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Effect of Chitosan Edible Coating on Quality Attributes of Pomegranate Arils During Cold Storage

Abdel Fattah, A.A. [1]; I. S. Ashoush[1] and B. A. Alnashi [2]
[1] Food Science Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt.
[2] Food Science and Nutrition Dept., College of Health Sci., Public Authority for Applied Education and Training, Kuwait
Corresponding author: Abdel Fattah A. A., Food Science Dept., Fac. of Agric., Ain Shams Univ., Shoubra El-Khema, Cairo, Egypt
E-mail address: abdeo1978@agr.asu.edu.eg

ABSTRACT

The aim of this study was to evaluate the effect of edible coating of chitosan solutions at different concentrations (0, 0.5, 1, 1.5 and 2%) on the extend the overall quality stability and shelf-life of pomegranate arils during cold storage at 4 ± 1 °C for 16 days. Physicochemical, microbiological and sensory quality attributes of the arils were determined during storage. The results revealed a general trend; slightly changes were detected in total titratable acidity, pH, total anthocyanin, total phenolic content and antioxidant activity values of all coated samples compared with uncoated samples. Chitosan coating helped keeping the visual quality of arils during storage as confirmed by their surface color measurement. Total Plate count as well as psychrophilic bacterial count and yeasts and molds counts were increased with the increasing of cold storage period in all the treatments. The uses of edible coating of chitosan solutions (1.5 and 2%) were more affective to preserve high quality and convenience of pomegranate arils during storage at 4±1 °C for 16 days, with good chemical and microbiological qualities as well as sensory attributes compared with uncoated samples.

Keywords: Pomegranate arils, Chitosan, Antioxidant activity, shelf life, Sensory evaluation.

INTRODUCTION

Pomegranate (*Punica granatum L.*) fruits are commonly consumed in both fresh arils and commercial processed forms, such as juice, dips and flavourings in the Middle East, China, India, and America. The fruit gives rise to three parts: seeds (about 3%), juice (about 30%) and peels, each of which has interesting pharmacologic activity (Lansky and Newman, 2007 and Palma *et al.,* 2015). Pomegranate juice possesses anticancer activities, including interference with tumour cell proliferation and improves lipid profiles in diabetic patients with hyperlipidemia (Lansky and Newman, 2007; Tezcan *et al.,* 2009; Caleb *et al.,* 2013 and Li *et al.,* 2014).

Pomegranate fruit contains many different kinds of polyphenolic compounds and commercial pomegranate juice has been shown to possess antioxidant activity three times higher than those of green tea (Gil *et al.,* 2000 and Caleb *et al.,* 2013). The soluble polyphenolic content of pomegranate juice includes anthocyanins, punicalagins, catechins, ellagic acids, tannins, and gallic acid (Aviram *et al.,* 2000; Gil *et al.,* 2000 and Caleb *et al.,* 2013). A linear relationship between total phenolic content, antioxidant capacity and antibacterial activity against several microorganisms has been established by Shan *et al.,* (2007) and Palma *et al.,* (2015).

Maintaining the quality of pomegranate arils is a critical challenge, they are very susceptible to textural and nutritional deterioration; this lead to reduce shelf life (Maghoumi *et al.,* 2013). Optimum cold storage condition is essential in order to minimize physiological disorders of pomegranate fruit (Caleb *et al.,* 2012). However, the commercial shelf life of arils differs to whole fruit. Whole fruit may be stored for 3- 4 months at temperatures below 10 °C (Ghafir *et al.,* 2010), while arils shelf life varies from 7 to 18 days at temperatures between 0 and 5 °C under different packaging conditions (Artés *et al.,* 2000; Ayhan *et al.,* 2009).

The antibacterial properties of pomegranate have been reported extensively for several pathogens such as *Escherichia coli*, *Staphylococcus aureus*, including several methicillin resistant strains like *Staphylococcus aureus*, *Vibrio cholerae* and *Bacillus subtilis* (Braga *et al.,* 2005; Melendez; Capriles, 2006 and Caleb *et al.,* 2013). Scientific community reflecting consumers demand for natural antimicrobials has made efforts to investigate the possibility to use natural sources of antimicrobials (Drosionon *et al.,* 2009).

Chitosan [poly-*β*-(1→4)- N- acetyl – D – glucosamine] is a natural nontoxic biopolymer produced by the deacetylation of chitin, a major component of the crustaceans shells such as shrimp, crab and crawfish and the second most abundant natural biopolymer after cellulose (Dahiya *et al.,* 2006; Kerch, 2015). Currently, chitosan and chitooligosaccharides have attracted considerable interest due to their excellent biological antimicrobial activities (Zhao and Xia, 2006; Chung and Chen, 2008 and Aider, 2010), bio-safe and biochemical properties (Lin *et al.,* 2008), hypocholesterolemic (Kim and Rajapakse, 2005; and Liao *et al.,* 2007), immunity-enhancing and antitumor effects (Xia, 2003).

The antimicrobial activities of chitosan has been reported and considered to be one of the most important properties, corresponding directly to their possible biological applications (Wei and Xia, 2003; Xia, 2003; and Zhao and Xia, 2006). The effect of chitosan coating on color preservation of some fruit products, such as litchi has been established (De Reuck *et al.,* 2009).

Chitosan based edible films and coatings have been applied by Dhall, (2013) and Elsabee and Abdou,

(2014); they concluded that chitosan has a good antibacterial and antifungal properties for food protection, but it is necessary to improve some of mechanical properties, such as, gas and water vapor permeability.

The objective of this study was to evaluate quality attributes (Physical, chemical, microbiological and sensory) to extend the shelf life of ready-to-eat pomegranate arils coated with chitosan solutions at different concentrations during cold storage at 4±1 °C.

MATERIALS AND METHODS

Materials:

Pomegranate (*Punica granatum L.* cultivar Munfaloty) fruits were procured during the summer season of 2014 from the local market, Cairo, Egypt. Methanol and sodium carbonate were obtained from El-Gomhoreya Co., Cairo, Egypt. 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and Folin-Ciocalteus phenol reagent was purchased from Sigma–Aldrich Inc. (St Louis, MO, USA). Chitosan with molecular weight (MW= 300 KDa), from a crab shells with 85% degree of deacetylation [poly (β - (1, 4) – 2 – amino – 2 – deoxy – D – glucose] (Sigma chemical company) was used in this study.

Methods:

Preparation of pomegranate arils coating with chitosan:

Outer skins of healthy fruits uniform in size and appearance were carefully cut at the equatorial zone with sharpened knife and the arils were manually separated. Negative control aril samples (T1) were dipped in distilled water without acetic acid and chitosan treatment, where positive control arils samples (T2) were dipped in 1% acetic acid solution without chitosan treatment. Pomegranate arils (T3, T4, T5 and T6) were dipped in different concentrations of aqueous chitosan solutions 0.5, 1.0, 1.5 and 2.0 % (w/v), respectively with 1% acetic acid (v/v). Arils samples were allowed to straining at room temperature for 30 min. Then arils were placed in high density polyethylene bags (1 kg per bags) and stored at 4 ± 1 °C for 16 days.

Analytical methods:

Total titratable acidity and pH measurement:

Total titratable acidity (TTA) and pH values were evaluated as quality indices. The pH values were measured using a pH meter (HANNA-Instrument, USA). Means of 3 replicates were reported for each treatment. TTA was determined potentiometrically by titrating 3.0 gm pomegranate juice using 0.1 M NaOH to the end point of pH 8.1, TTA expressed as grams of citric acid per liter according to AOAC (2012) method NO. 965.30.

Determination of total anthocyanin:

The total anthocyanin content (TAC) of samples was determined by a pH differential method described by Caleb *et al.*, (2013) using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M). Briefly, 1.0 mL of sample was mixed with 24 mL of the appropriate buffer and read against a water blank at 510 and 700 nm. Absorbance (*A*) was calculated as:

$$A = (A_{510} - A_{700})\ pH_{1.0} - (A_{510} - A_{700})\ pH_{4.5}$$

The total anthocyanin content of the samples (milligrams of cyanidin-3-glucoside per liter of pomegranate juice) was calculated by the following equation:

$$TAC = (A \times MW \times DF \times 1000)/MA$$

Where *A* is the absorbance; MW, molecular weight of anthocyanin (449.2 g/mol); DF, dilution factor (25); and MA (26900) molar extinction coefficient in liter per mol per centimeter for cyanidin-3-glucoside; 1000 = conversion from grams to milligrams.

Determination of total phenolic content:

The total phenolic content of the pomegranate juice was determined colorimetrically using the Folin-Ciocalteu method as described by Singleton *et al.*, (1999).

Antioxidant activity (DPPH˙ free radical scavenge):

The ability of the pomegranate juice to scavenge available free radical DPPH˙ (2,2-diphenyl-1-picrylhydrazyl) was determined by the method described by (Bondet *et al.*, 1997). The mixture containing 3 mL of a methanol solution of 0.16 mM DPPH˙ and 100 mL of sample was allowed to react for 15 min in a cuvette. The absorbance of the DPPH˙ solution was determined at 515 nm by a Hitachi U-1900 (UV/Vis.) spectrophotometer (Hitachi, Tokyo, Japan). The percentage inhibition of the absorbance of DPPH˙ solution added with sample was calculated using the following equation:

$$\text{Inhibition (\%)} = 100 \times [(A_{515}\text{ of control} - A_{515}\text{ of sample})/ A_{515}\text{ of control}]$$

Microbiological examination:

Samples were taken immediately after processing and during cold storage. Different prepared arils samples were prepared for microbiological analysis in accordance with ISO 6887-1 (2003) test method for sample preparation titled: (Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination). Different samples of 10 g were weighed out from the sterile stomacher zipped bag. Maximum recovery solution (MRD), of 90 ml was added. The sample and MRD solution were blended at low speed for 30 to 60 seconds in stomached machine. A dilution series was prepared by transferring 1ml of the previous dilution to 9ml of MRD) solution.

Different samples were examined for total aerobic bacterial count (ISO 4833-2003) as well as psychrophilic bacterial counts. Other methods for microbiological analysis were carried out for yeast and mold counts (ISO 21527-1, 2- 2008) and coliform group (ISO 4832-2006). All previous tests were used to reflect the microbiological quality of the prepared arils samples. The dilutions were plated onto duplicate plates. Plates were incubated at 30 °C for 2 days for total aerobic bacterial count and at 37 °C for coliforms bacteria, also at 7-10 °C for 5 days for psychrophilic bacterial counts, and at 21-25°C for 3-5 days for yeasts and molds. Results were expressed as (log cfu /gm. of arils samples).

Sensory evaluation:

Pomegranate arils samples were exhibited to sensory evaluation by ten panelists from staff members of

Food Science Department, Faculty of Agriculture, Ain Shams University. The panelists were asked to score the pomegranate arils for their freshness, color, texture, taste and overall acceptability; giving numerical scores to each of their attributes from 10, using a report sheet according to Watts *et al* (1989).

Statistical analysis:

Data were expressed as the mean values of three replicates and standard deviations were statistically analyzed by performing analysis of variance technique (ANOVA) using the statistical Analysis System according to SAS, (2008). Differences among means were compared using Duncan's multiple range test at significant level 95% (*P≤0.05*).

RESULTS AND DISCUSSION

Physicochemical analysis:

In general, significant difference (*P≤0.05*) was observed between the control samples (T1 and T2), and prepared coated samples at different concentrations of chitosan (T3, T4, T5 and T6) in terms of total titratable acidity TTA (%) as shown in Fig. (1). However, storage period had significant (*P≤0.05*) effect on TTA at all treatments, TTA significantly (*P≤0.05*) increased in the control sample without coating agent (T1) from 8.86 to 18.72 grams of citric acid per liter after 16 days of cold storage, and from 9.49 to 15.93 in the control sample within 1% acetic acid (T2), where the initial TTA slightly increased in pomegranate samples prepared at different concentrations of chitosan. The lowest increasing ratio was observed in prepared samples with 1.5 and 2% chitosan (T5 and T6). The increasing in TTA during cold storage of all samples might be imputed to the fermentation and breakdown of sugars with the formation of acids. The obtained results are in agreement with those obtained by Varasteh *et al.*, (2012) and Caleb *et al.*, (2013); they reported that TTA of pomegranate arils increased in the control sample without chitosan coating compared with treated samples.

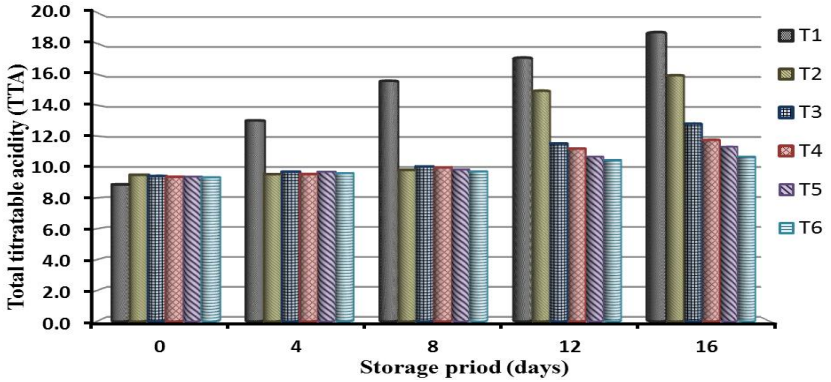

Fig. 1. Total titratable acidity (TTA) of pomegranate arils samples prepared with different concentrations of chitosan on at different cold storage periods (days).

There was not much difference observed in pH values between uncoated and coated samples during the first 8 days. The initial pH values decreased slightly until 8 days for all samples. The pH values of all samples ranged from 3.69 to 3.77 at zero time and from 2.56 to 3.51 after 16 days of cold storage; indicating a slight decrease during cold storage. The slightly decrease in pH values during cold storage of all samples might be attributed to the fermentation and breakdown of sugars with the formation of acids as seen in Fig. (2).

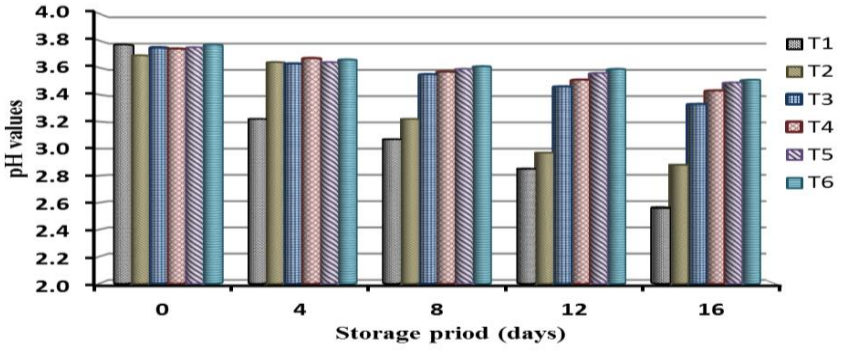

Fig. 2. pH values of pomegranate arils samples with different concentrations of chitosan on at different cold storage periods (days).

Table (1) presents the changes of chemical parameters of uncoated and coated pomegranate arils samples prepared at different concentration of chitosan during cold storage at (4±1 °C for 16 days).

Anthocyanins are important phenolic compounds responsible for typical red color of pomegranates. There were insignificant (*P≤0.05*) effects of chitosan coating application, on the total anthocyanin content in coated pomegranate arils during cold storage days. In general, total anthocyanin content was decreased as the cold storage time increased for all treated samples. Total anthocyanin content decreased from 341.20 mg cyanidin-3-glucoside equivalent/L of pomegranate arils juice at day 0 to 335.2, 338.08, 338.90, 340.71, 340.82 and 340.93 mg cyanidin-3-

glucoside equivalent/L of juice after 16 days of cold storage for T1, T2, T3, T4, T5 and T6, respectively. Pomegranate arils samples T4, T5 and T6 prepared at 1.0, 1.5 and 2% of chitosan had higher total anthocyanin contents than the uncoated samples during cold storage.

Table 1. Chemical composition parameters of pomegranate arils samples with different concentrations of chitosan during cold storage periods (days).

	Treatments	Storage period (Days)				
		0	4	8	12	16
Total anthocyanin (mg/L)	T1	341.2 ^{a} ± 0.76	341.09 ^{a} ± 0.01	339.26 ^{e} ± 0.02	337.2 ^{d} ± 0.10	335.20 ^{e} ± 0.10
	T2	341.2 ^{a} ± 0.76	341.15 ^{a} ± 0.01	339.53 ^{d} ± 0.01	338.2 ^{c} ± 0.10	338.08 ^{d} ± 0.04
	T3	341.2 ^{a} ± 0.76	341.18 ^{a} ± 0.01	340.85 ^{c} ± 0.08	339.73 ^{b} ± 0.02	338.90 ^{c} ± 0.15
	T4	341.2 ^{a} ± 0.76	341.19 ^{a} ± 0.01	341.04 ^{b} ± 0.02	340.83 ^{a} ± 0.04	340.71 ^{b} ± 0.01
	T5	341.2 ^{a} ± 0.76	341.2 ^{a} ± 0.01	341.15 ^{a} ± 0.02	340.91 ^{a} ± 0.03	340.82 ^{ab} ± 0.01
	T6	341.2 ^{a} ± 0.76	341.2 ^{a} ± 0.01	341.18 ^{a} ± 0.02	341.05 ^{a} ± 0.28	340.93 ^{a} ± 0.01
Total phenolic (mg GAE/L)	T1	1532 ^{a} ± 1.0	1532 ^{b} ± 0.50	1522 ^{c} ± 0.1	1520 ^{e} ± 0.15	1518 ^{e} ± 0.20
	T2	1532 ^{a} ± 1.0	1530 ^{d} ± 0.30	1529 ^{b} ± 0.5	1522 ^{d} ± 0.25	1519 ^{d} ± 0.20
	T3	1532 ^{a} ± 1.0	1532 ^{b} ± 0.50	1529 ^{b} ± 0.5	1528 ^{b} ± 0.5	1525 ^{c} ± 0.10
	T4	1532 ^{a} ± 1.0	1532 ^{b} ± 0.50	1529 ^{b} ± 0.2	1529 ^{a} ± 0.2	1527 ^{b} ± 0.50
	T5	1532 ^{a} ± 1.0	1531 ^{c} ± 0.40	1530 ^{a} ± 0.05	1528 ^{b} ± 0.5	1526 ^{b} ± 0.25
	T6	1532 ^{a} ± 1.0	1533 ^{a} ± 0.20	1530 ^{a} ± 0.05	1528 ^{cb} ± 0.5	1528 ^{a} ± 0.05
Antioxidant activity (%)	T1	45.89 ^{a} ± 0.01	43.56 ^{d} ± 0.01	41.89 ^{e} ± 0.02	38.44 ^{c} ± 0.02	36.15 ^{e} ± 0.02
	T2	45.89 ^{a} ± 0.01	43.66 ^{c} ± 0.01	41.59 ^{f} ± 0.04	38.19 ^{d} ± 0.23	36.14 ^{e} ± 0.01
	T3	45.89 ^{a} ± 0.01	43.56 ^{d} ± 0.01	42.81 ^{a} ± 0.03	42.09 ^{b} ± 0.27	41.77 ^{d} ± 0.01
	T4	45.89 ^{a} ± 0.01	43.42 ^{e} ± 0.01	42.32 ^{d} ± 0.01	42.22 ^{a} ± 0.04	41.95 ^{c} ± 0.02
	T5	45.89 ^{a} ± 0.01	43.69 ^{b} ± 0.02	42.51 ^{c} ± 0.01	42.42 ^{a} ± 0.02	42.28 ^{b} ± 0.02
	T6	45.89 ^{a} ± 0.01	43.77 ^{a} ± 0.01	42.58 ^{b} ± 0.01	42.45 ^{a} ± 0.04	42.33 ^{a} ± 0.01

Data are expressed as means ± SD (n = 3). Mean values in the same column within each parameter bearing the same superscript do not differ significantly (P ≤ 0.05).

The aforementioned results are in agreement with the results obtained by Varasteh *et al.,* (2012) they reported that chitosan coating delayed anthocyanin degradation in the pomegranate arils. Also Chiabrando and Giacalone, (2015) reported that, application of chitosan coating delayed the decrease in anthocyanin content in highbush blueberry (*Vaccinium corymbosum* L. cv Berkeley and O'Neal). Change in anthocyanins content of the chitosan-coated sweet cherry fruits were delayed (Petriccione *et al.,* 2015).

There were significant (*P≤0.05*) effects of coating application and cold storage period on total phenolics content. In general, total phenolics content of pomegranate arils (mg gallic acid equivalent/liter of fruit juice) decreased slightly during the 16 days of cold storage. The coating treatments with different concentrations of chitosan of the amounts of total phenolics content during cold storage were probably due to changes in total acidity, which in return affected the total anthocyanin content and total antioxidant activity.

Pomegranate arils appear good antioxidant capacity primarily due to its high levels of phenolic acids, flavonoids and other polyphenolic compounds (Aviram *et al.*; 2002). There was a significant (*P≤0.05*) effect of coating treatments and cold storage period for the total antioxidant activity of pomegranate arils. Total antioxidant activity decreased in uncoated samples (T1 and T2) prolonged through the 16 days of cold storage.

On the other hand, the total antioxidant activity of pomegranate arils coated with different concentration levels of chitosan slightly decreased during the 16 days of cold storage, and its activity was significantly (*P≤0.05*) higher than the uncoated samples.

While coated pomegranate arils samples had the lowest loss in antioxidant activity. There was a positive relationship between antioxidant activity (%), total phenolics content and concentration of chitosan coating treatments indicating the effect of polyphenol content and concentration of chitosan on antioxidant activity.

These results are in agreement with those of Gol *et al.,* (2015); who reported that the edible chitosan coatings submitted and maintain higher concentration of total phenolics in carambola (*Averrhoa carambola* L.) during could storage. Application of chitosan coating belated the decrease in phenolics content in highbush blueberry (Chiabrando and Giacalone, 2015). Changes in the total polyphenol content of the chitosan-coated sweet cherry fruits were delayed (Petriccione *et al.,* 2015). Chitosan coatings slow down decrease of total phenolics and flavonoid contents, catechin and caffeic acid, and total antioxidant activity (Kou *et al.,* 2014).

However, chitosan exhibited stronger antioxidant activity compared to uncoated arils samples. It is generally believe that the inhibition of lipid peroxidation by an antioxidant can be explained by various mechanisms, one is the free radical-scavenging activity. Park *et al.,* (2004) reported that, chitosan may eliminate various free radicals by the action of nitrogen on the C-2 position of the chitosan.

Xie *et al.,* (2001) found that, the scavenging mechanism of chitosan is related to the fact that the free radicals can react with the hydrogen ion from the ammonium ions (NH3+) to form a stable molecule. The

NH3+ has been formed by the amine group absorbing a hydrogen ion from the solution.

Microbial examination:

Microbial examination of coated pomegranate arils samples treated with different concentrations of chitosan were evaluated during cold storage at (4±1 °C) for 16 days and the obtained data are shown in table (2). The obtained results revealed that all treatments exhibited closely or similar initial total aerobic plate counts (TPC) at zero time of cold storage which ranged from 2.51 - 2.89 log cfu/g. This may be related to the good sanitary conditions followed during manually extraction of arils and preparation of coated samples. Increasing of cold storage period led to the progressive increase of TPC of negative control samples T1 overtime from 2.89 cfu/g at zero time to 9.68 log cfu/g after 16 days.

Table 2. Total plate count, psychrophilic bacterial count (PBC) and yeasts and moulds counts of coated pomegranate arils samples with different concentrations of chitosan solutions at different cold storage periods (days).

Storage Period (days)	T1	T2	T3	T4	T5	T6
	Total plate count TPC (log cfu /g)					
0	2.86	2.61	2.73	2.74	2.63	2.51
4	4.62	3.72	3.57	2.79	2.86	2.96
8	5.45	4.83	4.73	3.64	3.62	3.36
12	7.88	5.51	5.23	4.83	3.94	3.81
16	9.68	6.81	6.75	5.51	4.72	4.34
	Psychrophilic bacterial count PBC (log cfu /g)					
0	2.76	2.57	2.56	2.46	2.38	2.17
4	3.63	3.45	2.98	2.66	2.75	2.63
8	4.79	4.08	3.92	3.49	3.45	2.93
12	6.43	5.18	4.88	4.38	3.71	3.20
16	7.91	6.58	5.92	4.90	3.43	3.91
	Yeasts & molds counts YMC (log cfu/g)					
0	1.94	1.91	1.85	1.82	1.80	1.72
4	2.20	1.98	1.94	1.92	1.86	1.80
8	3.76	2.52	2.43	2.18	1.95	1.99
12	4.81	3.93	3.87	3.73	2.63	2.45
16	5.92	4.67	4.41	4.20	3.86	2.81

The same trend was observed for psychrophilic bacterial counts (PBC), yeasts and moulds counts (YMC) which revealed that all treatments exhibited closely initial counts at zero time of cold storage which ranged from 2.18 to 2.7 log cfu/g for PBC and from 1.72 to 1.94 log cfu/g for YMC. With the increase of cold storage period, PBC and YMC of negative control samples increased progressively overtime from 2.76 log cfu/g at zero time to 7.91 log cfu/g for PBC and from 1.94 to 5.92 log cfu/g for YMC after 16 days.

Moreover, the obtained data revealed that other treatments showed slightly increase in APC, PBC and YMC overtime during cold storage. Also it is very important to consider the antimicrobial effect of acetic acid 1% and chitosan. It could be observed that the coated pomegranate arils samples treated with chitosan solution (2.0%) was more effective in extending the shelf life of pomegranate arils until 16 day in cold storage which had the lowest APC, PBC and YMC (4.34, 3.91 and 2.81 log cfu/g; respectively) followed by coated pomegranate arils samples treated with chitosan solution (1.5, 1.0 and 0.5 %) and positive control samples compared to negative control samples. In addition, all investigated treatments were subjected to counting of Coliforms at zero time and every 4 days during cold storage until 16 days and the results were <10 cfu/ g sample.

These results might be due to a variety of phenolic compounds in pomegranate arils and their antimicrobial activities may involve multiple modes of action. Phenolic compounds can denature enzymes (Furneri *et al.*, 2002) but they can also bind to substrates such as minerals, vitamins and carbohydrates making them unavailable for microorganisms. Furthermore, phenols can be absorbed to the cell wall, resulting in a disruption of the membrane structure and function. The antibacterial properties of *Punica granatum* have been reported extensively for several pathogens such as *Escherichia coli*, *Staphylococcus aureus*, including several methicillin resistant strains, *Vibrio cholerae* and *Bacillus subtilis* (Melendez and Capriles 2006 and Braga *et al.*, 2005).

Antimicrobial activity of chitosan has been confirmed by previous studies on other fresh fruits and vegetables (Moreira *et al.*, 2011 and Alvarez *et al.*, 2013). Also Pushkala *et al.*, (2012) enhanced the microbiological quality of carrot shreds by using chitosan powder coating. Besides chitosan antimicrobial activity, the type of solvent could affect its antimicrobial action due to the pH of chitosan/solvent solution. This resulted in easy penetration of the chitosan molecule into the cytoplasmic membrane of bacterial cell wall and changing the internal pH of the bacteria (Cadogan *et al.*, 2014).

The most appropriate hypothesis of the antimicrobial effect of chitosan is a change in cell permeability due to interaction between the positively charged chitosan molecules and the negatively charged microbial cell membranes. This interaction leads to the leakage of proteinaceous and other intracellular constituent (Sudarshan *et al.*, 1992). Rabea *et al.*, (2003) and Li *et al.*, (2010) supposed that, the main mechanism that appears to govern the bacteriostatic and bactericidal effects of chitosan appears to involve binding of its positively charged amino ($-NH3^{+}$) groups to negatively charged carboxylate ($-COO^{-}$) groups located on the

surface of the bacterial cell membranes.

Sensory quality attributes:

Results of the effects of coating treatments of pomegranate arils with different concentrations of chitosan on the sensory attributes and overall acceptability of pomegranate arils during cold storage are presented in table (3).

Table 3. Mean values of sensory attributes of coated pomegranate arils samples prepared with different concentrations of chitosan solutions at different cold storage periods (days).

		Means Panelist scores				
	Treatments	**Day 0**	**Day 4**	**Day 8**	**Day 12**	**Day 16**
Freshness	T1	8.3 ^{c} ±0.67	8.3 ^{bc} ±0.82	7.1 ^{c} ±0.74	6.9 ^{d} ±0.57	6.2 ^{d} ±0.42
	T2	8.2 ^{c} ±0.92	8.1 ^{c} ±0.57	7.6 ^{bc} ±0.52	7.0 ^{d} ±0.67	6.0 ^{d} ±0.67
	T3	8.3 ^{c} ±0.95	8.2 ^{c} ±0.42	7.5 ^{bc} ±0.53	7.3 ^{cd} ±0.66	6.8 ^{c} ±0.42
	T4	8.5 ^{bc} ±0.71	8.6 ^{bc} ±0.70	7.7 ^{b} ±0.48	7.7 ^{bc} ±0.48	7.3 ^{b} ±0.48
	T5	9.1 ^{ab} ±0.32	8.9 ^{ab} ±0.57	8.7 ^{a} ±0.48	8.1 ^{ab} ±0.57	7.7 ^{ab} ±0.46
	T6	9.5 ^{a} ±0.53	8.6 ^{a} ±0.70	8.7 ^{a} ±0.48	8.3 ^{a} ±0.45	7.9 ^{a} ±0.32
Color	T1	8.5 ^{b} ±0.97	8.1 ^{c} ±0.57	7.4 ^{c} ±0.52	7.0 ^{b} ±0.47	6.0 ^{c} ±0.67
	T2	8.5 ^{b} ±0.97	7.4 ^{d} ±0.84	7.0 ^{c} ±0.67	6.5 ^{b} ±0.85	5.5 ^{d} ±0.53
	T3	8.5 ^{b} ±0.71	8.7 ^{b} ±0.48	8.1 ^{b} ±0.32	7.9 ^{a} ±0.32	7.0 ^{b} ±0.47
	T4	8.8 ^{ab} ±0.79	9.1 ^{ab} ±0.57	8.8 ^{a} ±0.63	8.3 ^{a} ±0.82	7.2 ^{b} ±0.63
	T5	9.0 ^{ab} ±0.67	9.2 ^{ab} ±0.63	8.7 ^{a} ±0.48	8.1 ^{a} ±0.99	7.7 ^{a} ±0.48
	T6	9.5 ^{a} ±0.53	9.6 ^{a} ±0.52	9.1 ^{a} ±0.57	8.5 ^{a} ±0.53	7.9 ^{a} ±0.32
Texture	T1	7.6 ^{c} ±0.52	7.9 ^{c} ±0.57	7.3 ^{bc} ±0.67	7.2 ^{d} ±0.42	6.5 ^{d} ±0.53
	T2	8.0 ^{c} ±0.82	8.3 ^{c} ±0.67	7.9 ^{ab} ±0.57	7.3 ^{cd} ±0.67	6.9 ^{cd} ±0.32
	T3	8.0 ^{c} ±0.94	8.3 ^{c} ±0.67^{c}	8.0^{ab} ±0.67	7.8^{bc} ±0.63	7.3 ^{bc} ±0.48
	T4	8.9 ^{b} ±0.88	8.9 ^{b} ±0.57	8.4 ^{b} ±0.52	8.2 ^{ab} ±0.63	7.6 ^{b} ±0.70
	T5	8.8 ^{b} ±0.63	9.2 ^{ab} ±0.63	9.1 ^{a} ±0.57	8.4 ^{a} ±0.7	7.8 ^{b} ±0.79
	T6	9.6 ^{a} ±0.7	9.6 ^{a} ±0.52	9.2 ^{a} ±0.42	8.7 ^{a} ±0.48	8.4 ^{a} ±0.52
Taste	T1	8.6 ^{b} ±0.84	8.4 ^{ad} ±0.52	8.1 ^{c} ±0.57	7.1 ^{d} ±0.57	6.3 ^{d} ±0.67
	T2	8.5 ^{b} ±0.71	7.9 ^{d} ±0.74	7.2 ^{d} ±0.79	6.7 ^{d} ±0.67	6.0 ^{d} ±0.82
	T3	8.5 ^{b} ±0.85	8.7 ^{bc} ±0.82	8.2 ^{c} ±0.63	7.8 ^{c} ±0.67	7.2 ^{c} ±0.42
	T4	9.4 ^{a} ±0.52	8.9 ^{abc} ±0.74	8.9 ^{ab} ±0.74	8.5 ^{ab} ±0.48	7.6 ^{bc} ±0.84
	T5	8.5 ^{ab} ±0.48	9.2 ^{ab} ±0.79	8.4 ^{bc} ±0.70	8.1 ^{bc} ±0.57	7.9 ^{ab} ±0.57
	T6	10.0 ^{a} ±0.23	9.5 ^{ba} ±0.71	9.1 ^{a} ±0.74	8.8 ^{a} ±0.48	8.4 ^{a} ±0.52
Overall acceptability	T1	8.0 ^{d} ±0.47	8.0 ^{c} ±0.47	7.4 ^{c} ±0.52	6.6 ^{c} ±0.70	5.7 ^{e} ±0.67
	T2	8.0 ^{d} ±0.47	7.4 ^{d} ±0.70	6.8 ^{c} ±0.79	6.3 ^{c} ±0.67	5.7 ^{e} ±0.67
	T3	8.6 ^{c} ±0.52	7.9 ^{c} ±0.57	7.4 ^{c} ±0.52	7.2 ^{b} ±0.63	6.4 ^{d} ±0.52
	T4	9.0 ^{bc} ±0.67	8.5 ^{b} ±0.53	8.1 ^{b} ±0.74	7.7 ^{b} ±0.48	6.9 ^{c} ±0.57
	T5	9.4 ^{ab} ±0.52	8.6 ^{b} ±0.52	8.4 ^{ab} ±0.52	7.9 ^{a} ±0.74	7.4 ^{b} ±0.52
	T6	9.8 ^{a} ±0.42	9.3 ^{a} ±0.48	8.9 ^{a} ±0.74	8.3 ^{a} ±0.67	8.0 ^{a} ±0.32

Data are expressed as means ± SD (n = 10). Mean values in the same column bearing the same superscript do not differ significantly ($P \leq 0.05$).

The minimally processed pomegranate arils were acceptable (the sensory score of 7 and above out of 10) in terms of product attributes such as aril freshness, color, texture, taste and overall acceptability at all samples until the end of the storage time. Chitosan coating helped to keep the visual quality of arils during storage as confirmed by their surface color measurement. Overall, the pomegranate arils treated with different concentrations of chitosan were acceptable by the panelists until 16 days of cold storage; however, it was limited to 12 days for the control samples.

CONCLUSION

The obtained results revealed that the potential effect of chitosan to make slight or no significant ($P \leq 0.05$) changes in chemical, microbiological and sensory quality during cold storage. The shelf life of pomegranate arils was suggested to be 16 days by coating of chitosan solution at levels 1.5 and 2%, which preserves high quality with good chemical and microbiological indices as well as sensory attributes.

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تأثير التغطية بالشيتوزان الصالح للأكل على خصائص الجودة لبذور الرمان العصيرية أثناء التخزين المبرد

عبد الفتاح عبد الكريم عبد الفتاح[١] و إيهاب صلاح عشوش[١] و بدر أحمد الناشي [٢]

[١] قسم علوم الأغذية ـ كلية الزراعة ـ جامعة عين شمس ـ شبرا الخيمة – القاهرة ـ مصر

[٢] قسم علوم الأغذية والتغذية ـ كلية العلوم الصحية – الهيئة العامة للتعليم التطبيقي والتدريب ـ الكويت

الهدف من هذه الدراسة هو تقييم تأثير إستخدام محاليل الشيتوزان كأغلفة صالحة للأكل عند تركيزات (صفر، ٠.٥ ، ١ ، ١.٥ و ٢%) على حفظ ثبات الجودة الكلية ومد فترة صلاحية بذور الرمان العصيرية خلال التخزين المبرد على ٤ ± ١ °م لمدة ١٦ يوم. وقد أظهرت النتائج إتجاه عام بتغيرات بسيطة فى قيم الحموضة الكلية، pH ، الأنثوسيانين الكلى، الفينولات الكلية والنشاط المضاد للأكسدة في كل العينات المغطاه مقارنة مع العينات غير المغطاه. التغطية بمحاليل الشيتوزان تساعد على الحفاظ على الجودة المرئية لبذور الرمان أثناء التخزين وهو ما أكده تقييم اللون. وأوضحت النتائج المتحصل عليها أن العدد الكلى للبكتريا وأعداد البكتريا المحبة للبرودة والخمائر والفطريات قد تزايد مع زيادة فترة التخزين المبرد فى كل المعاملات تحت الدراسة. وكان إستخدام محلول الشيتوزان عند مستوى (١.٥ و ٢%) كأغلفة صالحة للأكل الأكثر تأثير في الحفاظ على جودة وقبول بذور الرمان العصيرية بدرجة عالية خلال التخزين المبرد على ٤ ± ١ °م لمدة ١٦ يوم بخصائص جودة كيميائية، ميكروبيولوجية وحسية جيدة.