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Enhancing the Storability of Coated Broccoli Heads Using Irradiated Chitosan Combined with Olive Leaves Extract

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ABSTRACT

Broccoli is one of the most important non-traditional vegetable crops in Egypt. It gains its importance from its high nutritional value, but there are some obstacles facing broccoli storage, such as florets yellowing, high respiration rate and chlorophyll degradation, which lead to weight loss. So, this experiment was carried out to evaluate the effect of un-irradiated and irradiated chitosan (CS) with 25 kGy combined with 1 and 2% of olive leaves extract (OLE) as an edible coating to enhance the storage ability of broccoli heads under cold storage conditions (5°C and 90-95% RH). Results revealed that, broccoli heads coated by irradiated CS combined with 1% and/or 2% of OLE retained the highest quality and freshness for up to 28 days as manifested by the lowest weight loss percentage and decay score compared with uncoated broccoli heads. Particularly, irradiated CS combined with 1% OLE coated heads exhibited better general appearance, concomitant with higher chlorophyll, total soluble phenols and total free amino acids concentrations as well as higher antioxidant scavenging activity. Therefore, OLE incorporated with irradiated CS coatings; could presumably preserves the nutritional quality of broccoli heads up to 4 weeks cold storage period.

Introduction

Broccoli (*Brassica oleracea var. Italica*) is considered one of the most important vegetable crops of the family Brassicaceae. Egypt produces more than 120,000 tons of broccoli and cauliflower, and is 20th among the broccoli producing countries (FAO statistics, 2021). Consumers are increasingly incorporating broccoli into their diets, especially with increased awareness of its health benefits. It is a powerhouse of vitamins, minerals, fiber, and potent antioxidants like sulforaphane, making it an excellent supporter of immunity, heart, and bone health, and it may also contribute to cancer prevention (Guo et al., 2018). Although

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the high nutritional value of broccoli, its short shelf life, along with yellowing of florets are considered problems during the storage period (Hansen et al., 2001; Aubry et al., 2008). In the last few years different preservation methods had been developed to extend the shelf life of broccoli florets like controlled atmosphere, LED light, edible coating and chemical materials (Pintos et al., 2021 Zhang et al., 2022; Kabir et al., 2023).

Chitosan is a natural cationic polysaccharide; it is used as a food preservative and edible coating due to its biocompatibility, safety, safe degradation, antimicrobial effect and bio film characteristics (Sharkawy et al., 2020; Chen et al., 2024).

The irradiation of chitosan plays an important role in enhancing its solubility and elevating its role as an antioxidant agent (Ocloo et al., 2011). The reduction of chitosan molecular weight led to an increase in its antioxidant activity. Gamma irradiation breaks down the glycodic/acetal bond between consecutive positions and gives new active sites that scavenge the free radicals (Kheiri et al., 2016). Garcia et al. (2015) and Zahran et al. (2015) reported that the application of 25 kGy irradiation to chitosan coating pomegranate fruits enhanced fruit quality during cold storage at 5°C and 75% RH. Furthermore, the combination of chitosan and plant extract or essential oils showed an effective role in extending the shelf life of fruits like tomato and strawberries (Abdalla et al., 2023; Montes-Ramirez et al., 2024).

Egypt is a world leader in olive production, occupying a leading position in this field. Specifically, Egypt ranks first in the world in table olive production and seventh in overall olive production, with an estimated annual production of approximately 976,000 tons (Taha and Khalifa, 2025). The main byproducts of olive trees are leaves and branches obtained from tree pruning and harvesting (Molina-Alcaide and Yáñez-Ruiz, 2008). The total percentage of leaves obtained from pruning is about 25% and branches about 75% of the total dried weight of tree residues (Manzanares et al., 2017). The main component of olive leaves is phenolic compounds, which were obtained from leaves extraction. Therefore, it can be combined with some other natural materials to create edible coatings that help preserve food during storage, as they act as antioxidants and anti-fungal and anti-bacterial agents (Herrero et al., 2011; Khalatbary et al., 2012; Khalifa et al., 2016; Khwaldia et al., 2022; Selim et al., 2022). Edible coating obtained from byproducts like olive leaves have a good chance to decrease the environmental pollution. Additionally, the highest amount of antioxidants in olive leaves like hydroxytyrosol and tyrosol, can participate in the production of edible coatings enriched with antioxidant properties (Clodoveo et al., 2022). which protects the products from oxidative degradation and extends the shelf life and storage ability of fresh products.

Thus, this study aimed to evaluate the effect of un-irradiated and irradiated chitosan combined with olive leaves extract on the physical and chemical properties of broccoli heads stored under cold storage conditions (5°C and 90-95% RH).

Materials and Methods

Chemicals

Chitosan (CS) of high molecular weight, D.A.C. 81.2%, viscosity: 3600 cps, density: 0.15 g/ml, particle size: 95% pass 16mm, Mv 140,000-220,000 and degree of deacetylation >75% was obtained from El Alamia Company for Chemicals. Ethanol Absolute (99%) was obtained from Al-Gomhoria Company for medicines and medical supplies.

Broccoli cultivation

Broccoli (*Brassica oleracea var. Italica*) plantlets cv. Naxos F1 was obtained from a private nursery in Giza Governorate during the two successive seasons of 2023/2024 and 2024/2025. Plantlets were cultivated on the 1st of November in the experimental farm of the Natural Products Research Department, National Center of Radiation Research and Technology, Nasr City, Cairo, Egypt, during the two successive seasons.

All agricultural practices and pest management were carried out according to the recommendations of the Ministry of Agriculture and Reclamation Lands. Broccoli heads were harvested on 16th and 15th of January in both seasons at the marketable stage tightly closed, dark green buds with no signs of flowering. The head diameter was around 8 to 15 cm, and the stalks were firm and fresh in both seasons, and then were transported to the laboratory.

Olive leaves extraction

Leaves of olive cv. Picual were obtained from a private orchard in Sharkia Governorate. Leaves were collected during the spring season, then transported to the laboratory of plant physiology in the National Centre for Radiation Research and Technology, Nasr City, Cairo, Egypt. Leaves were washed with tap water, then distilled water and air dried at room temperature till the stability of their dry

weight. Dried leaves were ground in a blender, 10 and 20 g of leaf powder were macerated in 1000 ml of absolute ethanol and shaken for 48 hours using an electrical stirrer. The supernatants were filtered and concentrated using a rotary evaporator. The dried extract residues were reconstituted in distilled water to obtain 1 and 2% of olive leaf extracts. OLE was extracted according to Samad et al. (2019).

Irradiation process

Chitosan was irradiated with 25 kGy of gamma irradiation at the dose rate of: 0.66815 kGy/h (2023/2024) and: 0.58588 kGy/h (2024/2025) using the Indian gamma cell that uses ^{60}Co as a radiation source at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt.

Chitosan preparation

Chitosan solution was prepared according to the procedure described by Moreira et al. (2011)

GC-MS analysis

The chemical composition of the olive leaves' ethanolic extract was performed using a Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50°C and then increased by 5°C /min to 250°C held for 2 min. increased to the final temperature 300°C by 30°C /min and hold for 2 min. The injector and MS transfer line temperatures were kept at 270, 260°C, respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 4 min, and diluted samples of 1 µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source temperature was set at 200°C. The components were identified by comparison of their mass spectra with those of WILEY 09 and NIST 14 mass spectral database.

Broccoli heads coating and storage

Heads of broccoli were washed with tap water, distillated water and air died then were soaked in the mixture solution of un-irradiated and irradiated chitosan + incorporated with 1% as well as 2% of olive leaves extract for 5 minutes then air dried and packaged in clear and non-performed plastic bags, each bag contain one head under cold storage conditions at temperature of 5°C and 90 – 95% RH.

Weight loss percentage

Both uncoated and coated broccoli heads were weighed at the beginning of the storage period (zero time) and at 7, 14, 21 and 28 days of storage. Weight loss was calculated by taking differences between the initial and final weights, and thereafter they were expressed as a percentage.

Weight loss% =

$$\frac{\text{fruit initial weight} - \text{fruit weight on sampling date}}{\text{fruit initial weight}} \text{ x100}$$

Decay score

Decay score was measured on a scale of 1=non, 2=slight, 3=moderate, 4=severe and 5=extreme (Risse and Miller, 1986).

General appearance

It was determined as a score system of excellent> 9, good> 7 to 8.9, fair> 5 to 6.9, poor> 4.9 to 3, and unassailable> 2.9. The scale depends on morphological defects such as shriveling, fresh appearance, color change of heads and decay score. Heads rating (5) or below are considered unmarketable (Watada and Morris, 1966; Jimenez et al., 1998).

Chlorophyll a, b and total chlorophyll

Chlorophyll a, b and total chlorophyll (mg/g F.W.) were estimated by using a SP-V1000 spectrophotometer in broccoli heads according to Moran (1982).

Total soluble phenols

Total soluble phenols (mg/100g F.W.) were estimated in the methanolic extract using the colorimetric method. It was determined by the Folin-Denis reagent using a SP-V1000 spectrophotometer according to Swain and Hillis (1959).

Total free amino acids

Total free amino acids (mg/100g F.W.) in dry matter were estimated in methanolic extract using a colorimetric method. It was determined using a SP-V1000 spectrophotometer according to Jayaraman (1985).

Antioxidant activity

The antioxidant activity percentage of the sample extracts against 2,2-diphenyl-l-picrylhydrazyl (DPPH) radical was determined using a SP-V1000 spectrophotometer as described by Gulluce et al. (2004). Finally, antioxidant capa-

$$\text{Antioxidant capability (\%)} = 100 * \frac{\text{AbsDPPH} - \text{Abssample}}{\text{AbsDPPH}}$$

Scanning Electron Microscopy (SEM)

The outer peel (exocarp) of uncoated and coated heads with un-irradiated chitosan enriched with 1% and 2% of olive leaves extract as well as with irradiated chitosan en-

riched with 1% and 2% of olive leaves extract were fixed in 2.5% glutaraldehyde at 4°C for 24 hr and post-fixed in 1% osmium tetra oxide for one hour at room temperature (Harley and Fergusen ,1990). The specimens were then dehydrated with ascending concentrations of acetone, critical point dried, and finally sputter-coated with gold. The examination and photographing were done through a Joel Scanning Electron Microscope (JSM-30KV and 100X).

Experimental design and statistical analysis

The experiment was carried out in a completely Randomized Design with three replicates; each replicate contains five bags. The obtained data were statistically analyzed using MSTAT Computer Program Development Team, the MSTAT, (1989). To verify differences among means of various treatments, means were compared using Duncan's Multiple Range Test as described by Duncan (1955).

Results and Discussion

GC-MS analysis

The GC-MS analysis presented in **Table (1)** shows that around 28 compounds were detected in olive leaves ethanolic extract. The GC-MS chromatogram of olive leaves ethanolic extract shows that there were nepetalic acid, nepetalactone and phytol, which belong to terpenoid, iridoid mono terpenoid and diterpenoid. Fatty acids like tricinonoic acid, palmitic acid, oleic acid, margaric acid and 1-linoleoyl glycerol, as well as linoleoyl chloride, which belong to fatty acid chloride. L-3,4-dihydroxyphenylalanine as phenolic amino acid, 1, 25-Dihydroxyvitamin D3 and Vitamin E., Jasmone as ketone. D-glucose as Monosaccharide, Thyophyllidine as methylxanthine, Dibutylphthalate as benzoic acid esters. Methyl jasmonate as oxylipine phytohormone, gibberellic acid as phytohormone, carboxylic acid, like 10,12-Pentacosadiynoic acid. Arctiopicrin as germacranolide, dotriacontane as alkane and 2-Monolinoleoylglycerol trimethylsilyl ether as trimethylsilyl ether. Jasmone was the most abundant compound with an area peak percentage of 14.87%, followed by Linoleoyl chloride with an area peak percentage of 13.17%. Also, palmitic acid was present at an area peak percentage of 9.2%. Furthermore, Neophytadiene was presented at the percentage of 8.31%, followed by Dibutyl phthalate at 5.51%. Phytol at the percentage of 4.39%, Tricinonic acid at the percentage of 4%, Vitamin E at the percentage of 3.98% and Ambrein at the percentage of 3.63%.

Weight loss percentage

As presented in **Figure (1 a and b)**, broccoli heads coated with irradiated chitosan and 1% of olive leaves extract scored the lowest weight loss percentage during the storage period in the two successive seasons taking into consideration that there was an increase in weight loss percentage in all treatments at the end of storage period, the highest one was scored in un-coated heads. Same findings were obtained by Hussein et al. (2020), who found that broccoli florets coated with chitosan scored the lowest weight loss percentage during cold storage. Additionally, El Sayed et al. (2022) observed that green bean pods coated with 3% olive leaves and Zn scored a lower weight loss percentage than uncoated pods. This finding might be related to the effect of edible coating as a barrier against gas exchange, which creates a modified atmosphere around florets and delays the metabolic process such as enzymatic activity and respiration, as well as water loss.

Decay score

Regarding decay score, **Figure (2 a and b),** there was a gradual increase in decay score through the storage period and reaching its maximum at the end of storage period. Whereas broccoli heads coated with irradiated chitosan and 1% of olive leaves extract scored the lowest decay score during the storage period. This result agrees with that obtained by Mahmoud et al. (2017), who found that navel orange coated with chitosan combined with 4% of olive leaves extract scored the lowest decay score during the storage period. This decreasing in decay score might be related to the defensive effect of olive leaves extract on fruit surface which was referred to the phenolic compounds such as caffeic acid, verbascoide and oleuropein olive leaves extract (Pereira et al., 2007) which delay pathogenic infection, in addition to the positive effect of chitosan as a barrier against water loss and gas exchange.

General appearance

As for general appearance **Figure (3 a and b)**, results indicated that the lowest general appearance score was marked in uncoated heads during the storage period and marked the lowest score at the end of the storage period, with consideration that there was a decrease in general appearance score of all treatments through the storage period. On the other hand, it was marked that broccoli heads coated with irradiated chitosan with 1% of olive leaves extract scored the highest general appearance in both seasons. Never-

theless, there was no significant difference between heads coated with irradiated chitosan with 1% and 2% of olive leaves extracts in the second season. Similar findings were obtained by Hussein et al. (2020), who found that broccoli heads coated with chitosan scored the highest general appearance score. Further, Elsayed et al. (2022) observed that green bean pods coated with 3% of olive leaves extract with Zn showed the highest scores of general appearances through the storage period. Such results concomitant with those of weight loss percentage and decay score reflect the positive effect of irradiated chitosan and 1% of olive leaves extract in both seasons.

Table (1): GC_MS analysis of ethanolic extract of olive leaves extract.

No.	RT	Compound Name	Area %	Molecular Formula	Molecular Weight	Compound nature
1	12.49	Nepetalic acid	1.17	$C_{10}H_{16}O_3$	184	Terpenoid
2	13.48	Nepetalactone	2.34	$C_{10}H_{14}O_2$	166	Iridoid monoterpenoids
3	17.01	D-glucose	1.33	$C_6H_{12}O_6$	180	Monosaccharide
4	21.52	Tricinonoic acid	4.00	$C_{15}H_{24}O_3$	252	Fatty acid
5	22.06	l-3,4-Dihydroxyphenylalanine	2.45	$C_9H_{11}NO_4$	197	Phenolic amino acid
6	22.29	Theophyllidine	1.15	$C_6H_{10}N_4O$	154	Methylxanthine
7	22.61	Jasmone	14.87	$C_{11}H_{16}O$	164	Ketone
9	24.63	Dibutyl phthalate	5.51	$C_{16}H_{22}O_4$	278	Benzoic acid esters
10	24.88	Neophytadiene	8.31	$C_{20}H_{38}$	278	Diterpenoid
11	25.08	Methyl jasmonate	0.48	$C_{13}H_{20}O_3$	224	Oxylipin phytohormones
12	26.37	10,12-Pentacosadiynoic acid	0.75	$C_{25}H_{42}O_2$	374	Carboxylic acid
13	27.19	Palmitic acid	9.20	$C_{16}H_{32}O_2$	256	Fatty acid
14	29.56	Oleic acid	0.73	$C_{19}H_{36}O_2$	296	Fatty acid
15	29.70	1,25-Dihydroxyvitamin D3	0.54	$C_{30}H_{52}O_3Si$	488	Vitamin E
16	29.89	Phytol	4.39	$C_{20}H_{40}O$	296	Diterpenoid
17	30.35	Linoleoyl chloride	13.17	$C_{18}H_{31}ClO$	298	Fatty acid chloride
18	30.86	Thiosulfuric acid	1.66	$C_2H_7NO_3S_2$	157	Sulfurous acid
19	36.32	Eupatorin	1.07	$C_{18}H_{16}O_7$	344	Flavone
20	36.60	Diethylhexyl phthalate	5.45	$C_{24}H_{38}O_4$	390	Phthalates
21	38.99	Margaric acid	1.72	$C_{17}H_{30}O_2$	266	Fatty acid
22	39.58	Gibberellic acid	0.55	$C_{19}H_{22}O_6$	346	Phytohormone
23	39.58	Arctiopicrin	0.55	$C_{19}H_{26}O_6$	350	Germacranolide
24	40.70	Ambrein	3.63	$C_{30}H_{52}O$	428	Tricyclic triterpene alcohol
25	41.60	Dotriacontane	1.93	$C_{32}H_{66}$	450	Alkane
26	43.39	1-Linoleoyl glycerol	0.45	$C_{27}H_{52}O_4Si_2$	496	Fatty acid
27	43.83	Vitamin E	3.98	$C_{29}H_{50}O_2$	430	Vitamin E
28	44.93	2-Monolinoleoylglycerol trimethylsilyl ether	0.65	$C_{27}H_{54}O_4Si_2$	498	Trimethylsilyl ether

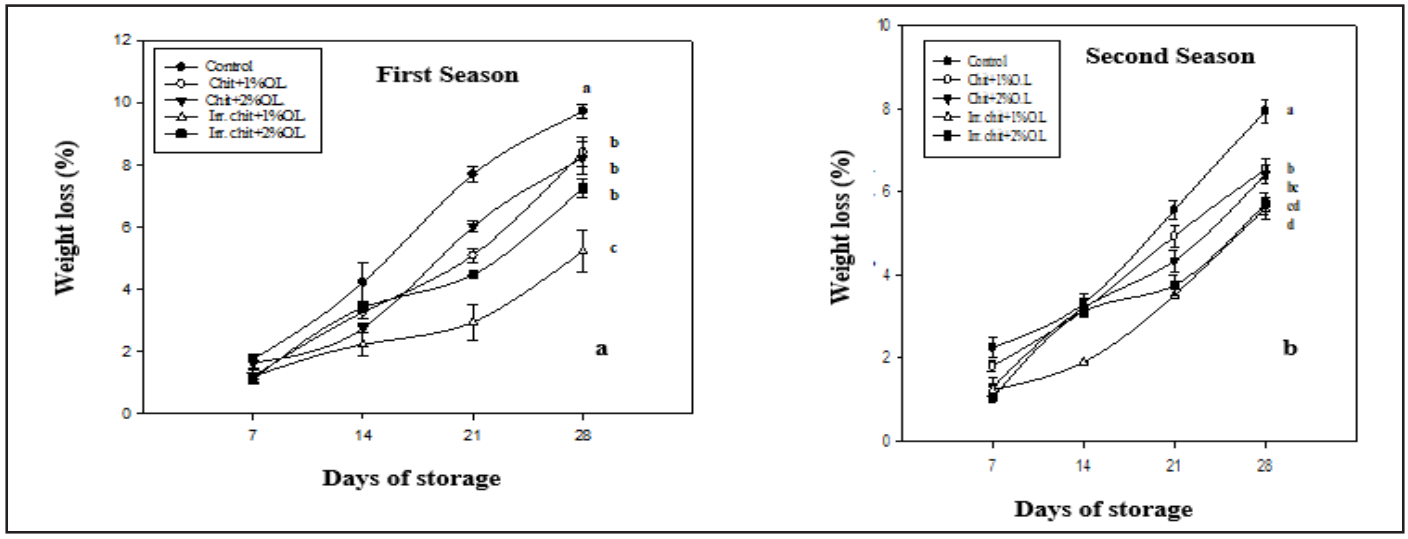

Figure 1(a) and (b): Effect of both un-irradiated and irradiated chitosan with 1 and 2% OLE on weight loss percentage of broccoli heads during storage period at (2023/2024) and (2024/2025) seasons.

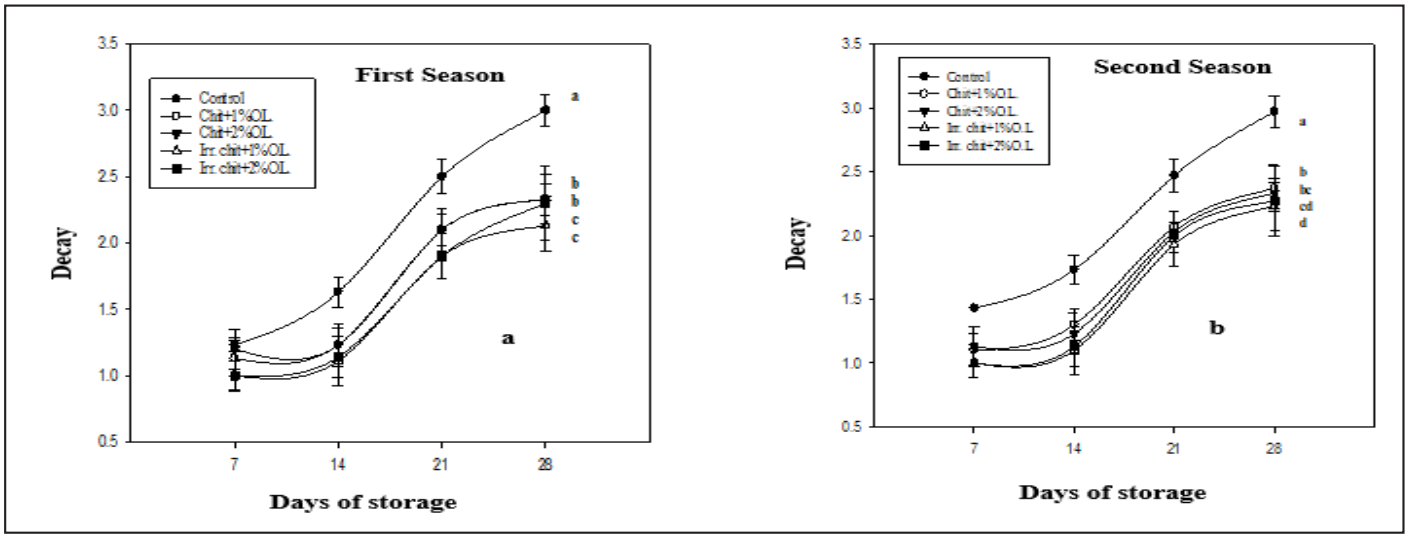

Figure 2 (a) and (b): Effect of both un-irradiated and irradiated chitosan with 1 and 2% OLE on decay score of broccoli heads during storage period at (2023/2024) and (2024/2025) seasons.

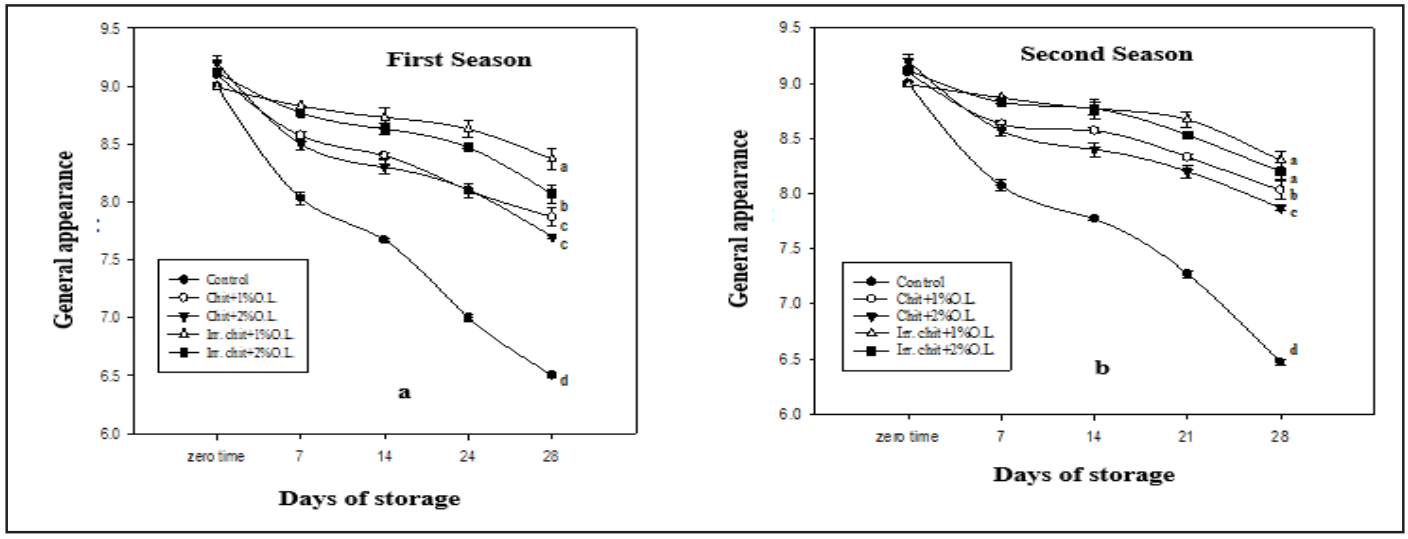

Figure 3 (a) and (b): Effect of both un-irradiated and irradiated chitosan with 1 and 2% OLE on general appearance of broccoli heads during storage period at (2023/2024) and (2024/2025) seasons.

Chlorophyll a, b and total chlorophyll

Data presented in **Table (2)** showed that there was a decrease in chlorophyll a concentration for all treatments by increasing the storage period in both seasons. The highest concentration of chlorophyll a was observed in broccoli heads coated with irradiated chitosan combined with 1% of olive leaf extract, while the lowest concentration was found at 28 days of storage period in un-coated heads. Same results were obtained by Hernández et al. (2017), who declared that the increment of storage period led to a decrease in chlorophyll a concentration of stored fresh cut broccoli. On the other hand, the increment of storage period led to a decrease in chlorophyll b concentration in both seasons, which agreed with previous findings obtained by Hernández et al. (2017). However, broccoli heads coated with irradiated chitosan and 1% of olive leaf extract scored the highest concentration of chlorophyll b till the end of the storage period (28 days) in both seasons **Table (2)**. Results also indicated that there was a decrease in total chlorophyll concentration through the prolongation of the storage period. Whereas broccoli heads coated with irradiated chitosan mixed with 1% followed by 2% of olive leaves extract scored the highest concentration of total chlorophyll till the end of the storage period compared with the other treatments. This result was in agreement with Hussein et al. (2020) who found that, broccoli florets coated with chitosan scored the highest value of total chlorophyll concentration during the storage period under cold storage conditions (0°C and 90-95% RH). The influence of chitosan in maintaining total chlorophyll content of broccoli heads might be attributed to its role in reducing the diffusion of oxygen and increasing carbon dioxide around the florets and as a gas barrier which delays the activity of enzymes like chlorophyllase which is responsible for chlorophyll pigment degradation (Kaewsuksaeng et al., 2006).

Table (2): Effect of un-irradiated and irradiated chitosan combined with 1% and 2% of olive leaves extract edible coating on chlorophyll a, b and total chlorophyll (mg/g F.W.) of broccoli heads stored during 2023/2024 and 2024/2025 seasons.

		Days after storage (2023/2024) and (2024/2025) seasons											
		0	7	17	21	28	Mean	0	7	17	21	28	Mean
Chlorophyll a (mg/g F.W.)	T_1	23.00^{a}	15.48^{g}	14.75^{h}	11.86^{j}	8.400^{k}	14.70^{E}	22.00^{a}	15.87^{h}	14.13^{i}	11.28^{k}	7.84^{i}	14.23^{E}
	T_2	23.00^{a}	18.08^{e}	16.26^{f}	15.24^{gh}	11.32^{j}	16.78^{D}	22.00^{a}	17.71^{f}	16.15^{gh}	13.50^{i}	12.25^{j}	16.32^{D}
	T_3	23.00^{a}	19.90^{d}	18.43^{e}	18.15^{e}	11.73^{j}	18.24^{C}	22.00^{a}	18.98^{e}	18.72^{e}	17.02^{fg}	12.31^{j}	17.81^{C}
	T_4	23.00^{a}	23.15^{a}	21.50^{bc}	20.95^{c}	16.77^{f}	21.07^{A}	22.00^{a}	21.40^{ab}	20.70^{bc}	20.36^{cd}	17.46^{f}	20.38^{A}
	T_5	23.00^{a}	21.71^{b}	21.27^{bc}	19.99^{d}	13.10^{i}	19.81^{B}	22.00^{a}	19.60^{de}	18.81^{e}	17.73^{f}	16.12^{gh}	18.85^{B}
	Mean	23.00^{A}	19.66^{B}	18.44^{C}	17.24^{D}	12.27^{E}		22.00^{A}	18.71^{B}	17.71^{C}	15.98^{D}	13.20^{E}	
Chlorophyll b (mg/g F.W.)	T_1	7.347^{a}	6.619^{b-d}	5.078^{gh}	4.420^{ij}	4.252^{j}	5.543^{D}	8.416^{a}	5.313^{de}	5.188^{e}	4.683^{fg}	4.193^{h}	5.559^{D}
	T_2	7.347^{a}	6.885^{a-c}	5.771^{ef}	5.244^{gh}	4.821^{hi}	6.014^{C}	8.416^{a}	6.264^{c}	5.626^{d}	4.998^{ef}	6.264^{gh}	5.951^{C}
	T_3	7.347^{a}	6.589^{cd}	6.141^{de}	6.049^{e}	5.544^{fg}	6.334^{B}	8.416^{a}	7.331^{b}	6.401^{c}	6.266^{c}	6.110^{c}	6.905^{B}
	T_4	7.347^{a}	7.260^{a}	7.110^{ab}	7.063^{a-c}	7.000^{a-c}	7.156^{A}	8.416^{a}	8.135^{a}	7.197^{b}	6.345^{c}	6.299^{c}	7.278^{A}
	T_5	7.347^{a}	7.144^{a}	6.193^{de}	5.912^{ef}	5.167^{gh}	6.353^{B}	8.416^{a}	7.261^{b}	6.401^{c}	5.213^{e}	4.301^{h}	6.104^{C}
	Mean	7.347^{A}	6.899^{B}	6.059^{C}	5.738^{D}	5.357^{E}		8.416^{A}	6.861^{B}	5.948^{C}	5.501^{D}	5.070^{E}	
Total chlorophyll (mg/g F.W.)	T_1	30.34^{a}	22.10^{h}	19.83^{i}	16.28^{l}	12.65^{m}	20.24^{E}	30.41^{a}	21.19^{gh}	19.32^{i}	15.96^{j}	12.04^{k}	19.78^{D}
	T_2	30.34^{a}	24.96^{f}	22.03^{h}	20.49^{i}	16.15^{l}	22.79^{D}	30.41^{a}	23.98^{ef}	21.78^{g}	18.50^{i}	16.70^{j}	22.27^{C}
	T_3	30.34^{a}	26.49^{e}	24.57^{fg}	24.19^{fg}	17.28^{k}	24.58^{C}	30.41^{a}	26.31^{c}	25.12^{d}	23.28^{ef}	18.42^{i}	24.71^{B}
	T_4	30.34^{a}	30.41^{a}	28.61^{bc}	28.01^{cd}	23.77^{g}	28.23^{A}	30.41^{a}	29.53^{a}	27.90^{b}	26.71^{c}	23.76^{ef}	27.66^{A}
	T_5	30.34^{a}	28.86^{b}	27.46^{d}	25.90^{e}	18.27^{j}	26.17^{B}	30.41^{a}	26.86^{c}	24.14^{de}	22.94^{f}	20.42^{h}	24.96^{B}
	Mean	30.34^{A}	26.56^{B}	24.50^{C}	22.98^{D}	17.62^{E}		30.41^{A}	25.57^{B}	23.65^{C}	21.48^{D}	18.27^{E}	

- T_1: Control, T_2: un-irradiated chitosan+1% olive leaves extract, T_3: un-irradiated chitosan+2% olive leaves extract, T_4: irradiated chitosan+1% olive leaves extract, T_5: irradiated chitosan+2% olive leaves extract.
- Duncan's multiple range test (DMRT) was used to compare the mean values between pairs of treatments. Means followed by the same letters are not significantly different at 5% level.

Total soluble phenols

As for total soluble phenols concentration of broccoli heads during storage period data presented in **Table (3)** show that, the increase of storage period led to a decrease in the concentration of total soluble phenols via the storage period. Meanwhile, broccoli heads coated with irradiated chitosan, 1% of olive leaf extract, scored a higher concentration of total soluble phenols than other treatments during the storage period in the two successive seasons. Such results were consistent with Hernández et al. (2017), who reported that fresh-cut broccoli showed a decrease in phenolic compounds through the cold storage period. Also, Khalifa et al. (2016) who found that strawberry fruit coated with chitosan incorporated with olive processing waste had higher concentration of total phenolic compounds than uncoated fruits.

The effect of chitosan on maintaining total soluble phenols might be related to its role in eliminating the reactive oxygen species scavengers that lead to an increase in phenolic contents (Jongsri et al., 2016), and also might be related to the high amount of phenolic compounds in olive leaves extract l-3,4-Dihydroxyphenylalanine. The lowest content of phenols in uncoated heads might be related to the high respiration rate and the degradation of cells (Zhang et al., 2018; EL-Bauome et al., 2022).

Data in **Table (3)** showed that there was a decline in total free amino acid concentration in both uncoated and coated heads throughout the storage period. Similarly, Hansen et al. (2001) found that the amino acids of stored broccoli florets decreased during storage. On the other hand, broccoli heads coated with irradiated chitosan with 1% olive leaves

Table (3): Effect of un-irradiated and irradiated chitosan combined with 1% and 2% of olive leaves extract edible coating on total soluble phenols (mg/100g F.W.), total free amino acids (mg/100g F.W.), and DPPH (%) of broccoli heads stored during 2023/2024 and 2024/2025 seasons.

		Days after storage (2023/2024) and (2024/2025) seasons											
		0	7	14	21	28	Mean	0	7	14	21	28	Mean
Total soluble phenols (mg/100gF.W.)	T_1	0.390^{j}	0.464^{ij}	0.661^{e-h}	0.681^{d-h}	0.865^{cd}	0.612^{c}	0.330^{i}	0.462^{hi}	0.608^{e-h}	0.723^{c-f}	0.841^{c}	0.593^{c}
	T_2	0.390^{j}	0.538^{h-j}	0.714^{c-h}	0.714^{c-h}	0.900^{c}	0.651^{bc}	0.330^{i}	0.535^{gh}	0.640^{d-h}	0.733^{c-e}	0.892^{c}	0.626^{c}
	T_3	0.390^{j}	0.543^{g-j}	0.753^{c-f}	0.727^{c-g}	1.214^{b}	0.725^{b}	0.330^{i}	0.550^{f-h}	0.717^{c-g}	0.781^{c-e}	1.209^{b}	0.717^{b}
	T_4	0.390^{j}	0.649^{f-i}	0.823^{c-f}	0.843^{c-e}	1.787^{a}	0.898^{a}	0.330^{i}	0.648^{d-g}	0.811^{cd}	0.817^{cd}	1.764^{a}	0.874^{a}
	T_5	0.390^{j}	0.648^{f-i}	0.792^{c-f}	0.738^{c-f}	1.688^{a}	0.851^{a}	0.330^{i}	0.640^{d-h}	0.743^{c-e}	0.782^{c-e}	1.670^{a}	0.833^{a}
	Mean	0.390^{d}	0.568^{c}	0.749^{b}	0.741^{b}	1.291^{a}		0.330^{d}	0.567^{c}	0.704^{b}	0.767^{b}	1.275^{a}	
Total free amino acids (mg/100gF.W.)	T_1	25.84^{a}	18.68^{ef}	8.27^{j}	15.78^{gh}	13.35^{hi}	16.38^{D}	26.20^{a}	17.19^{fg}	7.74l	15.09^{hi}	11.80^{k}	23.19^{A}
	T_2	25.84^{a}	18.68^{ef}	11.74^{i}	16.52^{fg}	13.53^{hi}	17.26^{D}	26.20^{a}	19.48^{de}	11.50k	16.01^{gh}	12.59^{jk}	21.16^{B}
	T_3	25.84^{a}	20.05^{de}	22.05^{b-d}	16.57^{fg}	13.56^{hi}	19.61^{C}	26.20^{a}	21.19^{d}	23.15c	17.42^{fg}	14.15^{ij}	20.42^{B}
	T_4	25.84^{a}	23.94^{ab}	23.58^{ab}	20.27^{c-e}	17.4^{fg}	22.21^{A}	26.20^{a}	25.60^{ab}	24.99^{a-c}	20.97^{d}	18.20^{ef}	17.15^{C}
	T_5	25.84^{a}	22.64^{bc}	22.75^{bc}	18.83^{ef}	13.98^{hi}	20.81^{B}	26.20^{a}	23.97^{bc}	23.62^{c}	17.63^{e-g}	14.38^{h-j}	15.60^{D}
	Mean	25.84^{A}	20.80^{B}	17.68^{C}	17.59^{C}	14.37^{D}		26.20^{A}	21.48^{B}	18.20^{C}	17.42^{C}	14.23^{D}	
D.P.P.H. (%)	T_1	94.00^{ab}	87.60^{f-h}	87.30^{gh}	83.70^{i}	50.40^{l}	80.60^{D}	92.00^{b}	86.10^{ef}	86.10^{ef}	83.40^{g}	50.10^{k}	79.54^{E}
	T_2	94.00^{ab}	90.00^{d-f}	88.80^{f-g}	84.00^{i}	57.20^{k}	82.80^{C}	92.00^{b}	89.40^{b-d}	88.20^{de}	84.80^{fg}	54.80^{j}	81.84^{D}
	T_3	94.00^{ab}	90.73^{c-e}	89.10^{e-g}	86.00^{hi}	59.00^{k}	83.77^{C}	92.00^{b}	90.60^{b-d}	88.40^{c-e}	85.80^{e-g}	58.40^{i}	83.04^{C}
	T_4	94.00^{ab}	95.70^{a}	92.40^{b-d}	90.90^{c}-^{e}	87.20^{gh}	92.04^{A}	92.00^{b}	94.80^{a}	91.20^{b}	90.30^{b-d}	86.60^{ef}	90.98^{A}
	T_5	94.00^{ab}	93.00^{bc}	92.10^{b-d}	87.30^{gh}	78.90^{j}	89.06^{B}	92.00^{b}	90.90^{bc}	90.60^{b-d}	86.70^{ef}	78.00^{h}	87.64^{B}
	Mean	94.00^{A}	91.41^{B}	89.94^{C}	86.38^{D}	66.54^{E}		92.00^{A}	90.36^{B}	88.90^{C}	86.20^{D}	65.58^{E}	

- T_1: Control, T_2: un-irradiated chitosan+1 % olive leaves extract, T_3: un-irradiated chitosan+2 % olive leaves extract, T_4: irradiated chitosan+1 % olive leaves extract, T_5: irradiated chitosan+2 % olive leaves extract.
- Duncan's multiple range test (DMRT) was used to compare the mean values between pairs of treatments. Means followed by the same letters are not significantly different at 5 % level

extract followed by irradiated chitosan with 2% olive leaves extract, compared to other treatments and uncoated heads. As presented in **Table (3)**, data revealed that there was a decrease in DPPH scavenging percentage through the storage period. While broccoli heads coated with irradiated chitosan with 1% olive leaves extract, followed by irradiated chitosan with 2% olive leaves extract scored the highest DPPH percentage compared with other treatments and uncoated heads. The same result was obtained by El-Sayed et al. (2022), who stated that coated broccoli heads with whey protein incorporated with mango peel extract scored the highest percentage of antioxidant activity during the storage period.

The high percentage of antioxidant compound in ethanolic olive leaves extract like Jasmone, Linoleoyl chloride, Palmitic acid, Dibutyl phthalate, Phytol, Tricinonoic acid, Vitamin E and Ambrein presented in **Table (1)** might be reflect on increasing the percentage of total soluble phenols, total free amino acids and total antioxidant activity, consequently decreasing chlorophyll degradation, scoring high general appearance, and maintain broccoli heads during storage period.

Microstructure observation

Broccoli heads coated with both un-irradiated and irradiated chitosan enriched with 1% and 2% olive leaves extract showed uniformity in coating distribution on the head surface, also, pores were not observed more than uncoated heads' pores. The homogenizations of edible coating on broccoli heads were marked more in irradiated chitosan mixed with 2% olive leaves extract **Figure (4e)** followed by 1% olive leaves extract **Figure (4d)** then un-irradiated chitosan combined with 1% olive leaves extract **Figure (4b)** and 2% olive leaves extract **Figure (4c**) than uncoated heads **Figure (4a).** Also, the coated head with irradiated chitosan combined with 1% of olive leaves extract was shiny and had more covering of irregular surface of broccoli heads. This indicates that there was more extensibility of the edible coating solution dispersion on the covered head surface, which plays an important role in decreasing water evaporation from heads than uncoated heads (Khalifa et al., 2016).

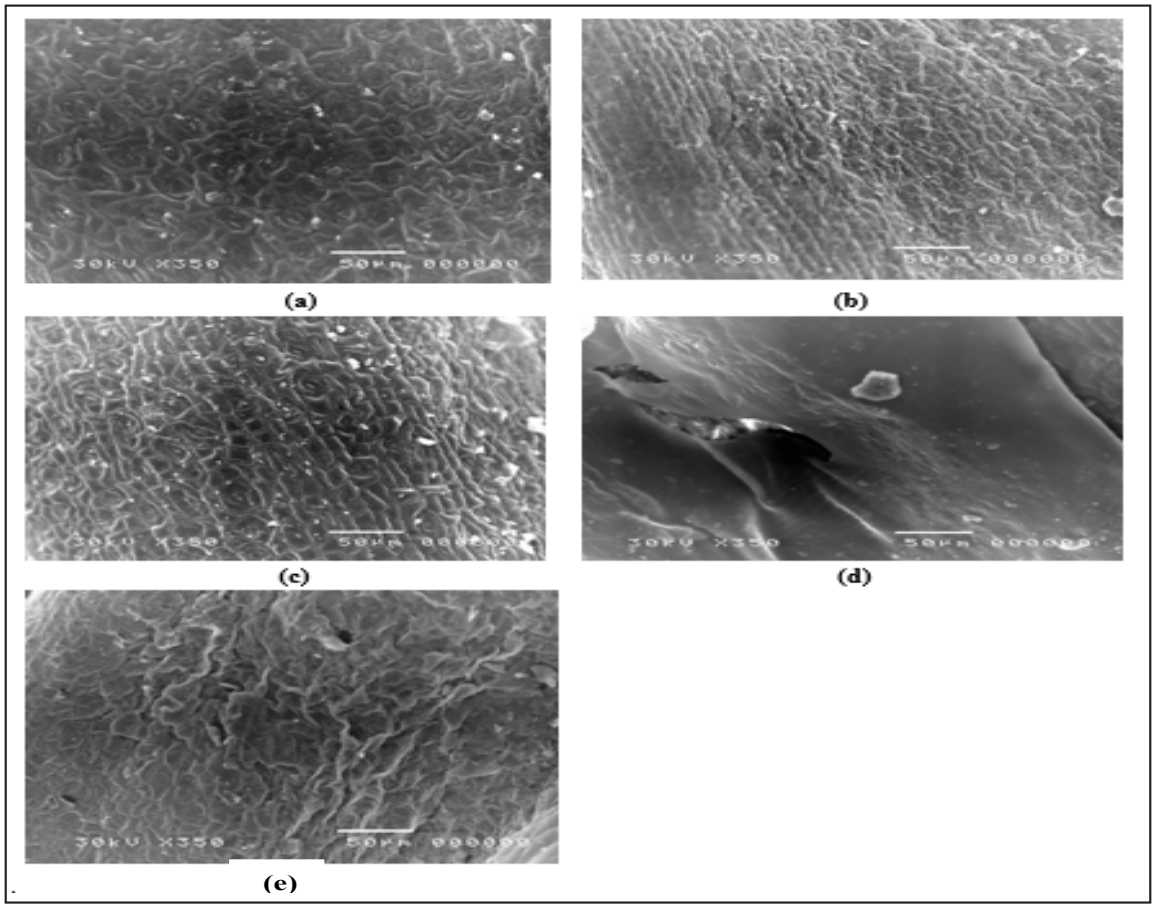

Figure (4): (a) uncoated broccoli head, (b)broccoli head coated with 1% OLE with un irradiated chitosan, (c) broccoli head coated with 2% OLE with un irradiated chitosan, (d) broccoli head coated with 1% OLE with irradiated chitosan and (e) broccoli head coated with 2% OLE with irradiated chitosan.

Conclusion

This study examined the effect of un-irradiated and irradiated chitosan combined with olive leaf extract on the physicochemical properties of broccoli heads stored under cold storage conditions (5°C and 90-95% RH). SEM showed that the usage of 25 kGy irradiation improved the edible film properties; the treated broccoli heads were shinier and had more coverage of the irregular surface compared with those of un-irradiated chitosan or uncoated. Further, the treated broccoli heads with irradiated chitosan combined with 1% of olive leaves extract remained at their optimal quality for 28 days at a temperature of 5°C and 90-95% RH. As reflected by the best general appearance, higher concentration of pigments (chlorophyll a, b and total chlorophyll), total soluble phenols and total free amino acids as well as total antioxidant percentage, compared with uncoated broccoli heads. Therefore, the study recommends using irradiated chitosan (25 kGy) incorporated with 1% olive leaf extract to preserve broccoli heads during storage or market display**.**

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