very slightly increased during storage and did not reach their initial count before irradiation even after 14 days of refrigeration storage, being 1.6 × 10^2 and 3.8 × 10^1 cfu g^{-1}, respectively.

Data in Table II shows almost similar trend for LAB as in TABC. The initial counts of lactic acid bacteria on fresh-cut pear samples immersed in only tap water and in tap water containing 2% ascorbic acid and 1% calcium lactate were 3 × 10^2 and 1.8 × 10^2 cfu g^{-1}, respectively. Irradiation dose of 1 kGy reduced the initial counts by 61.1%. Meanwhile the counts of LAB in fresh-cut pear samples exposed to 2 and 3 kGy were less than 10 cfu g^{-1}. During refrigeration storage, LAB increased in samples immersed in only water or samples immersed in water containing ascorbic acid and calcium lactate. Fresh-cut pear samples exposed to 3 kGy did not show any growth of LAB throughout their storage period.

Table II also shows that the initial total mould and yeast counts in fresh-cut pear samples immersed in only tap water or in tap water containing chemicals were 8.5 × 10^2 and 6.5 × 10^2 cfu g^{-1}, respectively. Irradiation at 1 kGy

Table I. Microbiological quality (cfu g^{-1}) of fresh-cut pears

Sample No.	Microbial load			Indicator microorganisms				Pathogenic microorganisms		
	TAPC	LAB	TM and Y	*Coliform	*E. coli	Ent. faecalis	*S. aureus*	*A. hydrophila*	*L. monocyt-ogenes*	*Salmonella spp*
1	3.7×10^4	3.2×10^3	4.7×10^3	<3	<3	<100	5.0×10^2	<100	<100	ND
2	5.1×10^3	4.0×10^2	3.9×10^3	<3	<3	<100	<100	<100	<100	ND
3	1.1×10^4	1.0×10^2	5.3×10^3	<3	<3	<100	<100	<100	<100	ND
4	1.1×10^4	2.5×10^3	4.8×10^3	20	9	<100	<100	<100	<100	ND
5	2.3×10^3	7.0×10^2	1.9×10^3	<3	<3	<100	<100	<100	<100	ND
6	2.6×10^3	7.8×10^2	2.0×10^3	<3	<3	<100	<100	<100	<100	ND
7	4.0×10^3	7.0×10^1	3.2×10^2	<3	<3	<100	<100	<100	<100	ND
8	7.5×10^1	<10	<10	<3	<3	<100	<100	<100	<100	ND
9	5.0×10^2	9.2×10^1	2.0×10^2	<3	<3	<100	<100	<100	<100	ND
10	9.0×10^2	3.7×10^1	2.0×10^2	<3	<3	<100	<100	<100	<100	ND
11	4.0×10^2	2.0×10^1	1.0×10^2	<3	<3	<100	<100	<100	<100	ND
12	2.0×10^3	4.5×10^2	1.7×10^2	<3	<3	<100	<100	<100	<100	ND
13	6.5×10^2	1.1×10^2	2.2×10^2	<3	<3	<100	<100	<100	<100	ND
14	1.1×10^2	1.9×10^1	3.0×10^1	<3	<3	<100	<100	<100	<100	ND
15	3.5×10^4	9.0×10^2	1.0×10^3	43	9	<100	2.2×10^2	<100	<100	ND

* = MPN/g, ND = Not detected, < 100 & < 10 = Below detectable level
< 3 = No positive tubes have been detected in the first three dilutions

Table II. Effect of combination treatment irradiation on the microbial counts (cfu g^{-1}) of fresh-cut pears during refrigerated storage

Microorganisms	Storage period (days)	Control immersed in water only	Immersion in water containing 2% ascorbic acid and 1% calcium lactate and irradiated with different doses (kGy)			
			0.0	1.0	2.0	3.0
Total aerobic bacterial count (TAPC)	0	6.4×10^3	6.0×10^3	2.8×10^2	2.5×10	1.3×10
	7	3.0×10^4 R	1.9×10^4 R	8.0×10^3	1.5×10^2	2.5×10
	14	R	R	1.5×10^4	1.6×10^2	3.8×10
Lactic acid bacteria (LAB)	0	3.0×10^2	1.8×10^2	7.0×10	< 10	< 10
	7	5.5×10^3 R	3.1×10^3 R	8.1×10	< 10	< 10
	14	R	R	9.0×10	2.0×10	< 10
Total mould and yeast (M&Y)	0	8.5×10^2	6.5×10^2	1.0×10^2	< 10	< 10
	7	9.8×10^3 R	5.0×10^3 R	1.2×10^3	1.7×10	< 10
	14	R	R	6.7×10^3	5.0×10	< 10

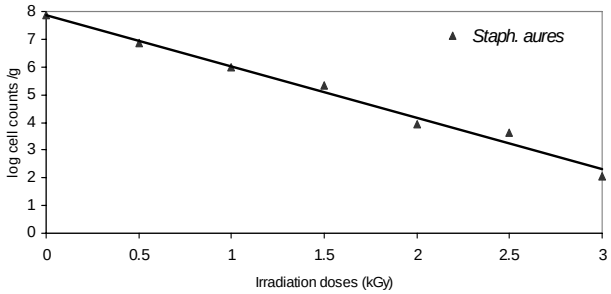
R = Samples sensorially rejected.

reduced the initial mould and yeast counts by 84.6%, meanwhile irradiation doses of 2 and 3 kGy reduced these counts to less than detectable level (< 10 cfu g^{-1}). Upon refrigeration storage, total mould and yeast counts in un-irradiated samples reached 3.5×10^4 cfu g^{-1} after two weeks, indicating that moulds and yeasts were the predominant micro-flora at the end of storage period. This is true and confirms the fact that the low pH of fruits restricted the growth of micro-flora rather than lactic acid bacteria and fungi. Moulds and yeasts in fresh-cut pear samples exposed to 3 kGy were below detectable level (< 10 cfu g^{-1}) all over the storage period.

Since none of the tested pathogenic bacteria was detected in non-irradiated (control) fresh-cut pears, the effect of irradiation on these pathogens was not studied.

Effect of combined treatment with irradiation on the amino acids content of fresh-cut pears. The profile of amino acids in fresh-cut pear and its change as a result of irradiation at the highest dose used (3 kGy) are shown in Table III. Un-irradiated fresh-cut pear contained 17 amino acids but at low concentrations. Total non-essential amino acids represented 69.6% from the total amino acids concentrations. Total essential amino acids represented only

Fig 1. Radiation dose response curves of *S. aures* in minimally processed fresh-cut pear



30.4%, indicating that pear fruits are poor in amino acids particularly essential amino acids. Aspartic acid was the predominant amino acid followed by glutamic acid. They were found in concentrations of 2.83 and 1.09 mg g^{-1}, respectively representing 27.7 and 10.7% from the total amino acids.

Irradiation at 3 kGy almost had no effect on cystine, methionine, isoleucine and leucine. This irradiation dose caused an increase in aspartic acid (9.2%), theronine (15.2%), serine (11.8%), glutamic (13.8%), praline (33%),

Table III. Effect of combination treatment irradiation on amino acids (mg/g sample on dry weight basis) profile in fresh-cut pear during refrigerated storage

Amino acid	Control	3 kGy	Amino acid	Control	3 kGy
Aspartic	2.83	3.09	Leucine*	0.71	0.71
Threonine*	0.46	0.53	Tyrosine	0.14	0.13
Serine	0.51	0.57	Phenyalanine*	0.40	0.39
Glutamic	1.09	1.24	Histidine*	0.19	0.18
Proline	0.97	1.29	Lysine*	0.36	0.32
Glycine	0.52	0.53	Arginine	0.30	0.28
Alanine	0.69	0.70	Total EAA	3.10	3.12
Cystine	0.05	0.05	Total non- EAA	7.10	7.88
Valine*	0.53	0.54	Total amino acid	10.20	11.00
Methionine*	0.01	0.01	EAA/NEAA	0.44	0.40
Isoleucine*	0.44	0.44			

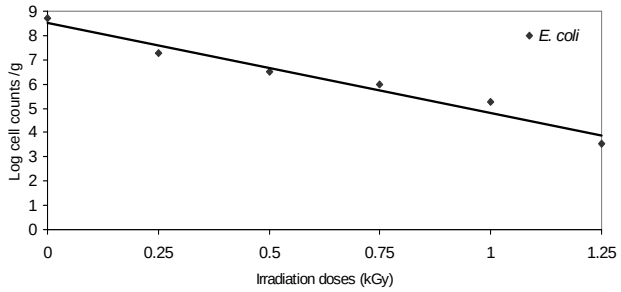
* Essential amino acids (Baxtre, 1996).

Table IV. Effect of combination treatment with irradiation on the ascorbic acid (mg/100g) content of fresh-cut pears during refrigerated storage

Storage period (days)	Control immersed in water only	Immersion in water containing 2% ascorbic acid and 1% calcium lactate and irradiated with different doses (kGy)			
		0.0	**1.0**	**2.0**	**3.0**
0	14.8 ±0.361^{gh}	16.0 ±0.101^{i}	15.4 ±0.400^{hi}	15.0 ±0.361^{ghi}	14.2 ±0.173^{g}
7	11.8 ± 0.153^{ef} R	12.2 ±0.265^{f} R	11.0 ±0.265^{de}	10.8 ±0.208^{de}	10.6 ±0.115^{ed}
14	0 ±0.00^{a}	0 ±0.00^{a}	9.7 ±0.265^{bc}	9.4 ±0.361^{b}	9.0 ±0.153^{b}

R = Samples sensorially rejected, Mean scores of three replicates ± standard deviation.
Means with different superscripts (a-i) are significantly different (P< 0.05).

Fig 2. Radiation dose response curves of *E. coli* in minimally processed fresh-cut pear



glycine (1.9%), alanine (1.4%) and valine (1.9%). On the other hand 3 kGy irradiation dose caused a decrease in tyrosine (7.1%), phenylalanine (2.5%), histidine (5.3%), lysine (2.8%) and arginine (3.3).

Effect of combined treatment with irradiation on the ascorbic acid (vitamin C) of fresh-cut pears. It is evident that irradiation at 1 and 2 kGy had no significant effect on the ascorbic acid content (AA) of fresh-cut pear (Table IV), while irradiation at 3 kGy significantly reduced ascorbic acid content. The percentage of loss in ascorbic acid was 3.8, 6.3 and 9.4% at dose levels of 1, 2 and 3 kGy, respectively. This loss could be attributed to oxidation of ascorbic acid (AA) by irradiation to dehydroascorbic acid (DHAA). This is expected due to the sensitive characteristics of ascorbic acid and irradiation takes place in the presence of air. Graham and Stevenson (1997) have shown that irradiation at doses of 1, 2 and 3 kGy reduced the AA content of strawberries and potatoes. Kilcast (1994) reported that ionizing radiation can cause a partial conversion of AA to DHAA.

Ascorbic acid in all un-irradiated and irradiated fresh-cut pear samples showed significant reduction upon refrigeration storage and the reduction increased with increasingly storage time. After 7 days of storage the loss of ascorbic acid reached 20 and 23.8% in control samples dipped in only water and in antibrowning and firmining agents, respectively. Meanwhile the loss in ascorbic acid content reached 28.6, 28 and 25.4% in fresh-cut samples irradiated at 1, 2 and 3 kGy, respectively.

Effect of combined treatment with irradiation on the polyphenol oxidase (PPO) activity of fresh-cut pears. The PPO activity (U g^{-1}) of pear peel, thin layer adjacent peel and pulp was measured and the results are shown in Fig. 3. The PPO activity was 2270, 2626 and 1910 U 100^{-1} g, respectively. This indicates that the highest PPO activity was found in the thin layer adjacent peel, while the lowest activity was found in pear pulp. Similar results have shown by Youssef *et al*. (2002) who found that PPO activity was 1468.0 U 100^{-1} g in mango pulp and was 7397.3 U 100^{-1} g in mango peel. This indicates that polyphenol oxidase in peel, beneath peel was higher than that in pulp. These results suggest removing of peel with the thin layer adjacent it (peeling of pear fruits) in the processing of fresh-cut pears to reduce browning.

The effect of treatment with ascorbic acid, as antibrowning agent, on PPO activity of peeled fresh-cut pear is shown in Table V. It is obvious that PPO activity of un-treated fresh-cut pears (dipped in only water) was 1907 U 100^{-1} g, while that of treated fresh-cut pears (dipped in water

Table V. Effect of combination treatment with irradiation on the polyphenol oxidase (PPO, U g^{-1}) of fresh-cut pears during refrigerated storage

Storage period (days)	Control immersed in water only	Immersion in water containing 2% ascorbic acid and 1% calcium lactate and irradiated with different doses (kGy)			
		0.0	**1.0**	**2.0**	**3.0**
0	1907 ±12.66^{g}	1821 ±28.02^{f}	1270 ±8.15^{b}	1148 ±8.145^{a}	1120 ±10.07^{a}
7	2075 ±9.851^{h} R	1838 ±10.82^{fg} R	1438 ±10.81^{d}	1349 ±10.81^{c}	1250 ±8.14^{b}
14	0 ±0.00^{a}	0 ±0.00^{a}	1670 ±22.91^{e}	1463 ±28.02^{d}	1320 ±10.81^{bc}

R = Samples sensorially rejected, Mean scores of three replicates ± standard deviation.
Means with different superscripts (a-h) are significantly different (P< 0.05).

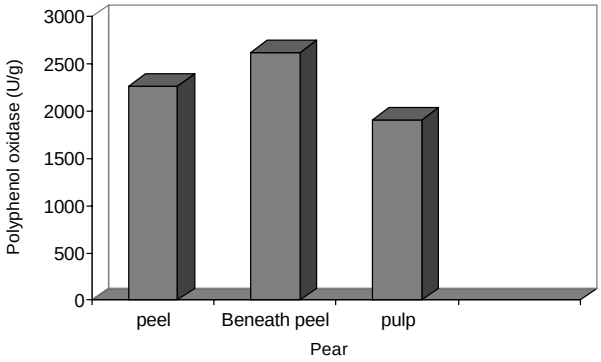
containing 2% ascorbic acid & 1% calcium lactate) was 1821 U 100^{-1} g. This indicates that the treatment of peeled fresh-cut pears with ascorbic acid significantly reduced PPO activity. Other investigators reported inhibition of PPO in fresh-cut apples and pears by treatment with ascorbic acid and calcium lactate or calcium chloride (Pizzocaro *et al.*, 1993; Soliva-Fortuny *et al*., 2002).

It is evident that irradiation reduced PPO activity and the reduction was proportional with irradiation dose (Table V). Irradiation doses of 1, 2 and 3 kGy significantly reduced PPO of fresh-cut pear samples by 30.3, 37.0 and 38.5%, respectively from the initial PPO activity in un-irradiated samples. This reduction in PPO activity could be attributed to the effect of irradiation on phenolic contents (substrates of PPO) of fresh-cut pears. Ahn *et al.* (2005) found that gamma irradiation at 1 kGy or above significantly reduced the phenolic contents in the cut Chinese cabbage. Reduction of phenolic compounds in the foods due to irradiation has been also reported by Villavicencio *et al.* (2000).

The PPO activity of irradiated fresh-cut pear samples significantly increased with increasing refrigeration storage period. This increase in PPO activity could be attributed to solubilization of PPO upon storage leading to higher activity as well as increasing concentration of total soluble phenols. Upon storage PPO becomes increasingly soluble and more extractable and the solubilization could occur as a result of membrane damage upon storage (Mayer & Harel, 1979). The results in Table V revealed that PPO activity of fresh-cut pear samples treated by ascorbic acid and calcium lactate was lower than that un-treated ones; this indicates that these chemicals significantly controlled PPO activity. Also, PPO activity of irradiated samples was lower than that of non-irradiated ones up to 14 days. Thus the enzymatic browning and changes in the color could be avoided and quality of fresh-cut pears would be maintained by using ascorbic acid (as an antibrowning agent) and calcium lactate (as a firmining agent) and irradiation at 1 or 2 kGy. Our results suggest that immersing of fresh-cut pears in 2% ascorbic acid + 1% calcium lactate solution for 3 min and irradiation at 2 kGy can be used to enhance the quality and safety of this fresh produce up to 14 days at 4°C ± 1.

Effect of combined treatment with irradiation on the firmness of fresh-cut pear. It is evident that firmness value (Table VI) of fresh-cut pear samples dipped in ascorbic acid and calcium lactate increased by 10.8%. Many investigators reported that calcium and its salts have been used to

Fig 3. Polyphenol oxidase (U g^{-1}) activity in pear peal, beneath peel and pulp



enhance firmness of a great variety of minimally processed fruits (Sapers & Miller, 1998; Soliva-Fortuny *et al.*, 2003).

Maintaining of firmness in fresh-cut fruits by application of calcium and its salts could be explained by interactions of calcium ions with pectic acid in the fruit cell wall to form calcium pectate, which firms molecular binding between constituents of cell wall as reported by Fennema (1985). Glenn and Poovaiah (1990) reported that infiltrated calcium in fresh-apples has been shown, by ultra-structural studies, to bind the cell wall and middle lamellae, where major influence on firmness is expected.

Table VI also shows that irradiation significantly reduced the firmness of fresh-cut pear samples and this reduction was proportional with irradiation dose. Firmness values of fresh-cut pear samples reduced by 19.2, 34.2 and 43.6% at irradiation dose levels of 1, 2 and 3 kGy, respectively. Gunes *et al.* (2001) found that firmness of fresh-cut apples decreased as irradiation dose increased beyond a 0.34 kGy threshold.

Upon refrigeration storage, the mean values of firmness were decreased in all fresh-cut pear samples. However, the rate of decrease was higher in control samples (immersed only in water) in comparison with those treated by chemicals where the rate of decrease was lower at the end of storage period (14 days). On the other hand, the rate of decrease in firmness values of irradiated samples was higher than that of non-irradiated ones. It is clear that the highest decrease in firmness values was observed with fresh-cut pear samples exposed to 3 kGy.

Effect of combined treatment with irradiation on the weight loss of fresh-cut pear. Table VII shows the

percentage of weight losses (%) of fresh-cut pear as affected by irradiation and refrigeration storage. There was a slight weight loss (not exceed 1%) in all the samples during storage. The maximum percentage of weight losses only reached 0.77 in fresh-cut pear samples exposed to 3 kGy.

Effect of combined treatment with irradiation on the sensory evaluation of fresh-cut pear. Table VIII shows that dipping of fresh-cut pears in water containing ascorbic acid (as antibrowning agent) and calcium lactate (as firmness agent) caused no significant changes in the mean scores of color, odor, texture and taste at zero time of storage. Many investigators reported that calcium salts are widely used as post harvest treatment to reduce softening (enhance firmness) of apple and pear fruits (Bangerth, 1979; Dong *et al.,* 2000).

Irradiation of fresh-cut pears had no significant effect on different sensory quality attributes. However, irradiation dose of 3 kGy decreased the mean sensory scores particularly texture scores (from 8.8 to 8.0) indicating that this dose had little pit an adverse effect on texture of fresh-cut pears. The use of irradiation to extend the shelf-life of raw fresh-fruits has been investigated extensively. However, many investigators reported that irradiation caused undesirable changes (softening) of fresh-fruits (Yu *et al*., 1996; Hegazi *et al.,* 2000).

During refrigeration storage, all sensory quality scores significantly decreased with increasing refrigeration storage period, but at different rates. According to visual observation the surface brown color of fresh-cut pear dipped only in water (control) developed at the same day of preservation and intensive complete browning was observed after 2 days of refrigeration storage (data not recorded). This means that the refrigeration shelf-life of fresh-cut pears was very short not exceeding 2 days indicating that the rapid onset of enzymatic browning is still a limiting factor for producing fresh-cut fruits such as fresh-cut apples and pears.

The color scores of non-irradiated (control) fresh-cut pears immersed in water containing 2% ascorbic acid and

Table VI. Effect of combination treatment with irradiation on the firmness (kg/cm^2) of fresh-cut pears during refrigerated storage

Storage period (days)	Control immersed in water only	Immersion in water containing 2% ascorbic acid and 1% calcium lactate and irradiated with different doses (kGy)			
		0.0	**1.0**	**2.0**	**3.0**
0	4.80 ±0.095^{fghi}	5.32 ±0.078^{i}	4.30 ±0.040^{efg}	3.50 ±0.100^{bcd}	3.00 ±0.275^{b}
7	4.53 ±0.137^{fgh} R	5.10 ±0.087^{hi} R	4.10 ±0.200^{def}	3.00 ±0.123^{b}	3.23 ±0.070^{bc}
14	0 ±0.00^{a}	0 ±0.00^{a}	3.82 ±0.167^{cde}	3.00 ±0.377^{b}	2.12 ±0.070^{a}

R = Samples sensorially rejected, Mean scores of three replicates ± standard deviation.
Means with different superscripts (a-i) are significantly different (P< 0.05).

Table VII. Effect of combination treatment with irradiation on the percentage of weight loss of fresh-cut pears during refrigerated storage

Storage period (days)	Control immersed in water only	Immersion in water containing 2% ascorbic acid and 1% calcium lactate and irradiated with different doses (kGy)			
		0.0	**1.0**	**2.0**	**3.0**
0	0.0 ±0.00^{a}	0.0 ±0.00^{a}	0.0 ±0.00^{a}	0.0 ±0.00^{a}	0.0 ±0.00^{a}
7	0.23 ±0.233^{b} R	0.26 ±0.152^{bc} R	0.36 ±0.021^{d}	0.33 ±0.012^{cd}	0.31 ±0.153^{cd}
14	0 ±0.00^{a}	0 ±0.00^{a}	0.59 ±0.026^{fg}	0.63 ±0.173^{g}	0.77 ±0.031^{h}

R = Samples sensorially rejected, Mean scores of three replicates ± standard deviation.
Means with different superscripts (a-i) are significantly different (P< 0.05).

Table VIII. Effect of combination treatment with irradiation on the sensory evaluation of fresh-cut pears during refrigerated storage

Parameters	Storage period (days)	Control immersed in water only	Immersion in water containing 2% ascorbic acid and 1% calcium lactate and irradiated with different doses (kGy)			
			0.0	**1.0**	**2.0**	**3.0**
Colour	0	8.8 ±0.349^{e}	8.8 ±0.258^{e}	8.6 ±0.210^{e}	8.6 ±0.394^{e}	8.3 ±0.258^{e}
	7	3.0 R ±0.624^{b} R	6.3 ±0.483^{d} R	6.5 ±0.471^{d}	6.3 ±0.587^{d}	6.0 ±0.527^{d}
	14	0 ±0.00^{a}	0 ±0.00^{a}	4.5 ±0.667^{c}	4.3 ±0.587^{c}	4.0 R ±0.577^{bc}
Odour	0	8.8 ±0.258^{f}	8.6 ±0.394^{f}	8.6 ±0.394^{f}	8.5 ±0.333^{f}	8.1 ±0.394^{f}
	7	3.3 R ±0.675^{b} R	5.7 ±0.753^{de} R	6.0 ±0.624^{e}	6.2 ±0.537^{e}	5.5 ±0.745^{cde}
	14	0 ±0.00^{a}	0 ±0.00^{a}	4.5 ±0.667^{bcd}	5.1 ±0.658^{cde}	4.3 ±0.483^{bc}
Texture	0	8.6 ±0.210^{f}	8.8 ±0.258^{f}	8.8 ±0.258^{f}	8.6 ±0.211^{f}	8.0 ±0.408^{f}
	7	6.3 ±0.421^{de} R	6.5 ±0.408^{e} R	6.5 ±0.408^{e}	6.0 ±0.408^{cde}	5.5 ±0.471^{bcd}
	14	0 ±0.00^{a}	0 ±0.00^{a}	5.3 ±0.578^{bc}	5.1 ±0.516^{bc}	4.9 ±0.516^{b}
Taste	0	8.9 ±0.211^{e}	8.7 ±0.258^{e}	8.7 ±0.258^{e}	8.5 ±0.333^{de}	8.5 ±0.333^{e}
	7	3.0 R ±0.333^{b} R	6.0 ±0.408^{d} R	5.8 ±0.258^{d}	6.2 ±0.587^{d}	5.8 ±0.422^{d}
	14	0 ±0.00^{a}	0 ±0.00^{a}	4.8 ±0.578^{c}	4.8 ±0.422^{c}	4.1 ±0.316^{c}

R = Samples sensorially rejected and therefore could not be used for the rest of the storage period
Mean scores of 10 panelists ± standard deviation. Means with different superscripts (a-f) are significantly different (P< 0.05).

1% calcium lactate reached the unacceptable level after 7 days of storage at 4°C ± 1 (Table VIII). Sapers and Miller (1998) observed minimal color changes for at least 14 days at 4°C in slightly under ripe Anjou and Barlett fresh-cut pears preserved with a combination of antibrowning agents and the use of modified atmosphere packaging (MAP). Although Gorny (1997) reported that the enzymatic browning of apple slices can be eliminated by use of modified packaging atmosphere with low oxygen levels, Luo and Barbosa-Canovas (1996) reported that such low levels could potentially result in anaerobic respiration and off-flavors. The antibrowning effect of ascorbic acid in fruits in concentration ranging from 0.5 to 4% have been widely demonstrated in several fruit fresh-cut products under a wide range of conditions (Agar *et al*., 1999; Soliva-Fortuny *et al.,* 2002). Generally, its inhibitory effect is due to the reduction of the ortho-quinones by the action of the polyphenol oxidase enzyme, back to the phenolic substances. Pizzocaro *et al.* (1993) reported that ascorbic acid has long been applied in combination with other acids calcium lactate to maintain firmness of fresh-cut fruits.

Sensory quality scores of fresh-cut pear samples exposed to 1 or 2 kGy still acceptable even after 14 days of refrigeration storage. The sensory quality scores of these samples were the best at any time of refrigeration storage, while the scores for those exposed to 3 kGy were the worst. This means that 3 kGy-irradiation dose was not suitable for irradiating fresh-cut pears. Combination treatment (dipping in 2% ascorbic acid & 1% calcium lactate) with irradiation (at 1 or 2 kGy) approved to be the optimum for shelf-life extension and maintain sensory quality of fresh-cut pears.

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