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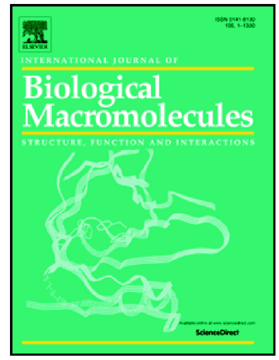

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Effect of Edible Co-polymers Coatings using γ -irradiation on Hyani Date fruit behavior During Marketing

Asmaa Ezz El-Dein[a], Ehab E. Khozemy[b], Serag A. Farag[a], N. Abd El-Hamed[c] And I. M. Dosoukey[c]

[a] *Food Irradiation Dept., National Centre for Radiation Research & Technology Atomic Energy Authority, P.O. Box. 8029 Nasr City, Cairo, Egypt*
[b] *Polymer chemistry Dept., National Centre for Radiation Research & Technology Atomic Energy Authority, P.O. Box. 8029 Nasr City, Cairo, Egypt*
[c] *Horticulture Dep., Faculty of Agriculture, Ain Shams University, P.O. Box. 68, Hadayek Shobra, Cairo, Egypt*

Abstract

The present work introduces a preparation of coating fruits film from natural biodegradable materials with evaluation of its efficiency in keeping the quality of fresh date fruits. Triple blend (Tb) which involved PVA, chitosan (Cs) and tannic (TA) acids was studied in preservation of Rutab (Hyani) date. Antimicrobial characters besides decay of fruits during a cold storage were determined. The blend solutions were exposed to the γ –irradiation (5.0 to 20 kGy) before casting or use. The effects of polymer composition and irradiation dose on the mechanical and thermo-mechanical properties were studied. The obtained results showed that γ- irradiation and the addition of tannic acid (TA) increased the mechanical properties of the films and the shelf-life of Rutab (Hyani) date during the marketing period (12±2 ^{0}C, 98 %, RH) from one week to one month of marketing period for consumers with accepted freshness and quality.

Keywords: Irradiation, dates, Hyani, edible coatings, PVA, chitosan, tannic acid

1. Introduction

Egypt is the largest date (*Phoenix dactylifera* L.) producer in the world with an average of 1.5 million tons per year with different varieties [1]. Hayani is the most important soft variety which harvested at rutab stage. At this stage the fruits are highly perishable because they are soft, squashy and have high moisture content. Fruits can be kept for 7-10 days at ambient temperature 15- 17 ºC in the markets [2]. The extending shelf-life of Hayani now is necessary, because of the increasing market demand. Therefore, the present work introduces a trial for preparation of edible co-polymers coatings and films by irradiation for keeping the quality and freshness of Rutab (Hyani) Date shelf life during the marketing. In general, Edible coatings or films should contain anti-bacterial, anti-microbial and anti-oxidant agents which act as barriers to reduce and/or prevent microbial attack on the fresh horticultural products while maintaining quality, freshness, and safety leading to extend of shelf-life and improved safety of the products [3, 4]. Poly vinyl alcohol (PVA) is a completely biodegradable polymer, harmless and non-carcinogenic. Poly vinyl alcohol (PVA) is a water soluble polymer and it has good biocompatible properties with other polymers, therfore, it was able to blend with different synthetic and natural polymers and form good films with highly chemical resistances. Poly vinyl alcohol (PVA) is useful in many industrial, medical applications and food packaging. Poly vinyl alcohol (PVA) was used with the mixture of Chitosan in the preservation of tomatoes and many of foods Because of the good biological activities of Poly vinyl alcohol (PVA) and Chitosan [3, 5, 6 and 7]. Chitosan is polysaccharides biodegradable polymer extracted from chitin have excellent properties in biomedical field due to their unique composition and its antioxidant properties which can minimize the growth of a wide variety of fungi and bacteria. Cs is a biocompatible polymer and can form non-soluble membranes with highly tensile strength. Cs has strong antimicrobial and antifungal activities that could effectively control perishable fruit decay. Chitosan coatings blended with bergamot oil produced strong antimicrobial activity [5]. Chien and his coworkers developed chitosan coatings in the maintaining quality and extending shelf-life of sliced mango [5]. Tannin or (tannic acid) is a polyphenolic compound has interest chemical activities, such as anti-inflammatory, antibacterial, antiseptic and antimicrobial. Tannic acid also acts as crosslinker between chitosan and PVA [8]. Tannins are produced by plants from different parts, therefore these special characters, tannic acid can be used in numerous applications such as packaging and pharmaceutical. Tannic acid blended with CMC and PVA can used in preservation of bananas [9-12]. Modification of polymers can be occurred by chemical or radiation methods. Radiation technique is the conventional tool for these improvements of polymer materials through crosslinked, grafting or degradation because it is a simple process to prepare biocompatible hydrogel, and there no residual chemicals that are harmful and toxic [10, 13].

2. Experimental

2.1 Materials

- Poly Vinyl Alcohol (PVA); degree of polymerization (1700-1800), fully hydrolized and it was purchased from Qualikems Fine Chemicals Pvt. Ltd., (New Delhi). Chitosan (Cs), molecular weight 100,000- 300,000 with degree of deacetylation more than 80%. was used as supplies Acros Organics, USA. Tannic Acid 99% (TA). $C_{76}H_{62}O_{46}$, molecular weight (1701.22), was obtained from Alpha Chem., (India), and was used without further purification. Glycerol was obtained from Algomhoria Company., (Egypt).
- Date fruits were obtained at the beginning of Rutabstage (Hyani variety). It collected after harvesting directly from the orchard near Cairo, Egypt. Samples were prepared by removing the injured or decayed one.

2.2 Preparation of (PVA/Cs/TA) Trible blend

The polymer blend solutions were prepared at different concentrations 2, 4 and 6 wt% from 85, 10 and 5 wt % (PVA, Cs and TA) respectively. The required amounts of PVA were added to 100 ml of distilled water at room temperature with continuous stirring for 2 h at 70˚C. The required amounts of Cs were dissolved in distilled water of 1% acetic acid solution. The solution was heated to 50˚C with continuous stirring. Required amount of Tannic acid Powder was dissolved in distilled water. After cooling of PVA solution, the solution heated raised at 50˚C, then the Cs solution was added with continuous stirring and temperature was gradually raised to 70˚C for 1 h then adding the tannic acid solution with continuous stirring, after complete mixing, the plasticizer (glycerol) was added and continuous stirring until complete homogeneity of the mixture. The mixture of PVA/Cs/TA was then irradiated with ɤ-irradiation. After exposures to ɤ-irradiation the mixture was poured on Petri dishes and dried for 48 h at 37˚C to form films. The films were finally removed from the dishes and placed in sealed containers at 4˚C to avoid moisture exchange. Also this mixture used after exposure to radiation as coating solution.

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2.3 Gamma Irradiation process

Irradiation to the required doses was carried out in the cobalt-60 gamma cell, at a dose rate of 1.66 kGy h^{-1} in the air. The irradiation facility is installed at the National Centre of Radiation Research and Technology (NCRRT), Atomic Energy Authority, Egypt.

2.4 Thermogravimetric Analysis (TGA)

The thermal characterizations of dried PVA/Cs/TA films have been investigated using thermogravimetric analysis (TGA). Shimadzu (TGA) system of type TGA-50 was used for the measurements of weight loss of the sample at a heating rate of 10 ^{o}C/min. in the presence of nitrogen gas to avoid thermal oxidation of the polymer sample.

2.5 Scanning Electron Microscopy (SEM)

The surface morphology of samples was investigated by (SEM) technique using a JSM-5400 Instrument, JOEL, (Japan)

2.6 Mechanical properties

The dumbbell-shaped samples of 40 mm long and 4 mm neck width were used for the measurements of the tensile strength and elongation percent using a tensile machine (model AG-1/50N-10KN, Japan) and the crosshead speed was 10 mm/min with pre-load of 0.5 N to determine load for each sample.

2.7 X-ray Diffraction (XRD)

The XRD measured using Shimadzu XRD 6000 diffractometer with Cu target. The XRD runs were carried out over the 2θ ranging from 10^{o} to 40^{o} at a scan speed of 8^{o}/min.

2.8 Fourier Transform Infrared (FTIR)

FT-IR spectra of the investigated films were recorded over the range 400–4000 cm^{-1}, in a Bruker, Unicom infra-red spectrophotometer (Germany).

2.9 Antimicrobial activity

The antimicrobial activity of the prepared blends was determined according to the reference [14].

2.10 Date quality parameters

Fresh date fruits were dipped in a solution of the coating process for one minute at room temperature, then left to dry completely as a coating thin layer. Each treatment was represented by six replicates each of them was packed in 6 carton boxes/ 1 kg. The coated fruits were stored at 12±2 ^{0}C and 98 % RH. Some parameters were calculated for evaluating the efficiency of edible coatings as a fellow:

2.10.1 Weight loss (%): Differences in weight between the initial of packaged dates samples at zero time and weight of same packaged dates at interval time were recorded weekly [15] by an electronic balance with sensitivity about 0.01g.

2.10.2 Decay (%): To calculate decay (%) for every treatment, four boxes per treated fruits were specified for that purpose, rejected fruit as injured, fermented and spoiled were removed at intervals, then calculate the decay (%) compared with the initial weight at zero time of storage [16].

2.10.3 Sensory evaluation: Sensory evaluation according to reference [17] for the appearance, color, taste, odor, texture, and overall acceptability were done in order to determine consumer acceptability. The tests were performed monthly. A scale ranging from 1 to 10 (1 is very bad and 10 for excellent) was used for sensory evaluation. Twenty panelists participated in the test [18].

2.10.4 Statistical Analysis

The experiment design was complete randomized with six replicates (500 g/replicate).The data were analyzed using analysis of variance technique for comparing the average value of the parameters [19]. Duncan's multiple range test (DMRT) was used to compare the mean values between a pair of treatments.

3. Results and Discussion

3.1 FTIR analysis

As shown in figure 1, the spectrum of pure Chitosan showed a band at 3357 cm^{-1} which is attributed to –OH stretching vibration. The band at 1576 cm^{-1} is assigned to the amide group –NH is bending, while a small peak (at 1644 cm cm^{-1}) is due to the C=O stretching. The bands at 2917, 2855, 1414, 1329 and 1260 cm^{-1} are assigned to CH_2 bending. The band at 1378 cm^{-1} is due to CH_3 wagging [20]. FTIR spectra of Chitosan and PVA blended films showed that, the presence of the band characteristics to the amide group at 1575 cm^{-1}, also an increase in the intensity and shifted of the -OH stretching at 3288 cm^{-1}. There was a noticed band shift at 1244, 1078 cm^{-1} with the adding of PVA corresponding to the –CH_2 bending and –C-O is stretching vibrations bands respectively.
FTIR spectra of the Trible blend (Tb) exhibited similar characteristics of PVA/Cs film. The intense band at 1714 cm^{-1} characteristics to C-O stretching of esters which suggested that the ester group in TA and Cs were detected in Tb films and the band at 1541cm^{-1} is attributed to the C=C aromatic ring stretching absorption from TA structure [21]. The band at 820 cm^{-1} is corresponding to the C-O in aromatic esters of TA [22] Therefore, the crosslinking between the PVA, Cs and tannic acid had successfully occurred using gamma radiation techniques.

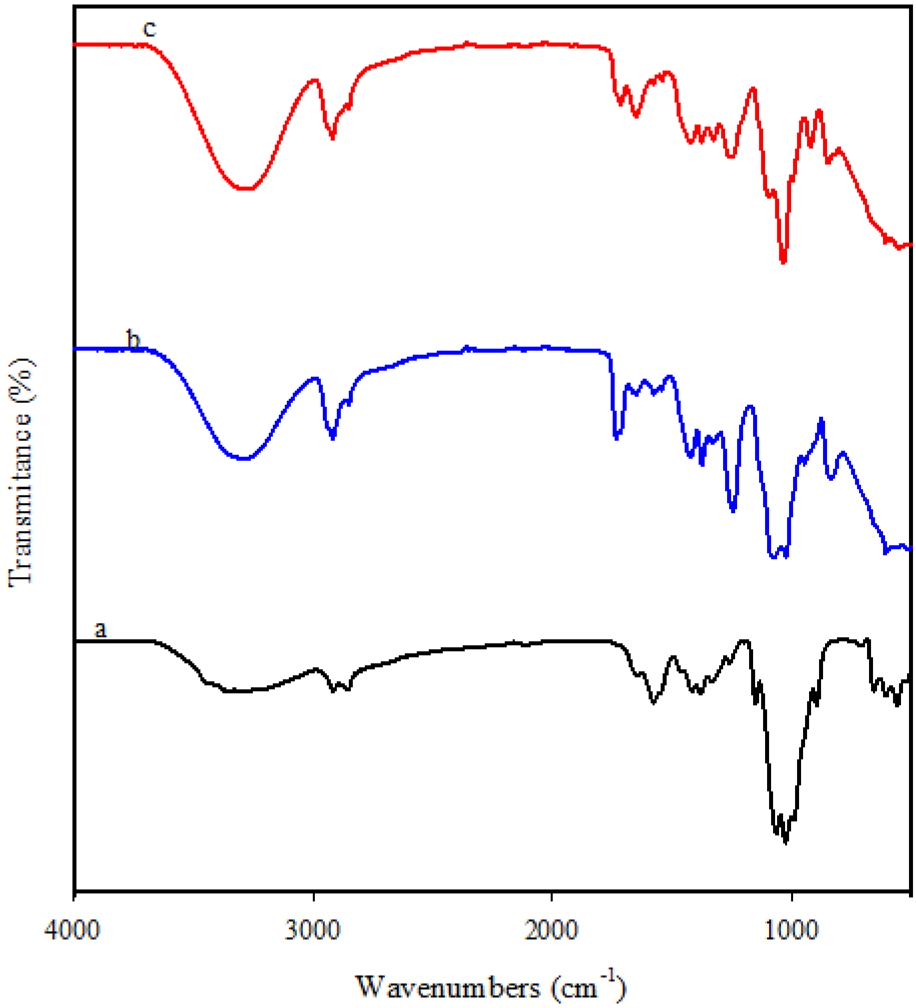

Fig. 1. FTIR spectra of (a) Chitosan, (b) PVA/Cs and (c) Tb

3.2 X-ray diffraction (XRD)

X-ray diffraction is a unique method of distinguishing between amorphous and crystalline of polymer samples. Figure 2 displays the XRD patterns of PVA, Chitosan and blend films of PVA/Cs and Tb. The sharp crystalline reflection with great intensity at around 19.7^o 2θ, was observed in the PVA film due to strong intermolecular interactions between the PVA polymer chains. The diffraction peak of Chitosan is at around 9^o and 19.1^o of 2θ. X-ray diffraction patterns of Chitosan: PVA show a rising shift in peak at 20.9^o of 2θ. From these difrograms, it is obvious that Chitosan: PVA is more crystalline than other two samples. The diffraction peak of Tb films shifted from 20.9 to 19.3^o of 2θ, this could be due to that the incorporation of tannic acid to the PVA/Cs crosslinking the samples during gamma irradiation [3].

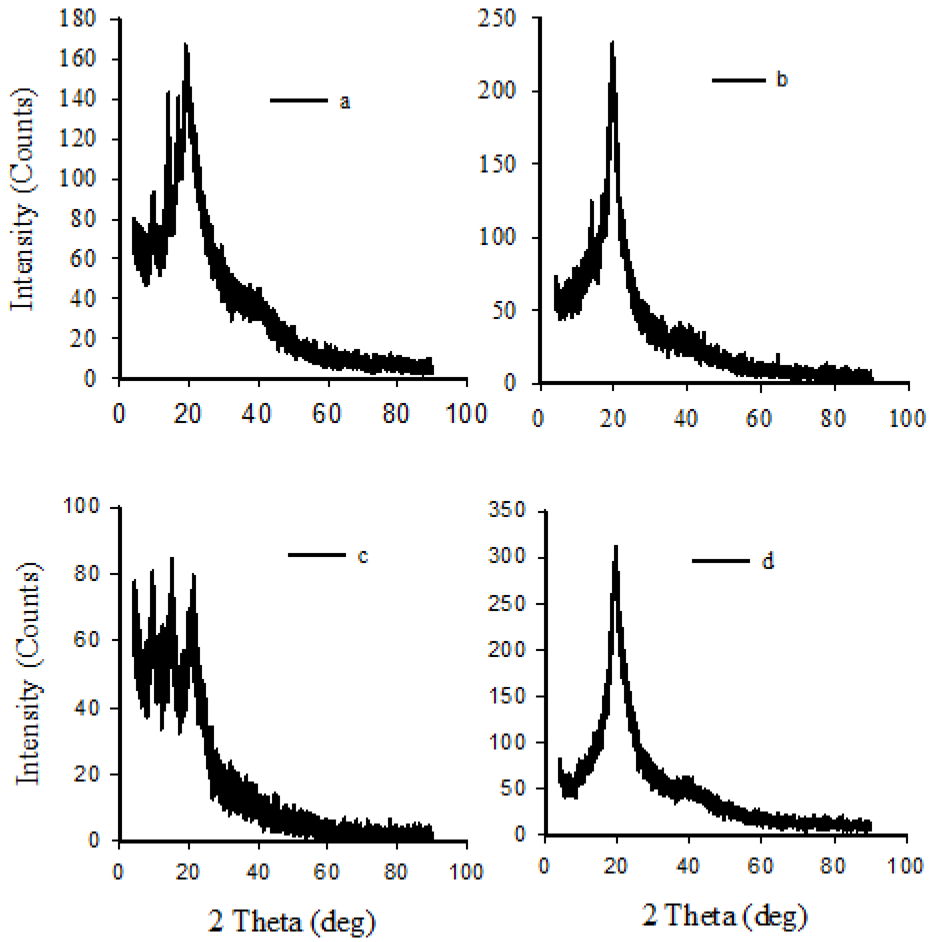

Fig. 2. XRD patterns of (a) PVA, (b) Chitosan, (c) PVA/ Cs and Tb irradiated films (d).

3.3 Scanning electron microscopy (SEM)

Scanning electron micrographs (SEM) of PVA, Cs, PVA/Cs and Tb films are shown in Fig. 3. These figures showed that, the pure Chitosan film shows smooth and homogeneous surface with small granules unlike that of pure PVA where no granules are seen. The surface of the blended films of Chitosan and PVA untreated with gamma irradiation represents pores and there is no homogeneity on the surface like the surface of Tb untreated with gamma irradiation there is also uniformity of particle distribution. The SEM micrographs of irradiated blended Tb films show small granules of Cs and TA dispersed in PVA matrix in the blend film with relatively good interfacial adhesion between the components. The surface of the film is relatively smooth and more homogeneous with a good structural conjunction. Advantages of g-ray polymerization are the free radicals mechanism, which enhances the polymerization between the molecules of the different polymers. G-ray polymerization tends to change PVA, Chitosan and Tannic acid hydrogels from microcell structure to a fibrous web structure with increase in the free radicals. The strong hydrogen bonding formed by the increasing gamma rays leads to decreased in the numbers of pore size and more homogeneity in the surface of the membranes of the triple blend polymer [3].

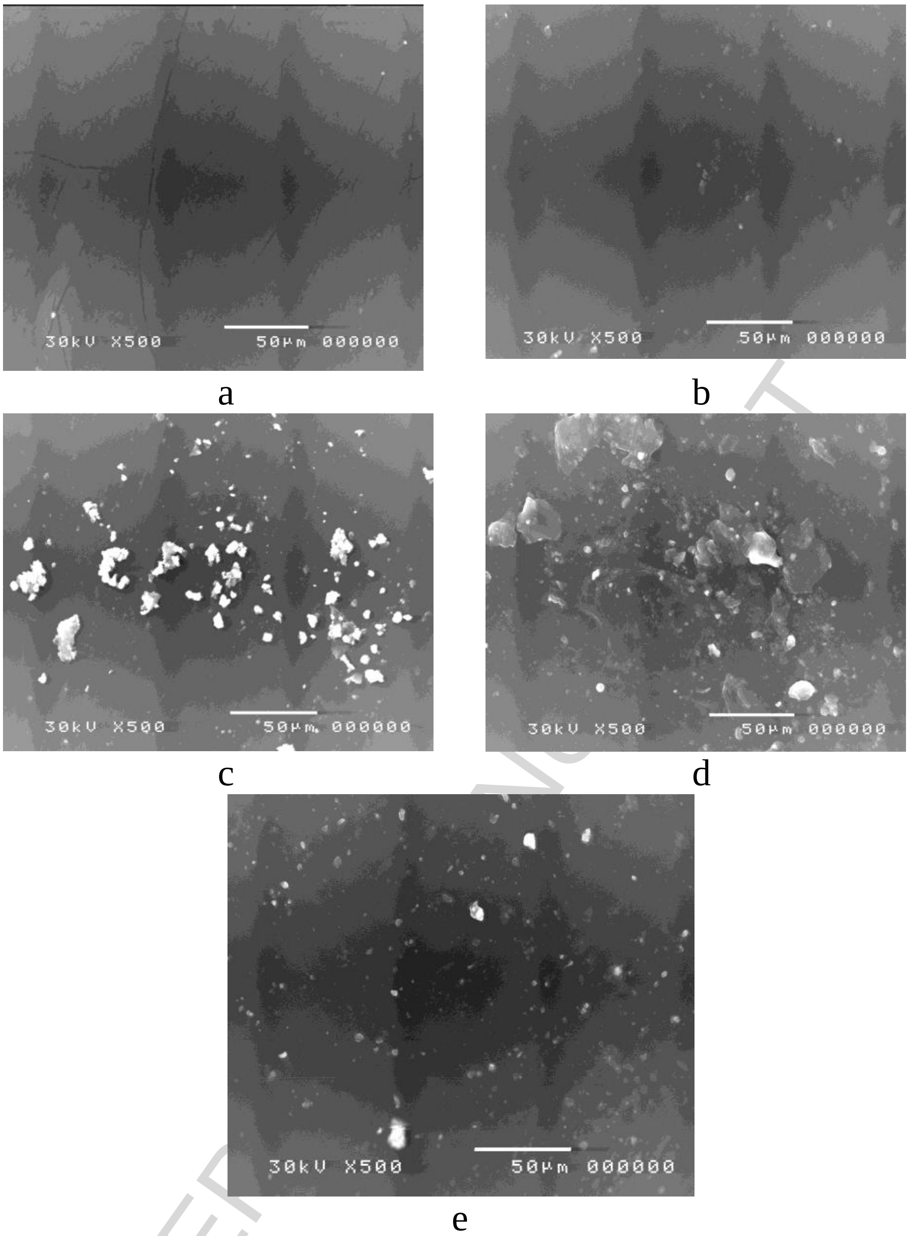

Fig. 3. Scanning electron micrographs of (a) PVA, (b) Cs, (c) PVA/Cs, (d) unirradiated Tb and (e) irradiated Tb

3.4 Mechanical properties

Table 1 shows the tensile strength and elongation of unirradiated PVA, PVA/Cs, Tb and irradiated Tb films. The addition of Cs increases the tensile strength and decreases the elongation as compared with the PVA film; this is attributed to the presence of Cs, which formed crosslinking with PVA content. The addition of TA increases the tensile strength and reduced the elongation as compared with the previous films; this may be due to that, the TA act as a good crosslinker between all components [20].

Table 1. Effect of polymer composition and radiation dose on tensile strength and elongation of PVA and blended films.

Sample	Concentration (%)	Rad. Dose (kGy)	Tensile strength (MPa)	Elongation (%)
PVA	4	-	25.18±1.07 **B**	279.63±12 **A**
PVA/Cs	4	-	26.70 ±0.80 **B**	216.97±6.5 **B**
Tb	4	-	29.93 ±1.40 **A**	176.08±5.1 **C**
Tb	4	10	30.96±0.56 **A**	133.00±3.0 **D**

*** Values following by the same letters (S) mean no difference significantly at 5%.**

Table (2) Show the effect of the total polymer concentration and the plasticizer (glycerol) on the tensile strength and the elongation of unirradiated and irradiated Tb blend films with different polymer content ratio. It can be seen that, the tensile strength of the irradiated films is higher than the unirradiated films and the tensile strength increases with irradiation dose, this is attributed to the formation of irradiation induced crosslinking in the matrix [6], and with the increasing of polymer concentration until 4 wt %. Above this percentage the tensile strength decreases and the elongation still the same values, this is due to that, the content of glycerol (plasticizer) is much more in 6 wt % with a polymer concentration which results in weak the tensile strength, because it is acting as a strong plasticizer which increases the elasticity [10].

Table 2. Effect of total polymer concentration on the tensile strength and elongation of the Trible blend with plasticizer.

Sample	Concentration (%)	Rad. Dose (kGy)	Tensile strength (MPa)	Elongation (%)
Tb+G	2	5	16.9± 0.75 **F**	214.6±7.5 **A**
Tb+G	2	10	21.0±0.70 **C D**	199.3±5.5 **AB**
Tb+G	2	20	22.9±0.55 **B C**	191.5±2.1 **B**
Tb+G	4	5	21.9±1.05 **C**	197.3±3.7 **B**
Tb+G	4	10	24.0±0.85 **B**	189.6±7.5 **B**
Tb+G	4	20	28.5±0.75 **A**	186.0±5.4 **B**
Tb+G	6	5	14.9±0.60 **G**	192.9±7.8 **B**
Tb+G	6	10	18.2± 0.50 **E F**	198.6±5.5 **AB**
Tb+G	6	20	19.4± 0.39 **D E**	190.3±3.5 **B**

*** Values following by the same letters (S) mean no difference significantly at 5%.**

3.5 Thermal gravimetric analysis

Figure 4 shows the thermogravimetric analysis (TGA) of irradiated PVA, PVA/Cs, Tb and (Tb +G) blend films. From the curve the PVA, PVA/Cs and Tb exhibited two stages of weight loss. The first stage of weight loss started at around 50 to 120 ^{o}C, due to the evaporation of absorbed water. The second stage started from 200 to 320, 300 and 310 ^{o}C respectively. This stage assigned to the decomposition of total polymer composition. However the decomposition of (Tb +G) film occurred in three stages. First stage started at around 30 to 120 ^{o}C followed directly by second stage at 120 to 200 ^{o}C which represents the decomposition of glycerol. The third stage at around 250 to 300 ^{o}C assigned for the decomposition of the total polymer remaining. The results concluded that, the addition of tannic acid (TA) arising the thermal stability of the blend nearly to the pure PVA.

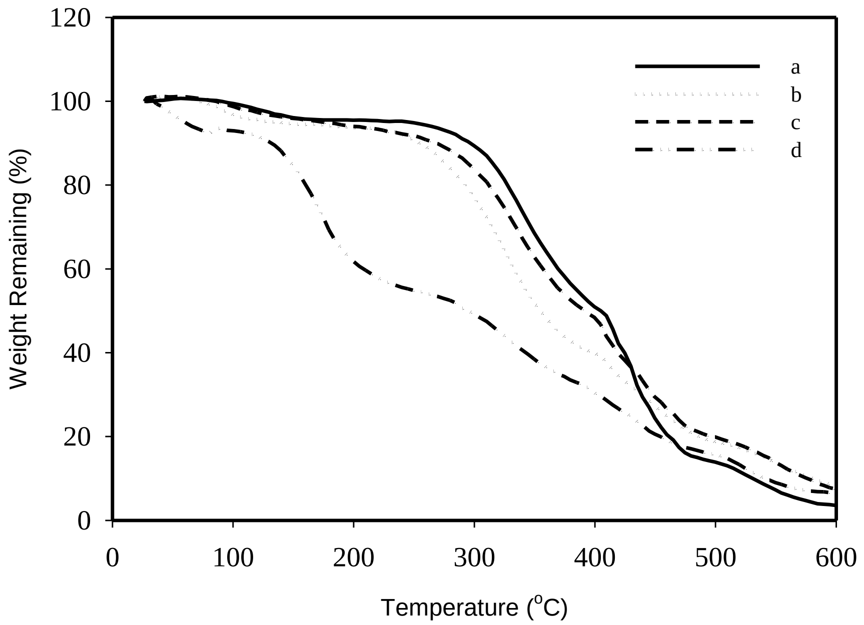

Fig. 4. Thermogravimetric diagram of (a) PVA, (b) PVA/Cs, (c) Tb and (d) Tb +G irradiated films.

3.6 Antimicrobial activity of Tb

The mean inhibition zones (MIZ) of the Tb suspension indicates high activity against *Acinetobacter baumal*, *Saccharomyces cerevislae* as shown in Table 3, Fig. 5. Trible blend (Tb) were prepared under two doses 5.0, 20 kGy, both of them introduced high MIZ as18,9 and 20,11mm respectively. Whereas, *E. coli* was resistant to tested (Tb) suspension may be need more concentration from chitosan or tannic acid in the future. Presence of tannic acid at the Tb activates the antimicrobial effects as reported in the reference [21] who recommended using of the tannin and phenolic compounds as antibacterial. The utilization of these constituents has also been reported to account for the exertion of antimicrobial activity of plants [23, 24]. Tannins and phenolic compounds have been found to inhibit bacterial and fungal growth and also capable of protecting certain plants against infection [25, 26]. The obtained results proved that Tb was effective against the tested organisms, but it needs more concentration to be effective against *E. coli*.

Table 3. Antimicrobial activity of Tb

No.	Strain	Result
5.0 kGy	*Acinetobacter baumannii*	20**
	Saccharomyces cerevisiae	11*
	E. Coli	Resistant***
20 kGy	*Acinetobacter baumannii*	18**
	Saccharomyces cerevisiae	9.0*
	E. Coli	Resistant***

* moderate inhibition (8-11 mm)
**high values of inhibition (18-20 mm)
***No response due resistant of microbes

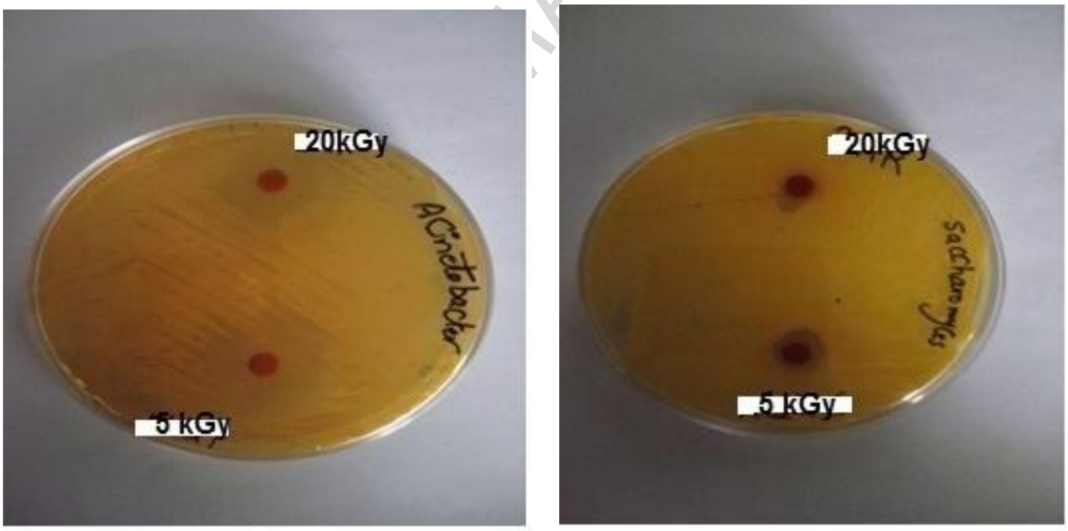

Fig. 5. Anti-microbial effect (inhibition zone) of irradiated combined with Tb.

3.7 Evaluation of the efficiency of edible tested coating during dates marketing

3.7.1 Weight Loss (%)

Concerning the effect of using a tested edible triple blend (Tb) on date fruits weight loss (%), as shown in the table 4 and fig. 6, the dates were treated with irradiated mixture of triple blend (Tb) recorded the lowest value in the weight loss 22.8 %. And there is no significant difference between the irr.Tb coating and PVA or Chitosan treatments on fruit weight loss % which recorded the same near values. While the uncoated dates and the dates treated with the un-irradiated mixture had a significant weight loss 34.22% and 31.37% respectively. The increasing of the weight loss % may be due to the highly evaporation and transpiration of the fruits through the bores of uncoated fruit beel or un-irradiated coatings. And the decreasing in the weight loss % may be due to the effect of γ radiation, which led to the crosslinking network structure that minimized the evaporation outside the fruit coating [27].

Table 4. Effect of treatments on weight loss (%) of Hyani dates after 28 days at (12±2°C, 98 %, RH).

Treatments	Weight loss (%)	
Control (uncoated fruits)	34.22±1.18	**A**
PVA	23.90± 0.23	**CD**
Chitosan	24.19±0.26	**CD**
Unirr.Tb	31.37±0.31	**B**
irr.Tb	22.81±0.79	**D**

*** Values following by the same letters (S) mean no difference significantly at 5%.**

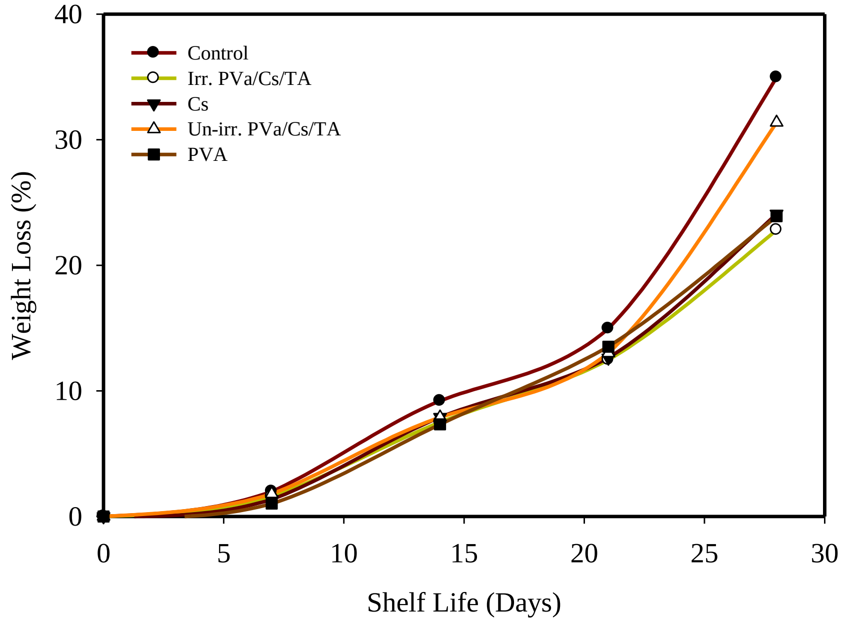

Fig. 6 Effect of treatments on weight loss (%) of Hyani dates during shelf life period at (12±2°C, 98 %, RH).

3.7.2 Fruits decay (%)

As shown in the Table 5 and Fig. 7, the marketing can extend to 28 days only by using (Tb) co-polymers. Whereas the control samples started to spoil during first week, then the spoilage increased gradually to reach 50% until control samples were decayed completely then discarded after two weeks. At the same time all coated fruits with different treatments recorded lower values of decay ranged between 18.33 to 40 %. Therefore, the obtained results proved that, the (Tb) co-polymer coatings improved and protected the dates for nearly one month during marketing comparing the control samples. And the decay value recorded lower percentage (%) of fruits near 18.33 %. Because of presence of the crosslinking network structure that minimized the evaporation and respiration rates around the coated fruits [27] whereas, the fruits were coated with un-irr. Tb recorded 40 % over the same period. The relevant factors of spoilage of fruits included an increase of respiration, evaporation which caused shrinkage then facilitate microorganism's attacks and spoilage. Chitosan films control spoilage due to its antimicrobial effect and reducing of respiration, then slowing of ripening process [28]. Also, it can be seen from table 5 and fig. 7, that, the presence of tannic acid increased the efficiency of (Tb) co-polymer coatings against microorganisms [21-23]. According to the reference [24], tannins and phenolic compounds have been found to inhibit bacterial and fungal growth and also capable to protecting certain plants against infection.

Table 5. Effect of treatments on decay (%) of Hyani dates during shelf life period at (12±2ºC, RH= 98 %).

Treatments	**Marketing period (days)**			
	7	**14**	**21**	**28**
Control (uncoated fruits)	38.6±1.50 **D**	58.6±1.31 **C**	83.3±0.93 **B**	100±0.60 **A**
PVA	-	-	-	39.0±0.53 **D**
Chitosan	-	-	-	38.0±0.62 **D**
Unirr.Tb	-	-	-	40.0±0.32 **D**
irr.Tb	-	-	-	18.33±0.15 **E**

*** Values following by the same letters (S) mean no difference significantly at 5%.**

Fig. 7. Date fruits at (A) zero time, (B) treated dates with irradiated (Tb) after 28 days and (C) non treated fruits (control)

3.7.3 Sensory evaluation

Sensory analysis was conducted by panelists weekly till four weeks (28 days). The evaluation included the appearance, color, taste, smell, texture and general acceptance of dates samples. In general, there were significant differences between tested samples. The sensory properties of control samples were significantly reduced after one week compared to the treated samples, and the results were obtained by panel members as shown in figure 7 and Table 6. Showed that, the coating treatments recorded a lower value in all sensory analysis characteristics, while the triple blend (Tb) co-polymer coatings has been distinguish in taste, smell, color, texture and all acceptability. Triple blend (Tb) has the priority due to the presence of Chitosan, and tannins that have the antibacterial characters [21, 23 and 24]. The irradiated edible coatings provide a semi-permeable barrier against oxygen, carbon dioxide, moisture and dissolved movement, thus reducing breathing, weight loss and oxidative reaction rate [29].

Table 6. Sensory evaluation after 28 days.

treatments	color	Appearance	Taste	odor	texture	Over all
control	00.0±0.0D	00.0±0.0C	00.0±0.0C	00.0±0.0C	00.0±0.0D	00.0±0.0C
PVA	6.60±0.57 B	7.33±0.57 A	5.33±0.57 B	4.33±0.57 B	7.33±0.57 AB	6.66±0.57 B
Cs	4.67±0.57 C	6.33±0.52 A	5.33±0.51 B	5.33±0.57 B	6.33±0.53 BC	5.66±0.57 B
Un-irra.Tb	5.33±0.56 BC	4.33±0.57 B	4.67±0.57 B	4.00±0.57 B	5.00±1.00 C	5.33±1.00 B
Irrad.Tb	8.33±0.57 A	7.67±0.57 A	8.33±0.57 A	7.33±0.56 A	8.33±0.57 A	8.33±0.57 A

*** Values following by the same letters (S) mean no difference significantly at 5%.**

***nil or very bad (0-2), low (4), medium (6), high (8) and excellent (10)**

4. Conclusion

The preservation of the ‘freshness’ quality of rutab date is relevant due to their economical impact. As an alternative to synthetic preservatives, natural antimicrobial agents have attracted the attention of modern consumers and the fresh produce industry. In the present work different edible polymeric coatings and films were prepared using poly vinyl alcohol (PVA), Chitosan and triple blend (Tb), which contained poly vinyl alcohol (PVA), Chitosan (Cs) and tannic acid (TA). Preservation or keeping the quality of the fresh date fruits which considered perishable products and usually rapidly deteriorate is the main object. The obtained results proved that the tested edible polymeric coating improved the quality of dates with superiority of the triple blend (Tb) co-polymer coatings, which recorded higher values of acceptability after four weeks of marketing period. Whereas, the control samples was rapidly deteriorating after a few days in contrast of triple blend (Tb) which extended to 28 days with the acceptability by the consumers with 80% more than other tested materials. Triple blend (Tb) coatings are thin layers of edible materials applied to food products that play an important role in their conservation, distribution and marketing. Some of their functions are to protect the product from mechanical damage, physical, chemical and microbiological activities besides its functional attributes, mechanical properties (flexibility, tension), optical properties (brightness and opacity), the barrier effect against gas flow, structural resistance to water and microorganisms and sensory acceptability. Therefore, it could be recommended that using the triple blend (Tb) co-polymer coatings, which is suitable for extended shelf life of highly perishable products as dates–hyani as fleshy fruits.

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