Development of guar gum based active packaging films using grape pomace

Chaturbhuj K. Saurabh¹ · Sumit Gupta² · Prasad S. Variyar²

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Abstract The objective of this study was to develop biodegradable active film to improve the shelf-life of minimally processed fresh-produce. Guar gum (GG) based films with improved properties were fabricated by employing tween-80 (0.88%) as emulsifier, nanoclay (13.9%) as reinforcement, beeswax (1.21%) for hydrophobicity, glycerol (3.07%) as plasticizer, and grape pomace extract (5%) as active ingredient (%w/w of GG). Active films had a tensile strength of 122 MPa and water vapor transmission rate of $69 \text{ gm}^{-2}\text{d}^{-1}$. Films demonstrated significant antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Salmonella Typhimurium*. The 2 kGy irradiated minimally processed pomegranate arils packed in film demonstrated a shelf-life of 12 days as compared to 4 days for unirradiated samples. The observed improvement in shelf-life was due to a radiation-induced release of antimicrobial volatiles from active films as confirmed by headspace analysis using GC–MS. Suitability of active films for food irradiation applications is thus demonstrated.

Keywords Guar gum · Active film · Grape pomace · Shelf-life · Gamma irradiation

Introduction

Guar gum (GG) is one of the suitable candidates for the production of biopolymer based plastics in order to counter the challenges of conventional petroleum based plastics. GG based films had excellent mechanical properties as compared to other biopolymers (Saurabh et al. 2013, 2015, 2016). Active films are packaging films incorporated with various functional additives such as antimicrobials or antioxidants which can result in improvement in safety and quality of food products (Piñeros-Hernandez et al. 2017). Addition of natural compounds as antimicrobial and antioxidant component in the active film is preferred due to the fact that synthetic additives can result in adverse effect on human health (Kechichian et al. 2010). There are several reports in the literature on use of herbal extracts for preparing active films (Piñeros-Hernandez et al. 2017).

Grape pomace consisting of the grape skins and seeds is the main by-product of wine industry. Even though most of the polyphenols from grape skin and seeds are extracted in wine during fermentation, grape pomace still has significant amount of bioactive metabolites of polyphenolic nature (Sanhueza et al. 2014). Especially, skins are rich in anthocyanins which are well known for their bioactive properties (Katsube et al. 2003). Grape pomace also possesses antimicrobial activity against various food borne pathogens (Sanhueza et al. 2014; Deng and Zhao 2011). Interestingly, antimicrobial activity of grape extract is also reported to be stable even at higher temperatures. Therefore, grape extract could retain its antimicrobial activity

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Chaturbhuj K. Saurabh and Sumit Gupta both the authors contributed equally.

✉ Prasad S. Variyar
prasadpsv@rediffmail.com

¹ School of Industrial Technology, Universiti Sains Malaysia, Penang, Malaysia

² Food Technology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400085, India

even at high temperatures encountered during the film formation (Cha and Chinnan 2004). Thus one possible use of grape pomace is to develop active film by incorporating it into film forming matrices. Very few reports exist on development of active packaging having grape pomace extract. Grape seed extract incorporated pea starch films reduced the bacterial growth on pork loins infected with *Brochothrix thermosphacta* (Corrales et al. 2009). It was also shown that incorporation of winery bio waste reduced the biodegradability of starch based films (Cerruti et al. 2011). Pomace extract incorporation into various biopolymers demonstrated antimicrobial activity against food pathogens like *Escherichia coli* and *Listeria innocua* (Deng 2011). Thus the use of grape pomace to develop active films appears promising. To the best of our knowledge there are no reports on use of grape pomace extract for development of GG based active packaging films.

Gamma-irradiation is another tool which can enhance shelf-life and ensure safety of the fresh-cut produce (Niemira and Fan 2006). The non-residual feature of ionizing radiation is a significant advantage in minimizing the use of chemicals applied to fruits and vegetables. There are various reports of radiation processing on shelf-life of fresh fruits (Arvanitoyannis et al. 2009). Removal of pomegranate cuticle to obtain the arils is a tedious process thus ready-to-eat pomegranate arils will be a convenient and desirable alternative as compared to the consumption of whole fruit. However, pomegranate aril as minimally processed agricultural produce is highly prone to microbial contamination because of the removal of protective cuticle. Thus combination of gamma-irradiation with active packaging films could be an effective method for shelf-life extension of minimally processed pomegranate arils.

PVC cling films are presently widely used for packaging of minimally processed products. In our previous study we have optimized process of preparing GG based films with similar mechanical and barrier properties to that of cling films using various additives such as beeswax, glycerol, tween-80, and nanoclay. In present study an attempt has been made to prepare GG based biodegradable active packaging by incorporating grape pomace extract into optimized GG films. The efficacy of developed pomace extract based active films was compared with cling film for the shelf-life extension of minimally processed irradiated pomegranate arils.

Materials and methods

Preparation and extraction of grape pomace

Red wine grapes (Shiraz variety), was chosen for the present study. Grapes were harvested at optimum maturity from vineyards located at Narayangaon, Maharashtra.

Samples were brought to laboratory within 12 h of harvesting. Wine preparation was carried out essentially as per procedure detailed earlier (Gupta et al. 2015). After completion of fermentation (200 h) seeds and skins (pomace) were separated from wine by straining through muslin cloth. 10 g of freeze dried grape pomace was powered for 30 s using a high speed homogenizer (B400, Buchi, Switzerland). Extraction of pomace powder thus obtained was carried out using pressurized solvent extractor (Speed Extractor E-914, BUCHI, Switzerland). 2 g of pomace powder was taken in a thimble (Whatman filter paper 42) and then placed in 40 mL extraction cell. The powder was extracted at 70 °C using 60% aqueous ethanol (100 bars, flow rate of 10 mL min⁻¹) using 2 cycles. First cycle had a heat up time of 3 min followed by 5 min holding time at 70 °C and 100 bars and then 5 min discharge time. Second cycle had heat up, holding, and discharge time of 1, 5, and 5 min respectively. Finally, samples were flushed for 2 min by solvent and then by N₂ gas for 3 min. Obtained extract was concentrated to dryness under vacuum using rotary evaporator (Rotavapor R-114, BUCHI, Switzerland) and stored at − 20 °C till further use.

Film preparation

GG was purified as per method described by Saurabh et al. (2013). We have earlier demonstrated that an optimized concentration of nanoclay, irradiated (50 kGy) beeswax, tween-80, and glycerol in GG films resulted in excellent mechanical and barrier properties (Saurabh et al. 2016). Thus in the present study optimized films were transformed into active packaging by addition of grape pomace extract with little modification. In brief, a dispersion of 107.45 mg of nanofil-116 (nanoclay) (Southern Clay Products, Inc., US) in 350 mL distilled water was prepared by using method previously reported by Saurabh et al. (2015). A 3.5 g of purified GG was added in nanofil-116 suspension along with 42.35 mg of 50 kGy irradiated beeswax (hydrophobic component), 30.8 mg of tween-80 (emulsifier), 486.85 mg of glycerol (plasticizer), and different amounts of grape pomace extract. Different concentrations of grape pomace including 0.5, 1, 2.5, 5, and 7.5% w/w of GG were used in film forming solution. Resulting solution was kept overnight at room temperature (25 ± 2 °C) on magnetic stirrer. Film forming solution was casted on a glass plate (20 × 20 cm) having removable boundary of insulating tape. Further heating, drying, and conditioning were done as per procedure described by Saurabh et al. (2016).

Mechanical and barrier properties of active film

Conditioned films were peeled and subjected to mechanical analysis as per procedure detailed by Saurabh et al. (2013).

To evaluate the effect of radiation processing on mechanical and barrier properties, films were subjected to radiation processing at dose of 0, 1, 2, 5, and 7.5 kGy using a ^{60}Co gamma-irradiator (GC-5000, BRIT, India) having dose rate of 3.6 kGy h^{-1} . Post irradiation films were conditioned again using same procedure as mentioned above prior to analysis of their mechanical and barrier properties.

Total phenolic content and antioxidant activity of active film

A 50 mg of film was dissolved in 5 mL water. Obtained solution was further analysed for total phenolic content by Folin–Ciocalteu method and antioxidant capacity by DPPH and FRAP assay using standard methods described previously (Paixão et al. 2007). Gallic acid (GA) standard curve was obtained in concentration range of $5\text{--}20 \mu\text{g mL}^{-1}$ and total phenolics in film was obtained using linear regression equation of GA standard curve and represented as mg GA equivalents (GAE) g^{-1} of film.

For DPPH assay, trolox standard curve was obtained in concentration range of $1\text{--}10 \mu\text{g mL}^{-1}$ and total antioxidant capacity in film was calculated using linear regression equation of trolox standard curve and expressed as mg trolox equivalents (TE) g^{-1} of film. For FRAP assay, trolox standard curve was obtained in range of $15\text{--}70 \mu\text{g mL}^{-1}$ and total antioxidant capacity of films was calculated using linear regression equation obtained for trolox standard and results expressed as mg trolox equivalents (TE) g^{-1} of film.

Total flavanoid, anthocyanin, and ascorbic acid content of active film

The AlCl_3 method reported by Luximon-Ramma et al. (2002) was used for determination of total flavonoid content. Solution of film for analysis was prepared as described above. Quercitin standard curve was obtained in range of $0\text{--}6.25 \mu\text{g mL}^{-1}$ and total flavonoid content of films was calculated using linear regression equation obtained for quercitin standard and results were expressed as mg quercitin equivalent (QE) g^{-1} of film. Total anthocyanin content (TAC) was estimated by diluting film solution to 100 times with 1% (v/v) of HCl. Optical density of resulting mixture was measured at 520 nm (Garrido and Borges 2011). TAC was calculated as mg malvidin-3-glucoside L^{-1} equivalents. Standard curve of malvidin-3-glucoside was prepared in concentration range of $0.0375\text{--}5 \mu\text{g mL}^{-1}$. Total vitamin C content of film was estimated in accordance with standard Association of Official Analytical Chemists (AOAC) official titrimetric method. Ascorbic acid content was expressed as mg 100 g^{-1} of film.

Antimicrobial activity of active films

Antimicrobial activity of films was tested both qualitatively and quantitatively by inhibition zone and viable cell count method, respectively as reported by Thomas et al. (2009) with slight modification. In detail, antimicrobial activity was assessed against five different food pathogenic bacteria i.e. *Staphylococcus aureus*, *Salmonella Typhimurium*, *E. coli*, *Bacillus subtilis*, and *Bacillus cereus*. Pure cultures of *E. coli* (ATCC 35218), *S. typhimurium* (MTCC 98), *B. subtilis*, *B. cereus*, and *S. aureus* (coagulase-positive strain isolated from poultry meat) were obtained from ICT (Mumbai, India). For qualitative assay 6 mm diameter disks were made from film samples, and the antimicrobial activity was determined using an agar diffusion assay (disk test). In plate count agar (PCA) plates 0.1 mL of inoculum suspension (10^6 colony forming units (CFU/mL)) was uniformly spread using a glass spreader. Four film sample disks were then placed on surface and the plates were incubated at 37°C for 48 h. The diameter of the clear zone that forms around the film disk on the agar plate was recorded as a measure of inhibition against the pathogenic microorganism.

To quantitatively assess antimicrobial activity of the films, viable cell count method was employed. All five pathogenic strains were separately inoculated in nutrient broth and incubated aerobically for 16 h at 37°C . After incubation inoculum of each strain was diluted with sterile broth to $1.0\text{--}2.5 \times 10^6$ CFU/mL. 100 mL of this inoculum was then added with 250 mg of film samples. An inoculum of cell suspension without film sample was used as a control. Samples were then incubated in a shaker at 37°C and 50 rpm. Aliquot of 0.1 mL of cell suspension was withdrawn after 24 h under laminar flow and diluted serially with 0.9% saline solution and plated in duplicate on PCA. The plates were then incubated under aerobic conditions for 48 h at 37°C and final microbial counts are reported as CFU/mL.

Packing, irradiation, and storage of pomegranate arils

Pomegranates (Bhagwa variety) were procured from local market. Fruits were washed manually with running tap water to remove adhered dust, bruised or damaged items were removed and healthy fresh fruits were finally selected. Pomegranates were cut open using sterile stainless steel knives at the equatorial zone and the arils were manually removed. Arils were packed (40 g) into polystyrene trays (inner dimensions: $9 \text{ cm} \times 9 \text{ cm} \times 2.5 \text{ cm}$) and then over-wrapped all around either with cling film (Klin wrap, Flexo Film Wraps Ltd., India) or GG based active films or GG based non-active films (not containing grape pomace

extract). Packaged samples were subjected to various radiation doses (0.5, 1.0, 1.5, 2.0, and 2.5 kGy) at room temperature ($25 \pm 2^\circ\text{C}$) using ^{60}Co irradiator (GC-5000, Brit, Vashi, India) having a dose rate of 3.6 kGy h^{-1} . Post irradiation samples were stored in the dark at $10 \pm 0.5^\circ\text{C}$. Samples were examined for various quality attributes including microbial, sensory, texture, and moisture parameters after 0, 4, 8, 12, and 16 days of storage. Three replicates were prepared for each dose, storage day, and type of packaging.

Microbial analysis of arils

Standard methods were used to enumerate microorganisms present in minimally processed pomegranate aril at each sampling time. Mesophilic bacterial and total yeast and mold counts were enumerated using PCA and potato dextrose agar (PDA) supplemented with 0.1% tartaric acid, respectively by pour plate method. In brief, arils samples (25 g) from each tray were taken in stomacher bag containing 225 mL sterile physiological saline within a laminar. The sample was homogenized in a stomacher instrument at 260 rpm for 1 min. After appropriate serial dilutions, the samples were pour plated on PCA and PDA. The bacterial colonies were counted after 24 h of incubation at 37°C while before counting yeast and molds plates were incubated for 48 h at 25°C . Microbial counts were expressed as $\log_{10} \text{CFU g}^{-1}$ of arils. Each analysis was performed in triplicate.

Sensory analysis of arils

Arils (non-irradiated and irradiated) were analyzed by the panellist in different sessions. 10 g of samples were served in white trays numbered randomly to the sensory panel. Sensory analysis at all doses was carried out by hedonic test employing a trained sensory panel comprising of 15 members (7 women and 8 men). 9-point hedonic scale with 1, dislike extremely or not characteristic of the product and 9, like extremely or very characteristic of the product for assessment of various parameters such as color, aroma, texture, taste, and overall acceptability was used. To determine the acceptability of the samples at different storage points, packaging, and treatment (radiation processing) panellists were also provided fresh control samples during analysis.

Texture analysis

The texture analysis for the sample was performed using a Texture Analyzer (TA. HD. Plus, Stable Micro Systems). Arils (12 g) were placed into a 28 cm^2 metal plate and maximum force (N) required for crushing using a 5 cm

diameter cylindrical probe for test distance of 7 mm at a speed of 5 mm s^{-1} was measured and expressed as firmness.

Moisture loss from arils

For measurement of water loss through packaging film, each tray was weighed regularly during storage. Further, moisture loss was calculated cumulatively by comparing the weights of trays subsequent to packing of the arils immediately and at various storage times. Results were expressed as a percentage of weight loss.

Head space analysis of active film

Film head space analysis was performed by Solid Phase Micro Extraction (SPME) and subsequent analysis using GC/MS (QP5050A, Shimadzu, Japan) equipped with special SPME glass liner (Supelco, USA) for injector and RTX-5 column (5% diphenyl dimethyl polysiloxane, $0.25 \mu\text{m I.D.}$, 30 M length, Restek Corporation, USA). 1 g of film (control or irradiated) was taken in 40 mL SPME vial (Supelco, USA) after a storage period of 0, 4, 8, and 12 days. Samples containing vials were equilibrated for 30 min at 30°C . Headspace was subsequently extracted by preconditioned PDMS/DVB/CAR fibres (Supelco, USA) for 20 min at 30°C . After extraction fibres were desorbed (270°C) in the injection port of GC/MS. Helium was used as a carrier gas. GC column temperature program was initial: 60°C for 5 min, increased to 200°C at rate of 4°C min^{-1} and hold for 5 min, then increased to 280°C at the $10^\circ\text{C min}^{-1}$ and final hold of 10 min. MS parameters were ionization voltage 70 eV, electron multiplier voltage, 1 kV and scan mode from m/z 40 to 350. The peaks were identified by comparing their mass fragmentation pattern and Kovats indices with that of standard compounds as well as from the data available in the spectral (Wiley/NIST) libraries of the instrument.

Statistical analysis

DSAASTAT ver. 1.101 by Andrea Onofri was used for statistical analysis of data. Three samples were taken for every treatment and each sample was further analyzed in triplicates. Volatiles release, sensory quality, microbial growth, gaseous composition, texture, and moisture loss were analyzed by Analysis of variance (ANOVA) and multiple comparisons of means were carried out using Duncan's multiple range test.

Table 1 Physical properties of films incorporated with various quantities of grape pomace extract

Grape pomace extract (% w/w of GG)	Tensile strength (MPa)	Young's modulus (GPa)	WVTR (g/m ² /d)
Control	120 ± 18 ^a	97 ± 8 ^a	40 ± 4 ^b
0.5	118 ± 15 ^a	96 ± 10 ^a	41 ± 4 ^b
1	113 ± 12 ^a	92 ± 9 ^a	44 ± 5 ^{ab}
2.5	109 ± 13 ^a	88 ± 7 ^a	46 ± 3 ^{ab}
5	104 ± 10 ^a	83 ± 7 ^a	48 ± 5 ^{ab}
7.5	80 ± 10 ^b	70 ± 6 ^b	53 ± 7 ^a

Row in same column bearing different superscript are significantly ($p < 0.05$) different

WVTR means water vapour transmission rate

Results and discussion

Mechanical and barrier properties of active films

In previous study (Saurabh et al. 2016), we have prepared GG based biodegradable films having various additives whose concentrations were optimized by response surface methodology to a value of 0.88, 1.21, 13.9 and 3.07% w/w GG for tween-80, irradiated beeswax (50 kGy), glycerol, and nanoclay, respectively. In present study grape pomace extract was added in this optimized film to prepare active packaging films.

Effect of addition of grape pomace extract on mechanical and barrier properties of optimized films is shown in Table 1. Incorporation of pomace extract up to 5% (w/w of GG) resulted in no significant ($p < 0.05$) change in mechanical and barrier properties of the optimized film (Table 1). However, at higher concentration (7.5%) extract resulted in significantly ($p < 0.05$) lower mechanical and higher WVTR. Optimized films had tensile strength and WVTR of 120 ± 18 MPa and 40 ± 4 gm⁻² d⁻¹, respectively, while film incorporated with 5% of pomace extract had values for these attributes as 104 ± 10 MPa and 48 ± 5 gm⁻² d⁻¹, respectively. Whereas films prepared with 7.5% grape pomace extract a significant ($p < 0.05$) reduction in tensile strength to 80 ± 10 MPa and increase in WVTR to 53 ± 7 gm⁻² d⁻¹ was observed. This might be due to increase in inhomogeneity of film matrix with high content of pomace extract which resulted in lower functional properties of film.

Since no significant ($p < 0.05$) effect of grape pomace (5%) was observed on mechanical and barrier properties of films it was used for all further studies.

Antioxidant and antimicrobial activity of active films

Grape pomace is known to possess significant amount of phytochemicals which can impart considerable bioactive properties to the active films. In the active films total antioxidant capacity was observed to be 1.1 ± 0.2 and 4.5 ± 0.5 mg TE g⁻¹ of film when analysed by FRAP and DPPH assay, respectively. Content of total anthocyanins was found to be 6.8 ± 1.2 mg 100 g⁻¹ of film while total flavonoid and ascorbic acid was found to be 2.6 ± 0.1 mg quercitin equivalent g⁻¹ of film and 1.6 ± 0.2 mg 100 g⁻¹ of film, respectively. Total phenolic content of active film was 1.6 ± 0.3 mg GAEg⁻¹ of film. On the contrary, no antioxidant activity, total anthocyanins, flavonoid, ascorbic acid, and phenolic content were observed in cling film and non-active GG films. Major constituents which could be responsible for antioxidant activity in active film due to addition of grape pomace extract are phenolic acids (gallic, protocatechuic, caferic, *p*-hydroxybenzoic, and syringic acids) and flavonoids (catechin, epicatechin, and trans-resveratrol) (Corrales et al. 2009). These compounds are also known to have significant antimicrobial activity. Therefore, antimicrobial activity of films was further analysed.

The antimicrobial activity was analysed both qualitatively (the diameter of inhibition zone developed) as well as quantitatively (log cycle decrease in bacterial population). For active film in qualitative assay the zone of inhibition for *E. coli*, *S. aureus*, *B. cereus*, and *S. typhimurium* was found to be 1.3 ± 0.2 , 1.2 ± 0.2 , 1.1 ± 0.1 , and 0.9 ± 0.1 cm, respectively. While, a 3.6 ± 0.3 , 3.3 ± 0.3 , 2.9 ± 0.2 , and 2.1 ± 0.1 log cycle decrease was observed for *E. coli*, *S. aureus*, *B. cereus*, and *S. typhimurium*, respectively in quantitative antimicrobial assay as compared to control. No antimicrobial activity for both the test was however observed in either cling films or non-active GG films. *E. coli* demonstrated highest susceptibility towards the active films followed by *S. aureus*, *B. cereus* and *S. typhimurium*. However, no activity was observed against *B. subtilis*. Both *Bacillus* strains demonstrated different susceptibility towards active film. Bernhard et al. (1978) also observed similar difference in vulnerability of both the *Bacillus* strains against antibiotics. Authors attributed it to presence of different types of antibiotic resistant plasmid in both the strains.

Observed antimicrobial activity of the developed active film could be attributed to the different constituents such as anthocyanins, phenolics, and flavonoids as reported earlier by Corrales et al. (2009). These results clearly demonstrate that developed active films have significant antimicrobial activity. Therefore, they can be suitable for packaging of minimally processed products. Apart from packaging

gamma-radiation can be another hurdle which can be used for shelf-life extension of minimally processed products. Usefulness of radiation processing for shelf-life extension of several minimally processed products such as French beans (Gupta et al. 2012), cauliflower (Vaishnav et al. 2015), ash gourd (Tripathi et al. 2013), and pumpkin (Tripathi et al. 2014) has been earlier demonstrated. In present study, usefulness of developed active film in combination with gamma-radiation for shelf-life extension of minimally processed pomegranate was studied. However, there could be significant changes in mechanical, antioxidant, and antimicrobial properties of films due to radiation processing. Therefore, effect of radiation processing on these properties of films was also analysed.

Effect of radiation on mechanical, barrier, antioxidant, and antimicrobial properties of films

No significant ($p < 0.05$) effect of radiation was observed on mechanical properties of GG based films up to a dose of 5 kGy (Table 2). Films subjected to radiation processing at dose of 5 kGy demonstrated tensile strength and Young's modulus of 88 ± 12 MPa and 73 ± 8 GPa, respectively as compared to control having corresponding values of 104 ± 10 MPa and 83 ± 7 GPa, respectively. However, at doses beyond 5 kGy significant ($p < 0.05$) decrease in mechanical properties was observed. Radiation dose of 7.5 kGy resulted in reduced tensile strength and Young's modulus of 77 ± 8 MPa and 65 ± 5 GPa, respectively. A marginal but statistically not significant ($p < 0.05$) increase in WVTR was observed due to radiation processing. WVTR of control, 5 kGy, and 7.5 kGy irradiated films was observed to be 48 ± 5 , 53 ± 4 , and 60 ± 7 $\text{gm}^{-2} \text{d}^{-1}$, respectively.

In present study it was observed that films were stable only up to a radiation dose of 5 kGy. This is in contrast to our earlier studies (Saurabh et al. 2013) wherein it was observed that mechanical and barrier properties of GG films (prepared without grape pomace) were unaffected up to a radiation dose of 25 kGy. This could be due to various organic additives added (beeswax, grape pomace extract) that can produce significantly higher number of reactive carbon centred radicals during radiation

processing thereby causing decreased film properties. In an earlier study by Naznin et al. (2012), a significant reduction in tensile strength was observed beyond a dose of 2 kGy in starch/PVA-based film incorporated with *Acacia catechu* extract.

Radiation treatment up to a dose of 5 kGy showed no significant ($p < 0.05$) change in qualitative (Supplementary Fig. 1, Table 1) and quantitative antimicrobial activity of film (Supplementary Table 1). Similar result was observed for polyamide coated LDPE film with active compounds (sorbic acid, carvacrol, thymol, and rosemary oleoresin) wherein the antimicrobial activity of the films was found to get retained up to a dose of 3 kGy (Han et al. 2007). Furthermore, no significant ($p < 0.05$) effect of irradiation was observed on antioxidant activity, total anthocyanins, flavonoid, ascorbic acid, and phenolic content of 5% grape pomace extract incorporated active film.

A maximum dose of 3 kGy is required for shelf-life extension of fruits and vegetables (Niemira and Fan 2006). Thus the films developed in the present study can be successfully employed for packing of food that is meant for radiation processing since it demonstrates stability up to a dose of 5 kGy.

Microbial analysis of minimally processed pomegranate

Effect of irradiation (0.5, 1, 1.5, 2, and 2.5 kGy) and types of packaging on microbial load at different storage days is shown in Fig. 1. A significant ($p \leq 0.05$) increase in bacterial load during storage was observed in the non-irradiated samples for all three packaging materials employed (Fig. 1; Supplementary Table 2). Bacterial load of unirradiated arils, at day 0 was 1.79 ± 0.15 $\log \text{cfug}^{-1}$ irrespective of packaging, which increased to 9.2 ± 0.6 , 9.1 ± 0.4 , and 8.1 ± 0.5 $\log \text{cfug}^{-1}$ for arils packed in cling, non-active film, and active film, respectively after storage of 8 days (Fig. 1a, c, e). Similarly total fungal count of unirradiated arils, at day 0 was 0.79 ± 0.01 $\log \text{cfug}^{-1}$ irrespective of packaging, which increased to 2.8 ± 0.1 , 2.76 ± 0.2 , and 2.5 ± 0.1 $\log \text{cfug}^{-1}$ for arils packed in cling, non-active, and active film, respectively after storage of 8 days (Fig. 1b, d, f). It was observed that

Table 2 Effect of gamma irradiation on mechanical and barrier properties of GG film having 5% grape pomace extract

Dose (kGy)	Tensile strength (MPa)	Young's modulus (GPa)	WVTR ($\text{g}/\text{m}^2/\text{d}$)
Control	104 ± 10^a	83 ± 7^a	48 ± 5^a
1	101 ± 8^a	82 ± 6^a	49 ± 4^a
2	95 ± 9^a	79 ± 8^a	51 ± 5^a
5	88 ± 12^{ab}	73 ± 8^{ab}	53 ± 4^a
7.5	77 ± 8^b	65 ± 5^b	60 ± 7^a

Row in same column bearing different superscript are significantly ($p < 0.05$) different

Fig. 1 Effect of irradiation (—●— Control, —■— 0.5 kGy, —▲— 1 kGy, —×— 1.5 kGy, —*— 2 kGy, —◇— 2.5 kGy) and packaging films. **a** Bacterial count for samples packed in active film. **b** Yeast and mold count for samples packed in active film. **c** Bacterial counts for

samples packed in non-active GG films. **d** Yeast and mold for samples packed in non-active GG films. **e** Bacterial count for samples packed in cling film. **f** Yeast and mold count for samples packed in cling films

during entire storage period the bacterial and fungal count of unirradiated arils packed in active films were significantly ($p < 0.05$) lower than samples packed in other films (Fig. 1), however, the difference observed was marginal. This might be due to the fact that active films were not in direct surface contact with pomegranate arils.

A radiation dose dependent decrease in bacterial and fungal population was noted irrespective of packaging at all doses just after treatment (Fig. 1). An approximately 1.5 log cycle reduction in bacterial counts was observed at radiation dose of 2 kGy for all packaging materials. Similarly, for fungal counts approximately 0.75 log cycle reduction was observed at a radiation dose of 2 kGy.

Similar results have been reported earlier for radiation processing of other minimally processed products (Vaishnav et al. 2015; Tripathi et al. 2013; Gupta et al. 2012). During storage a significant ($p < 0.05$) increase in microbial counts was observed irrespective of radiation dose applied, however, samples irradiated at dose of ≥ 1.5 kGy demonstrated significantly ($p < 0.05$) lower microbial counts as compared to unirradiated samples during entire storage period (Fig. 1).

Although irradiated samples demonstrated lower microbial counts during storage, but it was observed that in all the three packaging materials used the bacterial counts reached $> 10^7$ cfu g⁻¹ and yeast and mold counts $> 10^2$

cfug⁻¹ after 8 days of storage for unirradiated arils and samples subjected to radiation up to dose of 1.5 kGy (Fig. 1a, c, e). The acceptable limit of bacterial counts is 10⁷ cfug⁻¹ for fresh fruits and vegetables (Oms-Oliu et al. 2010). These results suggest that up to a dose of 1.5 kGy no increase in shelf-life of pomegranate arils could be obtained.

For irradiation dose ≥ 2 kGy it was observed that bacterial counts were lower than 10⁷ cfug⁻¹ after 8 days of storage for all three packaging films (Fig. 1). Furthermore, it was observed that for samples irradiated at ≥ 2 kGy and packed in cling or non-active films bacterial counts reached $> 10^7$ cfug⁻¹ after 12 days of storage (Fig. 1c, e). Tripathi et al. (2013) also observed no microbial spoilage until 12 days of storage for cling film wrapped ready-to-cook ash gourd when irradiated at 2 kGy or above. Interestingly, for samples packed in active films and subjected to radiation processing at dose ≥ 2 kGy bacterial counts were less than 10⁷ even after storage period of 12 days. Samples packed in active films and irradiated for 2 kGy had bacterial counts of 10⁵ cfug⁻¹ after storage period of 12 days (Fig. 1). Bacterial counts reached beyond 10⁷ - cfug⁻¹ at storage period of 16 days for samples packed in active films and irradiated at 2 kGy.

Longer shelf-life of irradiated arils packed in active films could be attributed to the radiation induced release of volatiles from active film as confirmed by head space GC–MS analysis of irradiated films. It was observed that several volatiles such as acetaldehyde, ethanol, acetone, acetic acid, chloroform, 2-butanone, acetoin, and decanal were released when active films were subjected to radiation processing at a dose of 2 kGy or higher. These volatiles have been already reported in grapes (Rosillo et al. 1999). Thus grape pomace extract also inherited these volatiles when incorporated in GG based films. Head space of non-irradiated active film was characterized by presence of only acetic acid, whereas, no volatile compounds were observed in non-active or cling films (irradiated and non-irradiated).

The radiation induced release of volatiles with storage was further analysed at different storage points. A gradual decrease was observed in volatile content with storage at 10 °C. Table 3 illustrated that at the end of 4th day chloroform, 2-butanone, and decanal was 100% percent lost from irradiated active film and after 8 days only acetaldehyde and ethanol was detected by GC–MS. However at the end of 12 days no volatiles were detected. Volatiles like acetaldehyde, ethanol, and acetic acid are antimicrobial in nature (Oh and Marshall 1993; Ryssel et al. 2009). Up to 1.5 kGy no volatiles except acetic acid were observed by GC–MS which further volatilized completely after 4 days of storage and this explain the ineffectiveness of lower doses on shelf-life improvement of arils packed in active films.

Table 3 Percent decrease in peak area of volatiles released from irradiated (2 kGy) active films during storage

Volatiles	Day 0	Day 4	Day 8	Day 12
Acetaldehyde	0 ^d	17.6 $\pm$ 2.1 ^c	48.9 $\pm$ 3.3 ^b	100 ^a
Ethanol	0 ^d	15.7 $\pm$ 0.3 ^c	43.8 $\pm$ 1.2 ^b	100 ^a
Acetone	0 ^c	41.5 $\pm$ 1.9 ^b	100 ^a	100 ^a
Acetic acid	0 ^c	58.4 $\pm$ 3.3 ^b	100 ^a	100 ^a
Chloroform	0 ^b	100 ^a	100 ^a	100 ^a
2-Butanone	0 ^b	100 ^a	100 ^a	100 ^a
Acetoin	0 ^c	54.3 $\pm$ 4.5 ^b	100 ^a	100 ^a
Decanal	0 ^b	100 ^a	100 ^a	100 ^a

Row in same column bearing different superscript are significantly ($p < 0.05$) different

Higher increase in shelf-life of irradiated (2 kGy) samples packaged in active films could be due to prolonged exposure of packed product to the released volatiles. Irradiated (2 kGy) samples packed in commercial cling films and GG based active films were further compared for their sensory and textural properties.

Sensory analysis

Sensory quality of control and irradiated samples at different storage points were analyzed by hedonic scores. Sensory analysis of control samples was not performed beyond day 4 because they were microbiologically unsafe. The different sensory attributes of control and 2 kGy irradiated pomegranate arils is tabulated in Table 4. Sensory quality of non-irradiated sample packed in either of the films was found to deteriorate within 4 days of storage owing to the blackening of the arils. Irradiated samples were sensorially acceptable during the entire storage. No significant ($p < 0.05$) difference in the aroma quality of samples was observed between control and irradiated sample immediately after irradiation. The irradiated samples were found to maintain the aroma quality throughout the storage period of 12 days when packed in active film and 8 days for cling wrapped samples. No difference was observed in the texture quality between control and irradiated samples throughout the storage period. The taste quality of irradiated samples packed in active and cling wrapped films was maintained up to a storage period of 12 and 8 days respectively. Results from hedonic testing demonstrated that the overall quality of the control samples lowered gradually with storage whereas the irradiated samples received good overall sensory scores throughout the storage period irrespective of packaging used. Sensory acceptability of 2 kGy irradiated arils was higher than 2.5 kGy irradiated arils due to radiation induced softness of

Table 4 Effect of radiation treatment and storage on sensory quality of arils packed in: (A) Active film; (B) Cling film

Days	Dose	Color	Texture	Taste	Aroma	Overall acceptability
A						
0	Control	7.1 ± 1.1 ^a	6.8 ± 0.4 ^a	7.9 ± 0.8 ^a	6.0 ± 1.0 ^a	7.6 ± 0.9 ^a
	2 kGy	7.3 ± 0.6 ^a	6.6 ± 0.7 ^a	7.7 ± 1.1 ^a	6.5 ± 0.6 ^a	7.3 ± 0.8 ^a
4	control	7.2 ± 0.4 ^a	6.3 ± 0.5 ^a	7.5 ± 0.3 ^a	6 ± 0.7 ^a	6.5 ± 0.6 ^a
	2 kGy	7.1 ± 1.1 ^a	6.5 ± 0.8 ^a	7.4 ± 0.7 ^a	6.1 ± 0.4 ^a	6.9 ± 0.9 ^a
8	Control	NA	NA	NA	NA	NA
	2 kGy	6.9 ± 0.8 ^a	6.3 ± 0.9 ^a	7.1 ± 0.7 ^a	6.0 ± 0.8 ^a	6.8 ± 0.7 ^a
12	Control	NA	NA	NA	NA	NA
	2 kGy	6.7 ± 0.7 ^a	6.2 ± 0.8 ^a	6.7 ± 0.6 ^a	6.0 ± 0.6 ^a	6.5 ± 0.3 ^a
B						
0	Control	7.2 ± 1.2 ^a	6.7 ± 0.5 ^a	7.7 ± 0.9 ^a	6.0 ± 1.0 ^a	7.7 ± 0.8 ^a
	2 kGy	7.3 ± 0.5 ^a	6.6 ± 0.7 ^a	7.6 ± 1.3 ^a	6.4 ± 0.6 ^a	7.4 ± 1.1 ^a
4	Control	7.0 ± 0.8 ^a	6.2 ± 0.5 ^a	7.4 ± 0.7 ^a	6.1 ± 0.6 ^a	6.7 ± 0.8 ^a
	2 kGy	7.2 ± 1.2 ^a	6.7 ± 0.9 ^a	7.2 ± 0.7 ^a	6.3 ± 0.5 ^a	6.8 ± 0.9 ^a
8	Control	NA	NA	NA	NA	NA
	2 kGy	7.1 ± 0.6 ^a	6.4 ± 0.7 ^a	7.0 ± 0.7 ^a	6.2 ± 0.8 ^a	6.6 ± 0.7 ^a

Row in same column bearing different superscript are significantly ($p < 0.05$) different

Fig. 2 Effect of irradiation and packaging material (—♦— Control, Active packaging; —■— Irradiated (2 kGy), Active packaging; —▲— Control, Cling film; —×— Irradiated (2 kGy), Cling film). **a** Texture of

minimally processed pomegranate arils. **b** Moisture loss of minimally processed pomegranate arils

fruits at 2.5 kGy. Therefore, 2 kGy was found to be the optimum dose required for maintenance of microbial safety during storage of ready-to-eat pomegranate arils.

Texture analysis

Firmness loss of fruits often affects consumer acceptability. Texture of minimally processed pomegranate arils was therefore analyzed by a texture analyzer. Figure 2a provides the firmness of both the control and irradiated (2 kGy) samples packed in packed in active and cling films. Firmness of control samples packed in active film increased from 156 ± 10 N at day 0 to 225 ± 12 N at the end of storage period. On the other hand a slight decrease

in firmness was noted immediately after irradiation (135 ± 10 N) which however gradually increased to the value of 236 ± 13 N by the end of the storage period. Increased texture during storage may be attributed to moisture loss of arils through the packaging. However, the extent of increase in firmness was significantly ($p < 0.05$) lesser for samples packaged in cling films as compared to those packed in active films. After storage period of 12 days irradiated samples packed in cling films demonstrated a texture of 163 ± 11 N. This might be due to high moisture loss through GG based active films. Observed increase in firmness as determined by the texture analyzer was, however, not perceived by the sensory panellists.

Moisture loss

Figure 2b demonstrates the effect of irradiation and storage on weight losses (%) of minimally processed pomegranate arils packed in cling and active films. There was a significant ($p < 0.05$) weight loss in all the samples during storage. Maximum weight loss of $3.2 \pm 0.18\%$ at the end of 12 days was observed for 2 kGy irradiated arils packed in active film. However, 2 kGy irradiated arils wrapped in cling film resulted in water loss of $2.2 \pm 0.2\%$. A similar observation was made by Hamid et al. (2012) wherein maximum weight loss of 1.38% was noted for pear fruit subjected to a dose of 1 kGy and packed in polyolefin shrink film for storage. The GG based films have been demonstrated to have higher WVTR than thin film of polyolefin or cling film. This can justify the higher percentage of weight loss in the present study.

Conclusion

In present study GG based active packaging films were prepared using grape pomace extract. Addition of grape pomace extract up to 5% w/w of GG did not result in significant changes in mechanical properties of films. Developed films demonstrated significant antimicrobial activity against various food pathogens. Furthermore, active films were stable to radiation treatment up to a dose of 5 kGy. Efficacy of developed active films was compared with commercial cling films for packaging of irradiated minimally processed pomegranate samples. Irradiated arils (2 kGy) packaged in active films demonstrated improved shelf-life of 12 days as compared to 8 days for samples packed in cling films. This was attributed to radiation induced release of various antimicrobial volatiles such as acetaldehyde, ethanol, acetone, acetic acid, chloroform, 2-butanone, acetoin, and decanal from active film as confirmed by GC–MS. Sensory attributes of all arils samples were acceptable during entire storage period. Thus potential use of grape pomace for development of active packaging films is demonstrated.

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