Effect of combined preservation techniques on the stability and microbial quality and retention of anthocyanins in grape pomace stored at low temperature

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Abstract Studies were carried out with an objective to assess the effect of various post harvest treatments with chemical preservative, ionizing radiation doses and low temperature (LT) storage on the storability and the microbial quality of the grape pomace and retention of anthocyanins in it. The grape pomace collected after processing of fully matured grape berries (var.Bangalore blue) was treated with 0.1% sodium benzoate(0.1% SB), packed and sealed in LDPE film pouches and exposed to γ -irradiation(0, 2, 4 and 6 kGy) and stored along with untreated control at LT (4 ± 1 °C, 90–95% RH)conditions. During storage, changes in physico-chemical and microbial quality attributes of these treated grape pomace were recorded. Among the treatments, combination of 0.1% sodium benzoate and 2.0 kGy dose of γ -irradiation (0.1% SB +2.0 kGy) was found to be more effective in improving the storability of grape pomace and in retention anthocyanins (34.70 mg/100 g) during storage. The storage life of above treated grape pomace stored at LT could be extended up to 16 days as compared to 8 days of control grape

pomace (31.8 mg/100 g). Results on microbiological studies indicated that 0.1% SB +2.0 kGy reduced the total aerobic bacterial count (1.4 log CFU/g) and the yeast and mould count (1.6 log CFU/g) in the treated grape pomace during 16 days of storage as compared to the total aerobic bacterial count (6.5 log CFU/g) and the yeast and mould count (6.3 log CFU/g) in control. Sodium benzoate and low irradiation (0.1% SB +2.0 kGy) was significant in controlling the multiplication of coliforms in grape pomace during LT storage and thus finds potential for commercial application in ensuring the microbiological safety and quality of grape pomace during its bulk storage at low temperature (4 ± 1 °C; 90–95% RH) conditions. This preservation method could be used by the wine making industries for bulk storage of grape pomace after processing of colored grapes during peak harvesting season for further end uses.

Keywords Grape pomace · Storability · Gamma irradiation · Chemical preservative · Anthocyanins · Microbiological quality

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World's largest commercially important fruit crop is Grape (*Vitis vinifera*), with an annual production of 42 million Tons (FAO 2005). The economical importance of grapes and products (Prabhakara Rao et al. 2009) obtained there from, such as wine, grape juice, jams to raisins, is therefore obvious. About 80% of the World's total grape is used for wine making (Patil et al. 2007), 13% is sold as table grapes and the balance is used largely for raisins, juice and other processed products(Kammerer et al. 2004). At present, India produces nearly 12.6 lakh Tons of total grapes being produced from a cultivated area of 60,000 ha in India (FAO 2004). Unlike grape flesh, grape skin is the potential source of antioxidant and anticarcinogenic phenolic compounds

(Cantos et al. 2002). Both skins and seeds of most red grape varieties constitute a promising source of polyphenolics. Grape skin is proved to be rich source of anthocyanins and flavonols, while seeds are rich in flavonols and to some extent, flavonol glycosides are only present in the grape skins (Kammerer et al. 2004). The grape waste generated from grape wine industries comprises of 5–12% grape peels, 2–6% grape stalk and stem waste to 0–10% seed waste. Ripe berries of red grape are reported to contain anthocyanins in terms of anthocyanidins of 30–750 mg/100 g of fresh weight and that these compounds are only present in the grape skin (Bridle and Timberlake 1997).

Grape pomace is the major source of anthocyanins. The availability of bulk quantities of grape pomace as byproduct from wine industry in the peak harvesting season is for a very short period. Its utilization for food industrial application of anthocyanins as natural colorants so far has not been fully exploited due to its short storage life resulted from its microbial spoilage, instability of anthocyanins during storage, difficulty in bulk handling and storage of grape pomace and lack of knowledge on improved methods of storage (Mahajan et al. 2009). Reported work on γ -irradiation preservation of grape pomace for improving its storability and maintaining its quality and retention of anthocyanins during grape pomace storage has been found scanty.

Accelerated microbial changes are an impediment to the usage of pomace for extraction of anthocyanins. So far reported research findings on the efficiency of γ -irradiation on microbiological status of grape pomace are very limited. A logistic approach for efficient utilization of grape pomace in improving its storability and maintaining its quality in terms of microbial quality and anthocyanins stability by application of γ -irradiation treatments in combination with low concentration of chemical preservative and packaging with LDPE film pouches under low temperature storage conditions. Keeping these factors in view, the objective of this study was to optimize the conditions for extending the shelf life of grape pomace and to ensure the microbial safety of grape pomace and to retain anthocyanins pigments during storage of grape pomace.

Materials and methods

Sample preparation Fully matured fresh blue grape berries of Bangalore Blue variety were procured from the local market of Mysore, India. The fruits were then sorted and graded for uniform maturity, color and size. These graded grapes were then washed thoroughly with tap water and kept in sieved trays for draining. The cleaned grapes were destalked, loaded into the fruit miller and fruit pulper equipments and the juice was separated and the grape pomace was used for experimentation.

Irradiation treatments As per the recommendations of Joint Expert Committee on Food Irradiation, irradiation of any food commodity up to an overall average dose of 10 kGy is safe for human consumption and does not pose any special nutritional, microbiological problems or toxicological hazards (WHO 1994). So far irradiation of grape pomace for commercial use in India is not permitted under PFA rules, 1955 (PFA 1955), however, in the present study from the research point of view, grape pomace was irradiated from 2 to 6 kGy doses, with an objectives of reduction of total microbial loads, prolonging storage life of grape pomace and better recovery of anthocyanins from the stored grape pomace. The dose range of 0–6 kGy was fixed based on reported work for achieving effective storage life of grape pomace and maximum recovery of anthocyanins yield from grape pomace at the dose of 6 kGy (Ayed et al. 1999). In the experiment, each sample of 100 g of grape pomace was thoroughly mixed with about 0.1% sodium benzoate (0.1% SB) packed in low density polyethylene (LDPE) pouches (as only packaging material) and were exposed to different doses of γ -rays (0, 2, 4 and 6 kGy) in Gamma Chamber (Model GC-5000) at dose rate of 6.834 kGy/h at 22.7 °C, using ^{60}Co as radio isotope as the main source of γ -irradiation. Both the treated and control samples of the experiment were subsequently stored at low temperature (LT) (4 ± 1 °C; 90–95% RH) conditions for 16 days. Samples were periodically analysed for various physico-chemical (0, 4, 8, 12 and 16 days of LT storage) and microbiological quality attributes (0 and 16 days of LT storage).

Details of treatments

- Experiment-1: The treatments were T_0 : untreated control, T_1 : 2.0 kGy, T_2 : 4.0 kGy and T_3 : 6.0 kGy.
- Experiment-2: The treatments were T_0 : untreated control, T_1 : 0.1% SB, T_2 : (0.1% SB +2.0 kGy), T_3 : (0.1% SB +4.0 kGy) and T_4 : (0.1%SB +6.0 kGy).

Fruit colour measurement It was done using colour measuring system (Model, Lab scan XE, Hunter Associates Inc., USA) and at wave length ranging from 400 to 700 nm and expressed in terms of Hunter colour 'L' value and a/b ratio (Hunter 1975). L is a measure of lightness on a scale terms ranging from zero (black) to 100 (white): + a denotes redness,—a indicates greenness when the components values are negative: + b denotes yellowness when the values are positive and—b indicates blue, when the values are negative respectively. Mean of 3 readings is reported. For all chemical estimates, homogenated samples from each treatment were used titratable acidity (in terms of% tartaric

acid) was analyzed as per the methods suggested by Ranganna (1986). All estimations were carried out in three replicates per treatment and were expressed on fresh weight basis.

Total anthocyanins Total anthocyanins in the sample were determined by colorimetric method suggested by Ranganna (1986) at 535 nm using UV visible spectrophotometer (Model: Shimadzu – 760A) and is expressed in mg of anthocyanins/100 g of fresh weight.

Total polyphenolics content Total polyphenolics in the sample were measured by colorimetric method by Folin ciocalteau reagent at 765 nm using UV visible spectrophotometer (Model: Shimadzu – 760A) and the tannic acid was used as standard for estimating total polyphenolic compounds (such as **anthocyanins**, tannins flavonols etc.).

Microbiological analysis Dehydrated media used were procured from Himedia laboratories pvt. Ltd, Mumbai, India. Triplicate samples were analysed for total plate count, coli forms and yeast and mould count. Yeast and mould count was determined by using standard spread plate method. Pour plate method was employed for total aerobic bacteria and coliform count. 10 g of sample was homogenized in a masticator with 90 ml of Butter field's phosphate buffer and further diluted as needed under aseptic conditions. 0.1 ml of appropriate dilutions was plated in case of spread plate and 1 ml for pour plate. Plating was done on Plate count agar for total aerobic count, yeast and mould count on Rose Bengal Chloramphenicol agar and on Violet red bile agar for coliforms. For coliform count, Violet red bile agar was over layed with 5 ml of the same agar. Plates were incubated at 37 °C for 48 h for total aerobic bacteria and coliforms count and at 30 °C for 96 h in the case of yeast and mould count. Colony forming units were counted manually.

Statistical analysis All the determinations were made in 3 replicates, using 3 different batches of samples. Statistical analysis of data was carried out by Duncan's Multiple Range Test procedures of Statistical Analysis System(SAS 1985) with a view to compare the treatments at $p < 0.05$ level of significance. The standard error values were within the acceptable limit range. Each microbial count was the mean of three determinations ($n=3$) and microbial counts were expressed as $\log_{10}$ CFU/g and standard deviation was calculated.

Results and discussion

Results of experiment-1 with respective to changes in physico-chemical quality attributes of grape pomace were

not significant except the results on microbiological quality attributes of grape pomace and hence it is not presented in the paper.

I. Physical characteristics of grape pomace

Fruit color characteristics of grape pomace It was observed from the Fig. 1 that among the treatments, T_0 (untreated control) registered significantly lower 'L' values during 16 days of LT storage, followed by T_1 (0.1% SB) and T_2 (0.1% SB + 2.0 kGy) as compared to other treatments and highest 'L' value was recorded in T_3 (0.1% + SB 4.0 kGy) at the end of LT storage. Results on changes in fruit colour in terms of hunter value 'a' of grape pomace indicated that all treated ones maintained ($p < 0.05$) higher 'a' during 8 days of LT storage as compared to Untreated

Fig. 1 Effect of gamma irradiation doses and sodium benzoate treatment on changes in Hunter color of grape pomace (var. Bangalore blue) during storage at 4 ± 1 °C and 90–95% RH conditions

control(T_0). However, only T_4 (0.1% SB + 6.0 kGy) recorded ($p < 0.05$) higher 'a' value (of 13.21 and 12.75 respectively) during 12 and 16 days of LT storage as compared to others. Results on hunter parameters (L, a) revealed that treating the grape pomace with 0.1% sodium benzoate(SB) in combination with gamma irradiation (6 kGy dose) was found to be most effective treatment in retaining the initial grape pomace colour for 16 days of LT storage. This could be due to enhancement in accumulation of anthocyanins in grape pomace irradiated at higher doses that resulted due to rapid increase in cell membrane and cell wall degradation to these pigments more efficiently during LT storage (Kwon et al. 1993; Ayed et al. 1999) and the presence of sodium benzoate (0.1% SB) as the chemical preservative to facilitate the high acidic medium that might had prevented the loss of accumulated anthocyanins with better retention of these pigments and prolonging the shelf life of T_4 (0.1% SB + 6.0 kGy) treated grape pomace up to 16 days at LT storage.

II. Chemical characteristics of grape pomace

Titrateable acidity (%) of grape pomace It is evident from Fig. 2 that titrateable acidity increased during 8 days of LT storage from 2.09 to 4.50 and declined thereafter. Among the treatments only T_2 (0.1% SB + 2.0 kGy) registered ($p < 0.05$) higher acidity of grape pomace during 12 days of LT storage as compared to untreated control and others. However on 16th day of LT storage, it was only T_3 (0.1% SB + 4.0 kGy) followed by T_4 (0.1% SB +6.0 kGy) and T_2 (0.1% SB + 2.0 kGy) were recorded ($p < 0.05$) higher acidity than others. Maintenance of higher acidity and retention of higher anthocyanin content in the above treated grape pomace during LT storage could be attributed to the high acidic medium nature of sodium benzoate (0.1% SB).

Total anthocyanins (mg/100 g) of grape pomace It showed (Fig. 2) that grape pomace initially had the highest total anthocyanin pigment content of 66.4 mg/100 g and it was declined with storage time to 20 mg/100 g on 16th day of LT storage. Among the treatments T_2 (0.1% SB + 2.0 kGy) recorded higher total anthocyanin pigment content ($p < 0.05$) during 8 days of LT storage as compared to others. This could be due to the fact that the activities of enzyme systems related to the biosynthesis of anthocyanin monomers such as catechol oxidase gets enhanced by several folds in irradiated perishables like mushrooms at low doses than the controls a few days after gamma irradiation (Beaulieu et al. 1999). A similar increase in flavonoid content after irradiation for anthocyanins in strawberries (Couture and Willemot 1989) and for flavonoids in Clementina (Oufedjikh et al. 1998). Subsequently, on LT

Fig. 2 Effect of gamma irradiation doses and sodium benzoate treatment on chemical changes in grape pomace (var. Bangalore blue) during storage at 4+1 ° C and 90–95% RH conditions

storage of these gamma irradiated T_2 (0.1% SB + 2.0 kGy) grape pomace might possibly increased the yields of anthocyanins extracted from grape pomace on 16th day of LT storage (34.70 mg/100 g). Apart from T_2 , T_1 (0.1% SB) recorded ($p < 0.05$) higher total anthocyanin pigment content ($p < 0.05$) (33.10 mg/100 g) than others and the least (20 mg/100 g) value was noticed in T_0 (control). Chemically the anthocyanin pigments of coloured grapes predominantly constitute anthocyanidins with cyanidin, delphinidin, petunidin, peonidin and malvinidin. These are water soluble and exhibit their stability with intense colour under acidic condition ($< \text{pH} - 3.5$) and found suitable for imparting blue, purple red and black colour to acidic food and processed food products (Singleton and Esau 1969).

Retention of higher anthocyanins in grape pomace treated with T_2 (0.1% SB + 2.0 kGy) and T_1 (0.1% SB) at the end of LT storage, could be due to maintenance of high acidic medium by sodium benzoate and less anthocyanins degradation with the use of these treatments. On the other hand, rapid degradation of anthocyanin pigments in control T_0 (66.4 to 20 mg/100 g) resulted from the progressive decline in acidity (1.75%) and increased microbial spoilage of grape pomace during LT storage that led to decline in storage life of grape pomace to a short period of 8 days under same storage condition. Similarly, Ayed et al. (1999) reported highest anthocyanins yield extraction in the presence of 2,000 ppm of SO_2 from modified atmosphere (MA) packed grape pomace after pretreatment with 0.1% sodium metabisulfite and irradiated with 6.0 kGy.

Total polyphenolics content in grape pomace The results (Fig. 2) revealed that the grape pomace showed initially highest total phenolics content of 0.62 mg/g and it was decreased during 12 days of LT storage to 0.12 mg/g. Among the treatments only T_2 (0.1% SB + 2.0 kGy) and T_3 (0.1% SB + 4.0 kGy) recorded higher total phenolics content ($p < 0.05$) than others during 12 days of LT storage and the least (0.08 mg/g) value was noticed in T_1 (0.1% SB). This might be due to the synergic effect of γ -irradiation (at 2.0 and 4.0 kGy dose levels) and 0.1% concentration of the sodium benzoate on better retention of total phenolics in the treated grape pomace. Total phenolics in grape pomace predominantly consist of anthocyanins, tannins and flavonols. The higher accumulation and better retention of anthocyanins in the above treated grape pomace could be possibly due to preventive action of these phenolics on degradation of anthocyanin pigments during 16 days of LT storage.

III. Microbiological quality of irradiated grape pomace

Microbial load of various fruits and fruit products including grape and grape pomace during low temperature storage can be reduced to below normal range of microbial load by exposing them to higher doses of γ -irradiation. But these doses are usually beyond the tolerance limit of fresh grapes and their pomace due to high water content.

Effects of γ -irradiation treatments on microbiological quality of grape pomace Initially fresh grape pomace exhibited large variations in microbiological quality indicating possible contamination during growth, harvesting, post harvest handling, transportation, juice extraction and storage condition. The data given in Table 1 depicts the microbial load of γ -irradiated grape pomace during LT storage. In grape pomace, the initial load of total aerobic

bacteria, yeast and mould was 3.9 log and 4.1 log CFU/g while the coliform was not detected ($< 1 \log_{10}$ CFU/g of pomace). There was gradual decrease in the microbial load with increasing dosage of γ -irradiation. The highest dose of 6 kGy (T_3) resulted in 1.7 log cycle reduction of total aerobic bacteria, 2.1 log cycle kill of yeast and mould as compared to the untreated grape pomace (T_0) on the initial day. However, on subsequent storage for 16 days at LT resulted in 2.5 log increase of total aerobic count in untreated pomace (T_0). On the other hand, samples irradiated with 6 kGy (T_3) exhibited slow growth of the bacterial survivors i.e., increase by 1.2 log during 16 days storage with reference to the initial day counterpart. Grape pomace exposed to 2 and 4 kGy dosage of γ -irradiation showed 3.1 log and 2.2 log increase over a storage period of 16 days when compared with its treated counterparts on the initial day.

Yeast colonies dominated the population of yeast and moulds. The effect of irradiation on this population was similar to that observed for the total aerobic bacterial population. The count of yeast and moulds in untreated pomace increased from log 4.1 to log 5.6 during 16 days study. While grape pomace irradiated with 2 kGy and 4 kGy dose resulted in increase in this population by 1.6 and 2.0 logs respectively with reference to the zero day counterpart. γ -irradiation of 6 kGy (T_3) dose initially decreased the count of yeast and mould by 2.1 log as compared to the untreated sample (T_0). Though the population did increase during storage period of 16 days from 2.0 to 3.3 logs, the count of yeast and mould remained lower than the untreated at each time point. In both untreated and irradiated grape pomace, the coliform was undetected ($< 1 \log_{10}$ CFU/g). There was no evidence of reoccurrence of coliforms in both the samples during LT storage of 16 days.

The mechanism of microbial inactivation by ionizing radiation is mainly due to the damage of nucleic acids, direct damage or indirect damage, affected by oxidative radicals originating from the radiolysis of water. The radiation sensitivity of many moulds is of the same order of magnitude as that of vegetative bacteria (Farkas 2006). Above results are in agreement with Ayed et al. (1999) also reported that irradiation at 6.0 kGy inhibited the growth of bacteria, yeast and mold on the grape pomace during storage at 24 °C.

Combined effect of γ -irradiation and sodium benzoate treatments on microbiological quality of grape pomace Sodium benzoate (SB) is a widely used chemical preservative and γ -irradiation in combination with sodium benzoate (0.1%) reduces the population of total plate count, yeast and moulds and coliforms in grape pomace effectively as illustrated in Table 1. With addition of sodium benzoate,

Table 1 Effect of different dosage of gamma irradiation and 0.1% sodium benzoate treatments on microbial loads (log₁₀ CFU/g) of grape pomace stored at 4 ±1 °C and 90–95% RH conditions

Treatment	Dosage (kGy)	Sodium benzoate	Total aerobic count		Yeast & moulds	
			0 day	16 days	0 day	16 days
With irradiation	0 ^a	0	3.9a±0.12	6.4b±0.00	4.1a±0.10	5.6b±0.10
	2	0	3.2a±0.05	6.3b±0.03	3.4a±0.10	5.0b±0.07
	4	0	3.0a±0.09	5.2b±0.06	2.9a±0.15	4.9b±0.06
	6	0	2.2a±0.10	3.4b±0.15	2.0a±0.03	3.3b±0.07
Treatment with irradiation and sodium benzoate	0 ^b	0	5.5a±0.00	6.5b±0.02	5.0a±0.02	6.3b±0.14
	0	0.1%	4.5a±0.03	3.7b±0.06	4.4a±0.00	3.5b±0.10
	2	0.1%	3.4a±0.06	1.4b±0.04	3.1a±0.10	1.6b±0.26
	4	0.1%	3.3a±0.06	1.2b±0.15	2.7a±0.06	1.8b±0.06
	6	0.1%	2.3±0.06	ND ^c	2.6a±0.06	1.9b±0.06

Means in a column with different letters differ significantly at $p < 0.05$ between the storage days

^a Control—Untreated grape pomace

^b Control—Untreated grape pomace without sodium benzoate (SB)

^c ND Not detected, detection limit for total aerobic count is 1 log₁₀ CFU/g

the total aerobic count reduced from 5.5 log cycle to 4.5 log cycle. Addition of sodium benzoate reduced the total plate count to 4.5 which gradually decreased with increase in dosage of γ -irradiation. Combined effect of γ -irradiation of 2 kGy and sodium benzoate (T₂: 0.1% SB +2.0 kGy) reduced the total plate count to 1.4 log CFU/g and not much difference was observed in 4 kGy irradiated pomace (T₃: 0.1% SB +4.0 kGy) during 16 days of LT storage. Combined effect of γ -irradiation and sodium benzoate on grape pomace gradually reduced the total aerobic count to 2.3 log CFU/g on exposure to 6 kGy (T₄: 0.1% SB +6.0 kGy) on the initial day. Over the storage period of 16 days, the count of total aerobes in control sample enhanced to 6.5 logs, while in sodium benzoate treated sample it further decreased to 3.7 logs. Combination of sodium benzoate and 6 kGy dose of γ -irradiation (T₄: 0.1% SB +6.0 kGy) on 16 days of storage of pomace in LT, reduced total aerobic bacteria to <1 log₁₀ CFU/g.

A significant reduction in the count of yeast and moulds from log 5.0 to log 3.1 was observed in combined treatment of γ -irradiation (2 kGy) and sodium benzoate in grape pomace (T₂: 0.1% SB +2.0 kGy) on the initial day. With increased exposure to 4 kGy (T₃: 0.1% SB +4.0 kGy), there was further reduction in count of yeast and mould to 2.7 log cycle. The control samples without γ -irradiation (T₀) and the absence of sodium benzoate favored growth of yeast and mould to 6.3 log during the storage period for 16 days at 4 ±1 °C. Under the same storage conditions, irradiated grape pomace (2 kGy) with sodium benzoate (T₂: 0.1% SB +2.0 kGy) restricted the growth of yeast and mould to 1.6 log CFU/g. As a preservative, sodium benzoate controls microbial spoilage effectively possibly by interfering with

the mechanism of cell division, permeability of cell membrane and activity of enzymes in aerobes (Srivastava and Sanjeev 1993). However, the increase in dosage to 4 and 6 kGy had no marked significance in reducing the yeast and mould count. It was observed that irradiation dosage of 6 kGy (T₄: 0.1% SB +6.0 kGy) restricted the count of yeast and mould to 1.9 log CFU/g over a storage period of 16 days at LT.

Microbial degradation resulting in changes such as browning and softening was clearly delayed in irradiated samples (Koorapati et al. 2004). From the above two experiments we can suggest that irradiation was effective in reducing the microbial count. However, the combined treatment of irradiation and addition of sodium benzoate was more effective in controlling the count of total aerobes and yeast and mould in grape pomace during storage at 4 ±1 °C. Similar observation was made by Kamat et al. (2003) in his studies on combined effect of low dose of (1 kGy) gamma irradiation and chlorination treatment (250 ppm) to improve the microbiological safety of fresh coriander leaves.

There has been increasing interest throughout the world to employ γ -irradiation to enhance the shelf-life of perishable fruits as well as to ensure the microbiological safety of the product and the same can be extended to its by-products like grape pomace. The effects of γ -irradiation on extraction of anthocyanin from grape pomace to microbiological quality of pomace during storage after treatment was studied by Ayed et al. (1999). The results revealed that gamma irradiation combined with acidic aqueous extraction could be used for large scale recovery of flavonoids and polyphenols from grape pomace. The

microbial and chemical changes during the spontaneous ensilage of grape pomace was examined by Pina and Hogg (1999) and results indicated that after initial growth, yeast and Lactic acid bacteria population underwent progressive inactivation at 20 °C. Neall (2003) revealed that wine waste be processed for tartrate, yeast and alcohol recovery, tartaric acid, insecticides and preparation of health supplements.

Conclusion

It may be concluded from the present investigation that among the treatments T₂ (0.1% SB + 2 kGy) was found to be most effective in maintaining anthocyanins stability, in improving the storability of grape pomace stored at LT conditions (4±1 °C; 90–95% RH). The treated grape pomace could be preserved under LT storage conditions up to 16 days as compared to 8 days of untreated control grape pomace, with the better recovery of anthocyanins from the grape pomace after LT storage period.

However, the microbial studies of grape pomace stored at LT storage suggests that among the treatments, γ-irradiation of 6 kGy was effective in reducing microbial loads of grape pomace during 16 days of LT storage, in terms of mesophilic aerobic bacterial count, yeast and moulds and coliforms count. γ-irradiation in combination with sodium benzoate as chemical preservative agent (0.1% SB + 2 kGy) finds potential application in ensuring the microbiological safety and quality of grape pomace during its bulk storage at low temperature (4±1 °C; 90–95% RH) conditions by the grape and wine making industries.

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