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Effect of chitosan on biochemical composition and antioxidant activity of minimally processed 'Wonderful' pomegranate arils during cold storage

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Summary

Developing postharvest protocols incorporating nonchemical compounds with the aim of extending shelf life and maintaining quality of fresh produce is of great importance. The potential of chitosan (irradiated and unirradiated) in preserving pomegranate arils (cv. Wonderful) during cold storage was investigated in this trial. Solid chitosan powder was gamma irradiated with 25 and 50 kGy doses, chitosan solutions were prepared and arils were treated prior to cold storage. Initial arils quality characteristics were assessed before refrigeration at 5 ºC and RH 75% for up to 15 day, during which studied characteristics were determined trice, with a 5 day interval. Different treatments were applied by immersion in one of the following for 2 min with: Control (distilled water); 0.5%, unirradiated chitosan; 0.5%, 25 kGy irradiated chitosan; 0.5%, 50 kGy irradiated chitosan; 1.0%, unirradiated chitosan; 1.0%, 25 kGy irradiated chitosan; 1.0%, 50 kGy irradiated chitosan solutions). Results revealed that chitosan treatments reduced weight loss and controlled reductions in total soluble solids, titratable acidity, vitamin C and anthocyanin contents. Increased total antioxidant activity was also detected at the end of the cold storage period and was accompanied with increased total phenolic compounds. Such findings suggest that chitosan can be beneficial in extending shelf life and maintaining biochemical quality of fresh cut fruits.

Introduction

Pomegranate (*Punica granatum* L.) belongs to the *Punicacea* family and is one of the oldest known edible fruits. It is an important and commercial horticultural fruit which originated in the Middle East before it spread in the Mediterranean region and subtropical areas. Its fruits are of great importance because of its biological properties (antimicrobial, antioxidant, anticancer, anti-inflammatory) attributed to bioactive compounds in extracts obtained from several parts of the plant. That's why pomegranate extracts have been used in therapeutics, such as in the prevention of infection, inflammation, cancer, among other applications, beside the nutritional value of arils. Pomegranate is a non-climacteric fruit that does not ripen after harvest, and that's why, it's harvested when fully ripened after showing optimal organoleptic characteristics.

Arils, the edible parts of pomegranates make up approximately 50% of the fruit weight and are made up of 76-85% juice and 24-15% seeds (VARASTEH et al., 2012). Among the factors that affect the composition of pomegranate juice and the content of bioactive compounds are, postharvest conditions, storage, and processing, besides genetics, fruit maturity, environmental and agronomic factors (MIGUEL et al., 2004; POYRAZOGLU et al., 2002). Over the past few years, drastic increases in world trading of pomegranate fruits were noticed as a result of the growing awareness to its nutritional value. Meanwhile, several physiological and enzymatic disorders occur during cold storage causing quality loss. Never the less, loss of aril color, vitamin C, and acidity were reported, which were accompanied by reduction of acceptability in terms of freshness, juiciness, and taste (ARTÉS et al., 1998 and NANDA et al., 2001). Thus, the development of appropriate postharvest protocols and treatments are required to maintain quality during handling and transport of fresh produce. Moreover, there has also been an increasing interest in the development of new pomegranate derived food products such as minimally processed pomegranate seeds ("ready-to-eat") and products derived from it. That's why, maintaining aril quality after peeling of low commercial value fruits (small size, cracked, bruised, sun-burnt, noncommercial varieties) is of great importance, since it allows the use of fruits that cannot be commercialized in the fresh-fruit market in spite of having arils with good quality juice and seeds.

There is a growing emphasis on environmentally friendly postharvest technologies that maintain produce quality, and among those, satisfactory results have been reported for using natural compounds such as chitosan as a safe alternative to hazardous chemicals with negligible risk to human health and environment. Moreover, from a biological perspective, chitosan and its oligomers are very attractive for agricultural applications. In this regard, it is well know that when chitosan is subjected to gamma irradiation, chain scissions occur through the breakage of β (1-4) glycosidic bonds bonding the glucosamine and N-acetylglucosamine units that constitute chitosan. In solid state, such scissions are mainly due to the direct effect of ionizing radiation (TAHTAT et al., 2012). The degradation occurs through the formation of macro radicals that produce low molecular weight polymeric chains (GRYCZKA et al., 2009).

Fresh-cut products are particularly susceptible to weight loss and pathogenic activity owing to the removal of plant protective tissues, which results in reduced bioactive constituents and shelf-life reduction due to speedy deterioration. The aim of this study is to investigate the effect of edible irradiated and unirradiated chitosan coating on keeping quality of 'Wonderful' pomegranate arils during cold storage, particularly nutritive (titratable acidity and total soluble solids) and functional properties (ascorbic acid, total anthocyanin, total antioxidant activity and total phenolic compounds).

Materials and methods

This study was conducted during the two successive seasons of 2013 and 2014 on pomegranate cv. 'Wonderful' grown in a commercial orchard located on Cairo/Alexandria desert road. Fruits were harvested at commercial maturity and transported immediately to the Central Laboratory in the Horticultural Research Institute, Giza Governorate, where intact, physically sound fruits of uniform size were selected for this investigation. After washing whole fruits in sterilized water with 200 $\mu L L^{-1}$ sodium hypochlorite (NaOCl) solution using a brush, fruits were cut in half with a sharpened knife along the equator and all fruits that showed physiological disorders

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(browning or pitting of arils and internal surfaces or arils paleness) were discarded, then fruits were manually peeled and arils were separated from the husk, combined, well mixed to assure uniformity, and divided to 7 portions, one for each treatment.

High molecular weight solid chitosan powder (>75% deacetylated) obtained from Sigma- Aldrich was gamma irradiated with 0, 25 and 50 kGy doses at the National Center for Radiation Research and Technology (NCRRT) using a self-contained dry-storage gamma irradiator (Indian gamma cell GE 4000A) that uses ^{60}Co as a radiation source. Chitosan treatments/solutions were prepared according to the procedure described by JIANG et al. (2005). Solutions prepared were Control (distilled water); 0.5%, unirradiated chitosan; 0.5%, 25 kGy irradiated chitosan; 0.5%, 50 kGy irradiated chitosan; 1.0%, unirradiated chitosan; 1.0%, 25 kGy irradiated chitosan; 1.0%, 50 kGy irradiated chitosan, solutions. Arils were treated with one of the previous solutions by immersion for 2 min before they were dried, packed in self-sealed 250 g PP trays and refrigerated at 5 ºC and RH 75% for up to 15 day, during which studied characteristics were determined trice, with a 5 day interval. Initial aril quality evaluation was conducted right after aril extraction and before treatments and cold storage were applied (zero time). For each treatment, (3 replicates * 250 g) were used to determine weight loss percentage. Juice samples required for biochemical evaluations were manually extracted from another (3 replicates * 250 g) using hand pressure and filtration through cheesecloth.

- Weight loss (%) was determined by measuring the difference between initial and final weight of each replicate and results were expressed as a percentage loss of initial weight.
- Total soluble solids (TSS) (%) was determined in juice directly extracted from arils with a Carl Zeiss hand refractometer (AOAC, 2003).
- pH was measured in juice directly extracted from arils with a pH meter (Model: Mettler-Toledo model, D6 101-SC, Switzerland) (AOAC, 2003).
- Titratable acidity (TA) (%) was calculated as citric acid percentage (dominant organic acid in pomegranates) as described in AOAC (1990), where 1 ml of fruit juice was titrated with 0.1 M NaOH using phenolphthalein as an indicator and the percentage was calculated as follows:

$$\text{Titratable acidity \%} = \frac{\text{ml of NaOH} \times \text{Normality} \times 0.067}{\text{ml juice used}} \times 100$$

- Vitamin C was determined and expressed in mg ascorbic acid 100 ml^{-1} fruit juice, according to AOAC (1990).
- Total anthocyanin content was determined by the pH- differential method described by LEE et al. (2005) using 2 buffer systems: potassium chloride buffer, pH 1 (0.025 M), and sodium acetate buffer, pH 4.5 (0.4 M). The sample was diluted with the corresponding buffer and the absorbance was measured at 520 and 700 nm. Total anthocyanin content was calculated as cyanidin-3-glucoside according to the following equation:

$$\text{Total anthocyanins (mg L}^{-1}) = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1}$$

Where A = (A520–A700) pH1–(A520–A700) pH 4.5; MW = 449.2 g mol^{-1} for cyanidin-3-glucoside; DF = dilution factor; I = path length in cm; ε = 26900 molar extinction coefficient in L mol^{-1} cm^{-1} for cyanidin-3-glucoside; 1000 = conversion from g to mg.

- Total antioxidant activity (mg ascorbic acid eq. 100 g^{-1}) and total phenolic compounds (mg gallic acid eq. 100 g^{-1}) : For each sample, 5 g of arils were homogenized in 10 mL of 50 mM phosphate buffer (pH 7.8) and then centrifuged at 10 000 g for 15 min at 4 °C. The supernatant was used for total antioxidant activity (TAA) and total phenolic compounds quantification in duplicate, as previously described (SERRANO et al., 2005). For Total antioxidant activity, L-ascorbic acid was used for the calibration curve, and the results were expressed as mg ascorbic acid eq. 100 g^{-1} FW. The total phenolic compounds were expressed as mg gallic acid eq. 100 g^{-1} FW.

Statistical analysis

The experiment was laid out using a Completely Randomized Block Design (CRBD). Three replicates per treatment were evaluated for aril quality attributes. Experimental data obtained was treated with Analysis of Variance (ANOVA) at confidence level of 95%, which is the procedure used for testing the differences among means of two or more treatments and the differences between means were detected using least significant difference (LSD) at $P \leq 0.05$. All data was analyzed using statistical software (MSTATC 2.10, Russell D. Freed).

Results

Weight loss (%): Results presented in Fig. 1 show that like other fresh produce, pomegranate arils lost weight during cold storage. Throughout the whole experiment, all chitosan treatments reduced weight loss compared to the control but differences were not always statistically significant. Unirradiated 1% chitosan was the only treatment which significantly reduced weight loss at all times. At the end of the storage period, all 1% chitosan- treated arils loss significantly less weight compared to control arils in both investigated seasons while 0.5% chitosan treatments were significantly effective in this regard in season 2014 only. On the other hand, results reveal that irradiating chitosan generally reduced its moisture-maintaining effect, but such negative impact was statistically insignificant most of the time.

TSS (%): As shown in Tab. 1, TSS in arils generally decreased as cold storage proceeded. It was also found that chitosan treatments played a positive role in maintaining TSS, though statistical significance was not always detected. In season 2013, all arils treated with 1% chitosan and unirradiated 0.5% chitosan recorded significantly high TSS contents compared to control arils after 10 days of refrigerated storage, though this effect did not persist till the end of the storage period. Contrarily, in the latter season, all chitosan treatments recorded significantly high TSS contents compared to untreated control arils and this effect persisted at the end of the storage period. It was also noticed that when treatments were of statistical significance, irradiation dose effect was trivial, but still, it's worth mentioning that irradiating chitosan reduced its effect in controlling TSS decreases.

pH: As show in Tab. 2, chitosan treatments significantly affected pH values of minimally processed pomegranate after 15 days of cold storage. A similar effect was detected, 10 days of cold storage in season 2014 only. In all three incidents of statistical significance, different treatments showed insignificant differences in-between. The only treatment which resulted in a significantly lower pH value compared to the control in both seasons at the end of storage period was the unirradiated 1% chitosan treatment. Other treatments controlled pH increments, but its effect was statistically insignificant.

TA (%): Results presented in Fig. 2 show that TA decreased gradually as storage proceeded. It was found that all investigated treatments controlled acidity drops compared to the control throughout the whole trial, though statistical significance was not always detected. Moreover, 1% chitosan treatments were more efficient in maintaining TA compared to 0.5% treatments. On the other hand, irradiating chitosan had a negative effect in this regard because it led to bigger TA drops compared to unirradiated chitosan.

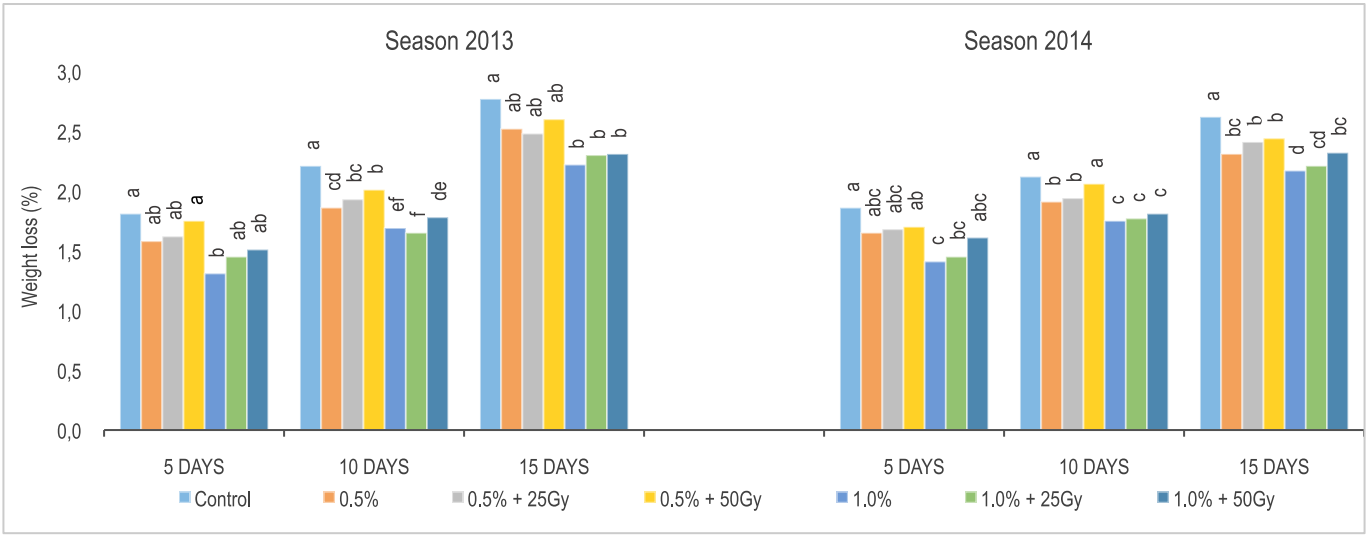

Fig. 1: Effect of irradiated and unirradiated chitosan on arils weight loss (%) during cold storage. Columns in the same group bearing a common letter are insignificantly different at P<0.05.

Tab. 1: Effect of irradiated and unirradiated chitosan on arils juice TSS (%) during cold storage.

Treatments	Storage period (Season 2013)				Storage period (Season 2014)			
	0 days	5 days	10 days	15 days	0 days	5 days	10 days	15 days
Control	18.2	18.0	17.0 c	15.8	17.9	17.2	16.3 b	15.5 d
Chitosan 0.5% + 0 kGy		18.1	17.7 ab	16.9		17.8	17.4 a	16.3 bc
Chitosan 0.5% + 25 kGy		18.0	17.6 abc	16.6		17.6	17.4 a	16.3 bc
Chitosan 0.5% + 50 kGy		18.0	17.4 bc	16.3		17.6	17.3 a	16.1 c
Chitosan 1.0% + 0 kGy		18.2	17.9 ab	17.2		17.9	17.6 a	16.8 a
Chitosan 1.0% + 25 kGy		18.1	17.9 ab	17.2		17.8	17.6 a	16.7 ab
Chitosan 1.0% + 50 kGy		18.0	18.1 a	17.0		17.6	17.6 a	16.6 ab

Means in the same column bearing a common letter/no letters are insignificantly different at P<0.05.

Tab. 2: Effect of irradiated and unirradiated chitosan on arils juice pH value during cold storage.

Treatments	Storage period (Season 2013)				Storage period (Season 2014)			
	0 days	5 days	10 days	15 days	0 days	5 days	10 days	15 days
Control	3.30	3.34	3.36	3.39 a	3.22	3.25	3.32 a	3.34 a
Chitosan 0.5% + 0 kGy		3.33	3.34	3.36 ab		3.24	3.26 b	3.29 ab
Chitosan 0.5% + 25 kGy		3.33	3.34	3.36 ab		3.25	3.27 ab	3.29 ab
Chitosan 0.5% + 50 kGy		3.34	3.34	3.37 ab		3.25	3.27 ab	3.31 ab
Chitosan 1.0% + 0 kGy		3.31	3.32	3.33 b		3.23	3.25 b	3.27 b
Chitosan 1.0% + 25 kGy		3.32	3.33	3.34 ab		3.24	3.26 b	3.27 b
Chitosan 1.0% + 50 kGy		3.32	3.33	3.34 ab		3.24	3.26 b	3.28 b

Means in the same column bearing a common letter/no letters are insignificantly different at P<0.05.

Vitamin C: As shown in Tab. 3, vitamin C reductions were recorded as storage proceeded. It was also found that all chitosan treatments controlled such reductions, though statistical significance was detected only in season 2014 after 15 days of cold storage. At that time, all treatments except the 50 KGy irradiated 0.5% chitosan treatment, resulted in significantly high vitamin C content compared to the control. As for the effect of irradiation on chitosan, it seemed to limit its effect in controlling vitamin C reduction during cold storage.

Total anthocyanin content: Results presented in Tab. 4 show that anthocyanin content dropped as storage proceeded. It also shows that chitosan played a positive role in controlling decrements and

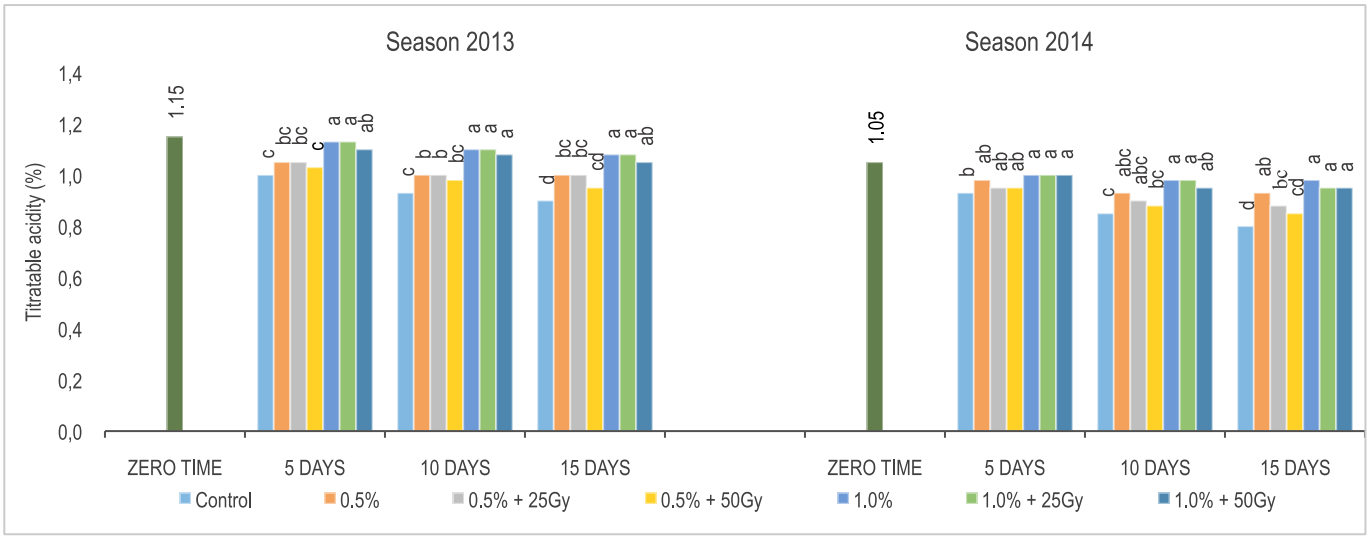

Fig. 2: Effect of irradiated and unirradiated chitosan on arils juice TA (%) during cold storage.
Columns in the same group bearing a common letter are insignificantly different at P<0.05.

Tab. 3: Effect of irradiated and unirradiated chitosan on arils juice vitamin C content (mg ascorbic acid 100 ml^{-1} juice) during cold storage.

Treatments	Storage period (Season 2013)				Storage period (Season 2014)			
	0 days	5 days	10 days	15 days	0 days	5 days	10 days	15 days
Control	125.2	118.4	110.0 c	100.1	118.6	110.7	102.3	95.0 d
Chitosan 0.5% + 0 kGy		120.1	117.9 ab	112.8		111.9	108.5	101.1 b
Chitosan 0.5% + 25 kGy		120.1	117.2 ab	111.2		112.1	106.4	99.0 bc
Chitosan 0.5% + 50 kGy		119.4	115.6 b	107.1		111.1	105.3	96.4 cd
Chitosan 1.0% + 0 kGy		123.3	121.8 a	116.6		116.5	111.7	107.2 a
Chitosan 1.0% + 25 kGy		121.4	120.8 a	114.2		116.0	111.1	105.6 a
Chitosan 1.0% + 50 kGy		121.0	121.6 a	113.5		114.4	107.5	101.4 b

Means in the same column bearing a common letter/no letters are insignificantly different at P<0.05.

Tab. 4: Effect of irradiated and unirradiated chitosan on arils juice total anthocyanin content (mg cyanidin-3-glucoside eq. L^{-1}) during cold storage.

Treatments	Storage period (Season 2013)				Storage period (Season 2014)			
	0 days	5 days	10 days	15 days	0 days	5 days	10 days	15 days
Control	312.5	301.4	296.5 c	292.0 b	318.1	307.1	300.7 b	295.3
Chitosan 0.5% + 0 kGy		306.2	301.4 abc	299.8 ab		312.0	306.6 ab	302.9
Chitosan 0.5% + 25 kGy		304.5	300.8 abc	298.9 ab		310.2	304.7 ab	299.9
Chitosan 0.5% + 50 kGy		305.1	299.5 bc	297.6 ab		307.6	303.0 ab	299.7
Chitosan 1.0% + 0 kGy		311.8	309.3 a	305.4 a		317.8	311.9 a	307.6
Chitosan 1.0% + 25 kGy		309.3	307.4 ab	305.3 a		315.1	311.8 a	305.6
Chitosan 1.0% + 50 kGy		307.5	307.0 ab	303.9 a		311.0	308.6 ab	304.1

Means in the same column bearing a common letter/no letters are insignificantly different at P<0.05.

maintaining anthocyanins in arils. Although this role was more pronounced when arils were treated with 1% chitosan compared to 0.5 % treatments, but statistically significant differences were not detected. Similarly, insignificant decreases in anthocyanins were recorded when chitosan was irradiated prior to aril treatments.

Antioxidant activity: Results presented in Fig. 3 show that total antioxidant activity increased as storage progressed. It also shows that all chitosan treatments resulted in increased antioxidant activity compared to the control. This effect was more pronounced when 1% chitosan treatments were used, compared to 0.5% chitosan treat-

ments. Moreover, irradiating chitosan prior to aril treatments proved to be beneficial in this regard.

Total phenols: Results presented in Tab. 8 show that total phenols increased as storage proceeded. All investigated treatments resulted in increased phenols but statistical significance was detected only after 10 days of cold storage in season in 2013 and 15 days of cold storage in season 2014. In these two incidents, increases resulting from 50 KGy irradiated 0.5% chitosan were insignificantly higher from the control, unlike the other five treatments which recorded significantly high phenol contents compared to control arils. As shown, higher chitosan concentration was correlated with higher phenol contents. It was also found that irradiating chitosan was correlated with lower phenol contents compared to unirradiated chitosan.

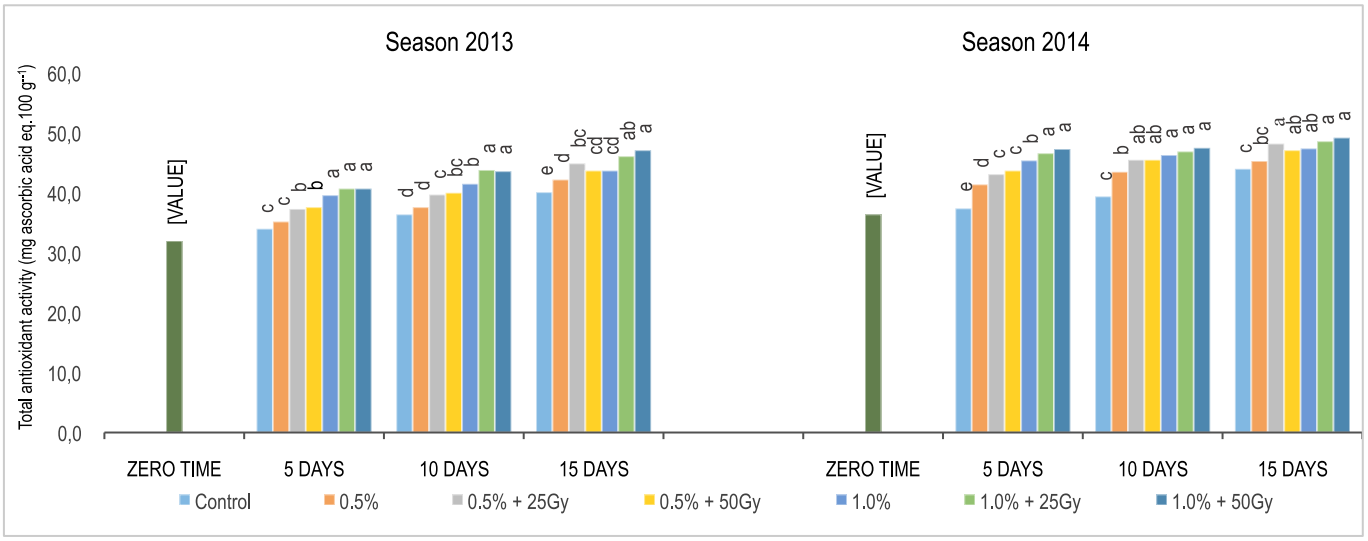

Fig. 3: Effect of irradiated and unirradiated chitosan on arils juice total antioxidant activity (mg ascorbic acid eq. 100 g^{-1}) during cold storage. Columns in the same group bearing a common letter are insignificantly different at P<0.05.

Tab. 5: Effect of irradiated and unirradiated chitosan on arils juice total phenols (mg eq. gallic acid 100 g^{-1}) during cold storage.

Treatments	Storage period (Season 2013)				Storage period (Season 2014)			
	0 days	5 days	10 days	15 days	0 days	5 days	10 days	15 days
Control	90.2	91.4	94.5 e	96.0	96.4	99.0	104.0	106.2 e
Chitosan 0.5% + 0 kGy		96.1	100.7 c	103.4		103.2	108.1	112.2 cd
Chitosan 0.5% + 25 kGy		95.4	99.8 cd	101.6		101.9	107.8	110.8 cd
Chitosan 0.5% + 50 kGy		92.6	96.8 de	98.6		100.2	104.4	108.4 de
Chitosan 1.0% + 0 kGy		102.3	106.9 a	111.3		105.8	113.8	119.1 a
Chitosan 1.0% + 25 kGy		101.9	106.3 ab	111.0		104.7	113.1	118.4 ab
Chitosan 1.0% + 50 kGy		98.5	102.7 bc	107.6		103.0	108.4	114.5 bc

Means in the same column bearing a common letter/no letters are insignificantly different at P<0.05.

Discussion

Gradual increases in arils weight loss as storage progressed agrees with results previously reported for pomegranates by Caleb et al. (2013) and Salama et al. (2012). This is probably due to arils respiratory activity, transpiration and some oxidation processes (Ayranci and Tune, 2003). Maximum moisture loss in untreated control arils might be due to high rate of respiration and transpiration (Abbasi et al., 2009) and the absence of the semipermeable coating formed as a result of chitosan treatments that blocks pores and lessens permeability to water vapour and gases. The positive role chitosan played in this regard was similar to its effect reported by Zhelyazkov et al. (2014) who found that chitosan retarded water loss and preserved effectively the quality and extended the shelf-life of fresh-cut apples. On the other hand, Jinasena et al. (2011) reported that both irradiated and unirradiated chitosan treatments which showed an insignificant difference in between, significantly reduced weight loss during cold storage of banana. Ibrahim et al. (2014) and Quynh' et al. (2003) also reported that irradiated and unirradiated chitosan-treated pineapple and apple fruits, respectively, maintained moister content significantly better than control fruits. Expected higher viscosity of 1% compared to 0.5% chitosan solutions investigated in this trial most probably formed a denser coat around ails, which might be the reason for better weight maintaining properties recorded for the higher chitosan concentration. Unlike our findings, Ghasemnezhad et al. (2010) attributed higher apricot fruit weight loss in response to high chitosan concentrations to probable anaerobic respiration. On the other hand, expected reduced viscosity of irradiated chitosan resulting from reduced MW in response to irradiation (Gryczka et al., 2009) might also explain its reduced efficiency in controlling weight loss.

Our findings are in line with results of SALAMA et al. (2012) who reported that TSS in pomegranate juice decreased as storage proceeded. Meanwhile, AYHAN and EŞTÜRK (2009) reported that TSS remained unchanged for the first nine days of cold storage, but they affirmed TSS reductions in arils afterwards. As for the effect of chitosan on TSS, SRITANANAN et al. (2005) reported that chitosan treatments did not affect soluble solid content in mangosteen fruits, which partially agrees with our results. Contrarily, ZHELYAZKOV et al. (2014) reported that chitosan coating controlled increases in soluble solid content in fresh-cut apple. Moreover, our results also contradict fresh-cut nectarines TSS increases in response to chitosan treatments reported by CHIABRANDO and GIACALONE (2013) who found that the untreated control treatment recorded a TSS decrease after 5 days of cold storage. Our obtained results might be indirectly attributed to chitosan's inhibitory effect on respiration and other bioactivities occurring in arils that consume sugars which are a main constituent of TSS. Accordingly, the expectedly higher inhibitory effect associated with 1% chitosan compared to 0.5% chitosan investigated in this trial maybe the reason for higher TSS records for juice extracted from 1% chitosan-treated arils.

pH increases found in this trial contradict slight pH decreases reported by AYHAN and EŞTÜRK (2009) for pomegranates. Such increases may be due to acids breakup with respiration. Moreover, the insignificant pH differences in response to different chitosan concentrations and irradiation doses which might be due to limited effects on respiration. Generally, pH change is associated with several reasons, i.e.; it might be due to a change in biochemical conditions and slower respiration rate and metabolic activity (JITAREERAT et al., 2007) and the consumption of acids in respiration during storage. On the other hand, chitosan's significant role in controlling pH increases was reported earlier in fresh-cut nectarines by CHIABRANDO and GIACALONE (2013). Contrarily, IBRAHIM et al. (2014) and JINASENA et al. (2011) reported that chitosan's effect on pH in banana and pineapple, respectively, was insignificant. Here, it is worth mentioning that as expected, since chitosan-treated arils in this study showed less variation in titrable acidity, the associated variation in their pH was also relatively lower than the control.

TA reductions found in this investigation were reported earlier for several other fruits including pomegranates (SALAMA et al., 2012) such as pine apple (IBRAHIM et al., 2014) and fresh-cut nectarines (CHIABRANDO and GIACALONE, 2013). In this regard, AYHAN and EŞTÜRK (2009) found that arils TA decreased, especially at the third day, but they reported that it stayed constant afterwards. Our results agree with the findings of CALEB et al. (2013) who reported significant decreases in TA. Acidity reductions may be due to the conversion of organic acids to sugars and their further utilization in respiration and metabolic processes (ABBASI et al., 2009; IBRAHIM et al., 2014). IBRAHIM et al. (2014) stated that chitosan coating can develop an oxygen barrier on fruit surface leading to reduced metabolic rates and consequently, less acidity variation in chitosan-treated fruits. It is assumed that such effect is concentration dependent because better TA maintenance was always correlated with 1% rather 0.5% chitosan treatments. CHIABRANDO and GIACALONE (2013) attributed acidity retention in response to chitosan to the expected lower respiration rate, and hence, reduced organic acids (substrates) consumption for many reactions during aerobic respiration. Moreover, higher acidity values are also due to the effect of acid utilized in the film forming solution. On the other hand, although statistical significance was mostly undetectable, irradiated chitosan was found to be less efficient in maintaining aril juice TA compared to unirradiated chitosan. This contradicts what has been reported by LAN et al. (2000) who stated that irradiated chitosan delayed fruits internal changes more than unirradiated chitosan. They attributed that to increased deacetylation and reduced molecular weight of irradiated compared to unirradiated chitosan (KUME and TAKEHISA, 1982).

Our results related to the amount of vitamin C are supported by the findings of IBRAHIM et al. (2014) who reported decreases in pineapple vitamin C during storage, but in their study, increases in vitamin C were recorded before the decreases occurred. They also reported a higher ascorbic acid reduction rate for uncoated fruits compared to chitosan coated fruits. The reason for high vitamin C content in chitosan treatments can be attributed to limited oxygen supply caused by the barrier effect imposed by chitosan, which causes oxidation of ascorbic acid (MALUNDO et al., 1997). Such effect was more pronounced in juice extracted from arils treated with 1% chitosan compared to that extracted from 0.5% chitosan-treated arils. The results also convene with the findings of JIANG and LI (2001) who found that ascorbic acid content decreased when longan fruit was coated with chitosan at low temperature. It is also worth mentioning that RUOYI et al. (2005) reported that 1% chitosan associated with other treatments significantly inhibited ascorbic acid oxidases (ASA-POD) and kept vitamin C in refrigerated peach fruits at a high level. On the other hand, irradiation reduced chitosan's efficiency in controlling vitamin C reductions but statistical significance was rarely detected. This might be due to the abundance of reducing functional groups (primary –OH, secondary –OH and $–NH_2$) of chitosan resulting from the breakdown of high MW to lower MW chitosan by irradiation which provided inappropriate conditions for the oxidation of ascorbic acid.

Apparent anthocyanin drops recorded in this study were similar to those reported earlier by AYHAN and EŞTÜRK (2009), SALAMA et al. (2012) and CALEB et al. (2013). Although 1% chitosan and unirradiated chitosan treatments showed better anthocyanin-keeping properties compared to 0.5% and irradiated chitosan treatments, but neither concentrations nor irradiation doses studied showed significant differences in-between. In this regard, GIL et al. (1996) found that anthocyanins were insignificantly altered during the first week of cold storage and LOPEZ-ROBIRA et al. (2005) recorded steady anthocyanin content after 13 days of refrigerated storage of early harvested pomegranates. Here, it is worth mentioning that Cyanidin 3-glucoside was reported to be the major anthocyanin pigment in wonderful and other several pomegranate cultivars (GIL et al., 1996). On the other hand, JIANG et al. (2005) observed that 2% chitosan delayed the decrease in anthocyanin content and the increase in PPO activity in litchi fruits. It has also been demonstrated to have beneficial effects in maintaining anthocyanin content in several fruits such as longan fruit (JIANG and LI, 2001) and peeled litchi fruit (DONG et al., 2004). ZHANG and QUANTICK (1998) reported that this might be attributed to the barrier effect the chitosan coating imposes on the surface of the produce resulting in the modification in its endogenous CO_2 and O_2 levels, which could result in a reduced O_2 supply required for the enzymatic oxidation reaction of anthocyanin.

Here it is worth mentioning that anthocyanin synthesis continues in harvested fruit even at low storage temperatures, and postharvest treatments may affect anthocyanin biosynthesis, degradation, or both (HOLCROFT et al., 1998; HOLCROFT and KADER, 1999; GONCALVES et al., 2007). That's why adopting protocols and procedures that enhances anthocyanin content in arils is of great importance because in addition to its colourant properties, DA COSTA et al., 2000 reported that it exhibits a wide range of biological, pharmacological, anti-inflammatory, antioxidative, and chemoprotective properties.

In contrast with our general findings, VARASTEH et al., 2012 recorded minor anthocyanin content increases in chitosan-coated pomegranates after the first 45 days of refrigerated storage. Moreover, they found that chitosan treated pomegranates showed less anthocyanin content compared to control fruits at this stage. In this regard, EL GHAOUTH et al. (1991) indicated that using chitosan coating decelerated anthocyanin synthesis in treated strawberries. MIGUEL et al., 2004 added that increases in anthocyanin levels might be related to changes in fruit internal atmospheric conditions. Such in-

creases in anthocyanin concentration after harvest during cold storage have been reported for fruits other than pomegranates such as sweet cherry (Goncalves et al., 2007) and raspberry (Han et al., 2004). Miguel et al., 2004 stated that this was correlated with the activity of the anthocyanin biosynthesis enzymes.

Ayhan and Eştürk (2009) stated that there was a positive relationship between antioxidant activity (%) and total phenolic content, indicating the effect of polyphenols on antioxidant activity. Mirdehghan et al. (2006) reported that total antioxidant activity was correlated primarily to the high levels of total phenolics and to lesser extent to ascorbic acid and anthocyanin contents (which recorded decreases as storage progressed in this trial). In this regard, Ocloo et al. (2012) reported that irradiated chitosan in solutions exerted slightly faster bacterial inhibition compared to unirradiated chitosan. Moreover, Kumar et al. (2007) reported higher antibacterial activity and Feng et al. (2008) reported higher antioxidant activity for lower molecular weight chitosan, which is in harmony with our findings. On the other hand, 1% chitosan treatments recorded better results compared to 0.5% treatments. In this regard, Ghasemnezhad et al. (2010) reported that 0.50% chitosan was the most effective concentration in increasing total antioxidant capacity in apricots compared to control, 0.25 and 0.75% treatments. The authors attributed that result to high total phenolic content. This might be attributed to what Ruoyi et al. (2005) reported regarding the positive effectiveness of chitosan associated with other treatments on the inhibition of polyphenol oxidase, peroxidase (POD), ascorbic acid oxidases (ASA-POD) and polygalacturonase (PG) activities to some extent, in peach fruits.

Our results related to total phenols are also in accordance with results reported by Ayhan and Eştürk (2009) who found that phenols slightly increased till the 12th day before it started to decrease. They stated that the diversity in total phenolic content was probably due to changes in acidity and TSS which in turn, influenced total anthocyanin content and total antioxidant activity. Generally, higher phenolics were recorded for 1% compared to 0.5% chitosan treatments and for unirradiated compared to irradiated chitosan. In this regard, Benhamou (1996) reported that chitosan has a potential of inducing phenolic contents in plants. Among 0.25, 0.50% and 0.75% chitosan treatments, 0.50% was reported to be the most active in increasing total phenolic compounds in apricots (Ghasemnezhad et al., 2010). As for the effect of chitosan coating, irrespective of concentration (1 and 2% dissolved in 2% glutamic acid), it delayed changes in contents of anthocyanins, flavonoids, and total phenolics. It also delayed the increase in polyphenol oxidase (PPO) activity, and partially inhibited the increase in peroxidase activity (Caro and Joas, 2005; Zhang and Quantick, 1997).

Conclusion

Chitosan edible coating application to minimally processed pomegranate proved to be beneficial in reducing water loss and presumably, respiration and microbiological problems. Integrating it with a 1% concentration in adopted protocols used in extending shelf life of pomegranate arils seems to be promising due to it positive role in maintaining or delaying senescence of several functional compounds with antioxidant activity such as phenolic compounds, anthocyanins and ascorbic acid. It was also useful in maintaining characteristics that contribute to organoleptic quality such as titratable acidity, total soluble solids (mainly sugars) and pH. However, future studies are needed in order to determine the usefulness of irradiating chitosan or using low molecular weight chitosan in such protocols because beneficial effects on different biochemical attributes are not persistent.

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