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Effect of Antioxidant and Ozone Treatment on The Postharvest Quality of Minimally Processed Pomegranate Arils Cv. 'Wonderful'.

Omayma M. Ismail[1*], Khaled S. A. Nagy[2] and Moustafa M. Zohair[1].

[1]Horticultural Crop Technology, National Research Centre (NRC), Giza,12311, Egypt.
[2]Food Technology Research Institute, Agriculture Research Center, Giza, 12619, Egypt.

ABSTRACT

This study investigated the effects of antioxidant and ozone treatment on the quality of minimally processed pomegranate arils cv. 'wonderful' behavior during cold storage at 4 °C as affected by washing the seeds with chlorinated water (CW) (2 ml /L) followed by antioxidants (ascorbic acid 5 g. /L for 30 sec. then the citric acid 5 g. /L for 30 sec (CA)), ozone treatment for (1, 5 and 10 min) in packages made of PET. The color parameters of arils, the chemical characteristics as soluble solids contents (SSC), pH, titratable acidity (TA), vitamin C content and anthocyanin content were investigated also Fruit decay, weight loss, respiratory activity and microbial evaluation were determined. The mean of arils color change (ΔE) ranged from 10.43 to 14.71 of (CW+CA) and (O_3/1min.) treatments respectively, pH mean values were not significant between ozone and antioxidant treatments. The anthocyanin content mean was insignificant differences among the treatments. After 19 days the decay percentage of the ozone treatments were 66.67 % while the control and CW+CA treatments were 100% decay; The best results were obtained from ozone treatments but there were no definite trend among the ozone doses with the different parameters. under these conditions, minimally processed pomegranate arils Under cold storage at 4 °C maintained, good quality and appearance for 19 days Without microbial visual defects.
Keywords: *Punica granatum*, post-harvest treatments, citric acid, ascorbic acid, O3 and sanitizer.

Corresponding author: omaymaismail@yahoo.com

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INTRODUCTION

Due to the increase in consumer demand worldwide for nutritious and therapeutic this is back to the important role of pomegranate as an antioxidant where many clinical studies demonstrated that it`s consumption contributes to prevent diseases such as coronary heart diseases and some types of cancer [1-3]. 'Wonderful' is the main plant cultivar in the new areas. However, the consumption of pomegranates is not very widespread mainly due to the difficulty of extracting the arils. For this reason, the minimally fresh processing of pomegranates to obtain ready-to-eat arils, with intact sensory and nutritional properties, represents a real possibility to increase the production and consumption of pomegranates [4-6].

There are several reasons why fresh-cut produce is relatively safe when compared to other foods. Conditions used with fresh produce are usually unfavorable for the growth of most pathogens (refrigeration temperatures, relatively low nutrients available in some types of vegetables, e.g., leafy vegetables, low pH of fruits, short shelf life). The spoilage microorganisms in refrigerated produce are usually psychrotrophic and therefore have a competitive advantage over most pathogens. Sometimes, this competition prevents the growth of pathogens [7-10].

Proper washing of fresh-cut produce immediately after cutting, is one of the most important steps in fresh-cut processing, but it`s effectiveness depends on the quality of the wash water. Sanitizers, despite their limited direct microbiological benefits on produce, are necessary in vegetable wash water to minimize the risk of cross-contamination from water to produce and among produce overtime [11].

To prevent microbial development, washing the processed products lightly with chlorine solutions has proven essential [12]. Since the color of pomegranate seeds is the most important quality attribute for consumers, its stability must be preserved [13]. Washing with antioxidant solutions might therefore prove useful [14].

Ozone, is a highly reactive form of oxygen where three molecules are bonded together. It has potent antimicrobial activity and other characteristics [15]. Beltrán et al., [16] reported that, ozone as an aqueous disinfectant, was declared to be generally recognized as safe (GRAS) for food contact applications in 1997 [17,18]. Ozonated water and gaseous ozone O_3 are applied to fresh-cut vegetables for sanitation purposes, reducing microbial populations, preventing browning, and extending the shelf life of some of these products [19]. The O_3 is a gas authorised in several countries for direct contact with harvested produce. The recent recommendation to the FDA by an expert panel supporting their classification as a generally recognised safe disinfectant for foods, offers a way for the industry to favour safety of products when applied along with good manufacturing practices [20].

Modified Atmosphere Packaging (MAP) is a dynamic process for altering gaseous composition inside a package. It relies on the interaction between the respiration rate (RR) of the produce, and the transfer of gases through the packaging material, with no further control exerted over the initial gas composition [21]. Sepúlveda et al. [22] observed that minimally

processed pomegranate arils cv. 'Wonderful' were storable for 14 days at 4 °C±0.5 in semi-permeable films.

Microbial decay can be a major source of spoilage of fresh-cut produce [8]. It may occur much more rapidly than in vegetable products due to high levels of sugars found in most fruit. However, the acidity of fruit tissue usually helps suppresses bacterial growth, but not the growth of yeast and molds. Little research has been performed on food-borne human pathogens on fresh-cut fruits. Recently, Conway et al, determined that Listeria monocytogenes survived and proliferated on 'Delicious' apple slices stored at 10 or 20 °C (50 or 68 °F) in air or CA (0.5% O_2 + 15% CO_2), but did not grow at 5 °C (41 °F).

The aim of this research was to study the effect of some postharvest treatments and package on chemical, sensory, microbial quality and shelf life of minimally fresh processed arils extracted from 'Wonderful' pomegranates.

MATERIALS AND METHODS

Plant material

The fruits of cv. 'Wonderful' were harvested in the end of October 2011 and ported to the laboratory (kept at 4 °C until used in the next day). The fruits were washed by tap water followed by distilled water and dried by towels then peeled manually to obtain arils.

Color Parameters

The fruits characteristics were measured as external skin color (three different measurements at three equidistant points on the equatorial region of each individual fruit) and color of external arils and the juice of 100 g. arils by using a Minolta CR-400/410 Chroma-meter (Minolta, Japan). Hunter scale (L, a, and b) system was used. The Chroma meter was calibrated with a white standard tile with illuminant D65(Y=93.3, x= 0.3161 and y= 0.3328) equivalent to HL system: HL = 97.10, a = -0.17 and b= 1.80. Calibration was made at each experiment. The parameter L; represents the brightness of the color, a; the hue range of the colors red (+) and green (-) and b; hue range of colors yellow (+) and blue (-). Total color change (ΔE), chroma (C), hue angle (h) and browning index (BI) were calculated using equations described by [23]:

$$\Delta E = ((L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2)^{½} \quad (1)$$

$$\text{Chroma} = (a_2 + b_2)^{½} \quad (2)$$

$$\text{Hue angle} = \tan^{-1}(b/a) \quad (3)$$

Where; subscript "$_0$" refers to the color reading of fresh arils and juice was used as a reference and a larger ΔE denotes greater color change from the reference material Maskan (2001) [23].

$$BI = [100 (x - 0.31)] / 0.17 \quad (4)$$

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Where:

$$x = (a + 1.75L)/ (5.645L + a –3.012b) \quad (5) [24].$$

Chemical characteristics as soluble solids contents (SSC) were measured using Digital Refractometer (ATAGO Pr-32). pH was determined by pH meter. The Titratable Acidity (TA) was expressed as percent citric acid according to [25].

The anthocyanin content was measured at 535 nm, using spectrophotometer JENWAY Ltd model 4600 according to [26]. The vitamin C ascorbic acid content was determined as [27].

Sanitizer, Antioxidant and Ozone Treatments

The arils were washed with distilled water and batched to the different treatments as follows:

- Control
- Arils washed with chlorinated water (CW) 2 ml Sodium hypochlorite /L. (NaOCl), then dipped in the ascorbic acid 5 g. /L for 30 sec (CA), then in the citric acid 5 g. /L for 30 sec., then dried and packaged (CW+CA).
- The arils are exposed to ozone only for (1, 5 and 10 min). usingMatraOzonator Model no.GL-2186 at a flow rate of 81L/hr with concentration 560 ppm for (1, 5 and 10 min), (CA+O_3 doses).

Arils were spread on the sanitized plastic sheets for drying by air fan for 10 min. before packaged. Each package filled by 300 g.dried arils. The packing frame was closed by adhesive tape and stored in the cold room storage at 4° C and relative humidity 90 ± 5%.

Package characteristic

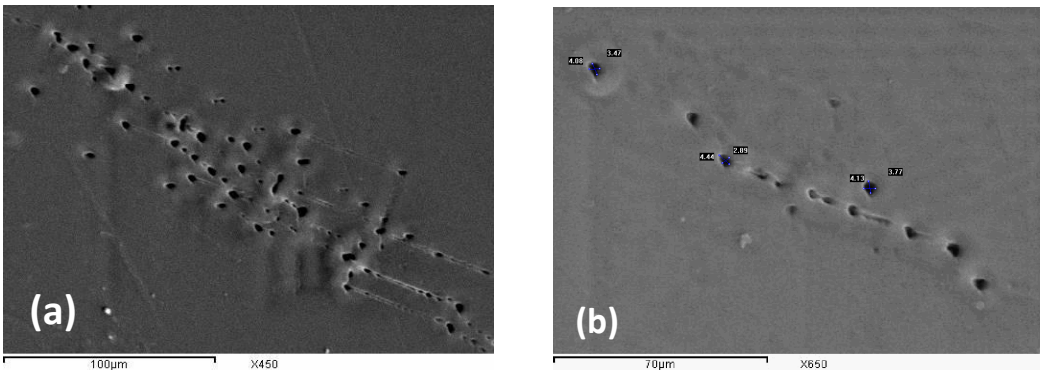

Fig.1. (a) scanning electron micrographs of package pores with magnification of 450x, (b) pores dimensions with magnification of 650x.

The packages characteristics are transparent and had permeability based on pores by laser as shown in (Fig 1). Cleared that it had many pores with different diameter. The package

Dimensions was 180 x 215 x 75 (mm), volume was 810 ml, and it made of PET raw materials, package temperature sensitivity is -20/+ 65ºC.

Fruit Decay and Quality Evaluation

The packages examined to physical characteristics, weight lost and determine decay. Chemicals analyses and microbial evaluation at each period storage a new three packages replicates of each treatment were examined then discarded..

Upon receipt, each bag of pomegranates was weighed and the percentage of weight lost during storage calculated according to the following equation:

$$WL=(W_o-W_f)/W_o \times 100 \quad (6)$$

Where WL is the weight loss (%), W_o is the initial weight (g) and W_f is the final weight (g) prior to package analysis.

The entire sample of fruit was visually inspected for decay and physiological disorders.

Decay Percentage was calculated according to the following equation:

$$\text{Decay \%} = (\text{Number of decay packeges})/(\text{Total number of packages}) \times 100 \quad (7)$$

Respiratory Activity

The respiration rate (RR) was determined by the food package analyzer Servomex, model No.01450 CIDMA results were expressed as mg. CO_2/kg/h. Initial gas concentrations (17% O_2 and 0.25 % CO_2). Changes in concentrations of O_2 and CO_2 within the packages were monitored of different treatments during storage periods. The effect of the package on CO_2 and O_2 concentrations was determined every 4 days of packaged arils without treatments which were stored at 4º C.

Microbiological Evaluation

Immediately after taking the gas analysis, packages were opened and used for microbial analyses, 5 g. arils from each replicate kept until analysis. Total plate counts were carried out as the method described by [28]. Each sample of arils was 2 g., then added 20 ml of sterile distilled water to obtain a 10-1 dilution, after shaking for 10 min. 1 ml of 10-1 dilution pipette by sterile Pipette to sterile Petri dishes in triplicate, then 10 to 15ml of the melted nutrient agar medium of between 44°C was poured. The plate was gently rotated for thorough distribution of inoculum through the medium. [29]. The plates were allowed to solidify, incubated in inverted at 37°C for 24 - 48 h and then were enumerated. Similarly, Total fungi were carried out in triplicate using pour plate method on plates of Potato Dextrose Agar (PDA) medium. Plates

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were incubated in inverted at 25°C±1 for 3 - 7 days [30].and then colonies of fungi were enumerated.

Experimental Design

The statistical design was completely randomized. For physical, chemical and microbial evaluation at each period storage a new three packages replicates of each treatment were examined then discarded.

Data Analysis

The data were subjected to ANOVA and were evaluated by MSTATC program. Least significant difference (LSD) test were performed to identify specific differences in factor levels between the treatments at 5% level of significance.

RESULTS AND DISCUSSION

Changes in Arils Color

Data in (fig.2) showed, that generally there was no much changes in the arils color (ΔE) among the different treatments during storage periods, and that, there was no specific trend of the treatments effect on BI during storage.The mean of arils color change (ΔE) ranged from 10.43 to 14.71 of (CW+CA) and (O_3/1min.) treatments respectively, also cleared that the antioxidant treatment was the least color changes whereas the BI mean was insignificant among the ozone treatments and antioxidant except O_3/1min. was the highest significant value (78.04) . The BI mean values during storage period had no definite trend, it ranged from (35 to 84.84) at 19 days and 8 days respectively.

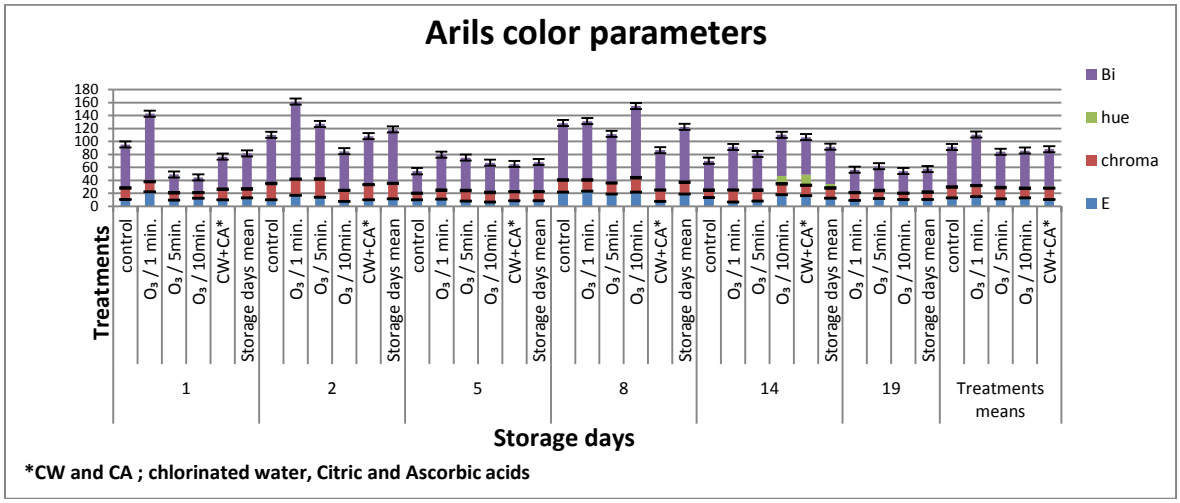

Fig.2.Pomegranate arils color parameters of antioxidants, sanitizing and ozone treatments.

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Changes in Chemical Quality Attributes

The highest significant mean value of Soluble Solid Content (SSC) was control 17.06, where the other treatments ranged from (16.40 to 16.90) of O_3/min. and antioxidant treatment respectively. Generally, the SSC mean decreased during storage periods. (Fig.3).

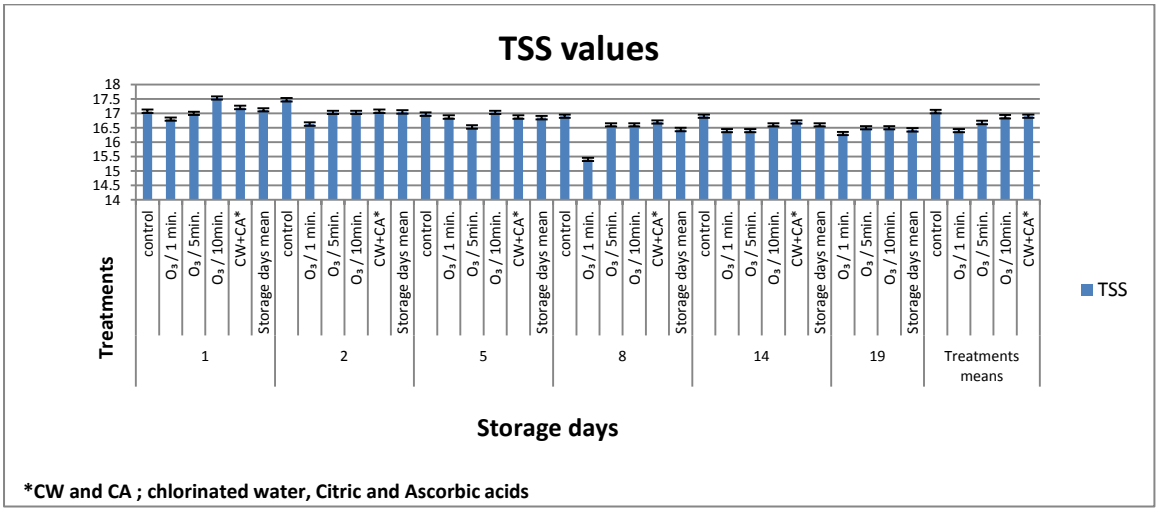

Fig.3. SSC values of antioxidants, sanitizing and ozone treatments.

Nanda et al., 2001 recorded a slight decrease in total sugar content during storage at different temperatures. When compared with values at harvest, a slight but non-significant decrease in SSC was found after storage in all the treatments in agreement with [31,32,33] in 'Ganesh' and 'Mollar' cultivars respectively.

pH mean values were not significant between ozone treatments and antioxidant treatment..Control pH was the highest mean value 3.32. During storage periods the titratable acidity (TA%) mean values increased during one and two days then decreased significantly with nonspecific trend. (Data not shown).

Caleb et al., [21] reported that the variability of pH, TSS, and TA values could be explained by several factors such as cultivar differences and the relative solubility effect of CO_2 in water molecules surrounding the freshly packed pomegranate arils.

The anthocyanin content mean was insignificant differences among the treatments, also it did not show a definite trend during storage periods but noticed that after one and two days were the highest mean values 5.49 and 5.06 (mg./100 g. fresh arils) respectively (Fig. 4).

The vitamin C ascorbic acid content mean was the highest value with control (7.3 mg. V.C /100 g. juice). Generally, treatments had no clear effect on vit.C content, and also during storage periods as in (Fig. 4).

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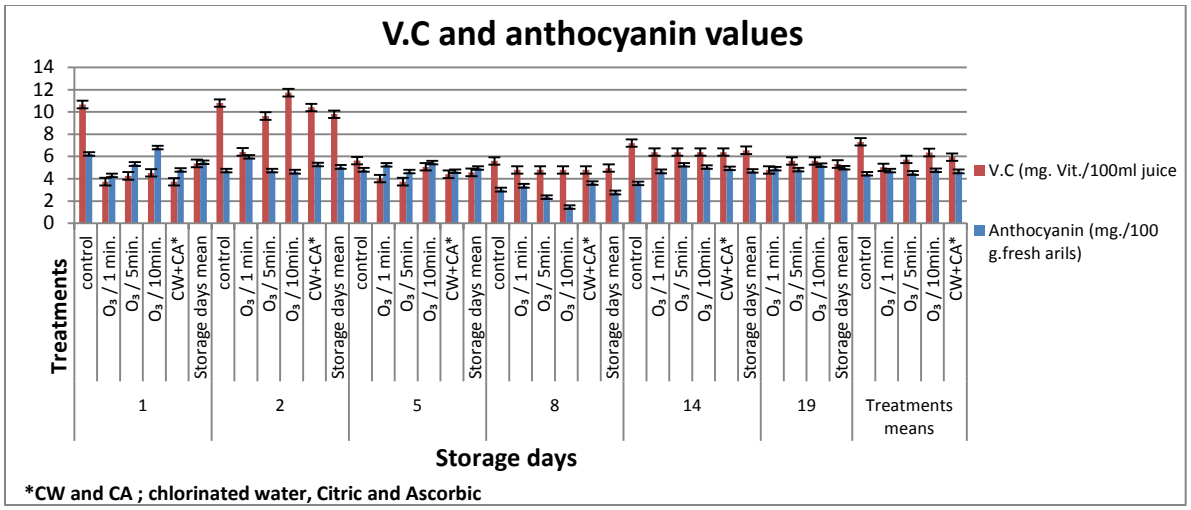

Fig. 4.V.C and anthocyanin values of antioxidants, sanitizing and ozone.

Fruit Decay Percentage, Weight Loss and Juice volume

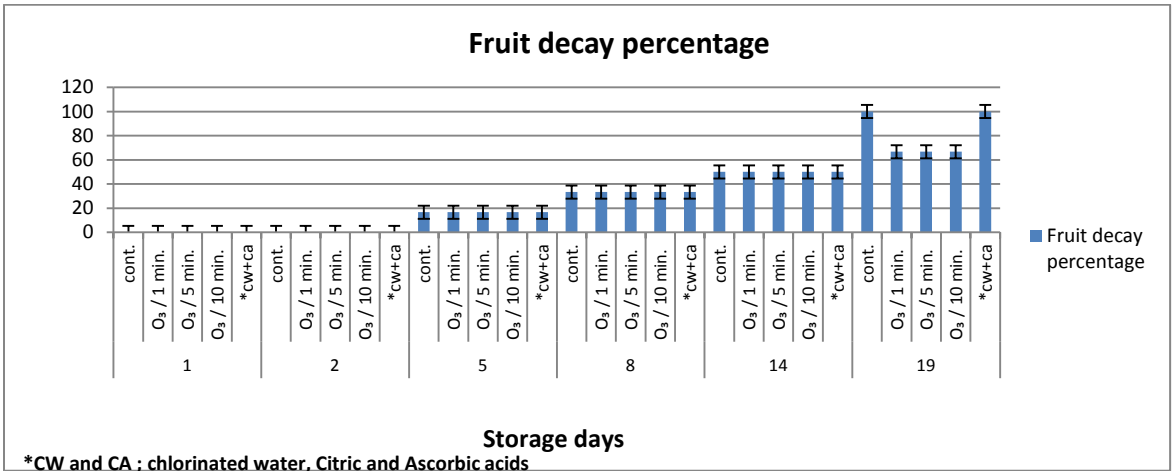

Fig. 5.Fruit decay percentage of antioxidants, sanitizing and ozone treatments.

In general, fruit decay increased with long storage periods, (Fig. 5) showed that insignificant difference between the treatments under the same storage period except after 19 days the fruit decay percentage of ozone treatments were 66.67 % while the control and CW+CA treatments were 100% decay; Whereas, [22] observed that minimally processed pomegranate arils cv. 'Wonderful' were storable for 14 days at 4 °C± 0.5 in semi-permeable films. These results showed that ozone had a good effect on reducing decay percentage. There was no weight loss during all storage periods of all treatments. The juice volume means ranged from 57.22 to 69.78 ml of control and O_3/5min. respectively, also noticed that the storage period had no specific effect on the arils juice volume means that due to no leakage from the arils observed (data not shown).

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Respiration

Pomegranate being a non-climacteric fruit with a low respiration rate [34], therefore, the respiration rate was not much affected by treatments.

The respiration rate decreased during the storage periods. The highest respiration rate values were 1.39 of (CW+CA) treatment then 1.19 and 1.19 mg. CO_2/ Kg./h. of (O_3/1min. and O_3/ 5min. treatments) after one storage days respectively. Most of the treatments caused decrease in the respiration rate especially ozone treatments compared with control where insignificance among each others as in (Fig. 6).

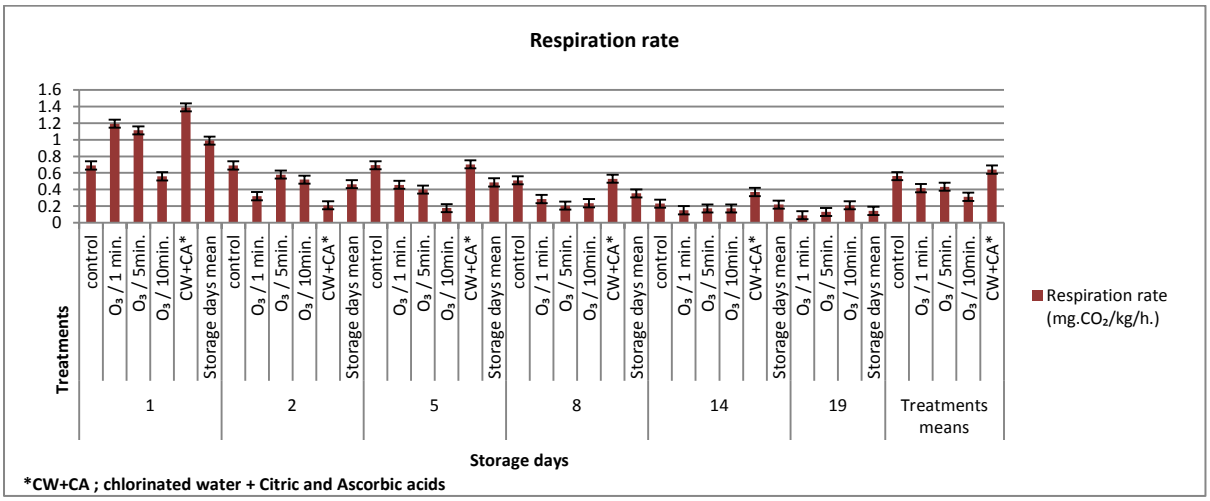

Fig. 6.Respiration Rate of antioxidants, sanitizing and ozone treatments.

The effect of package on the arils respiration rate without treatments showed that it decreased with time. The results shown in (Fig. 7) proves that the kind of package had an effect on gas permeability during storage. This observation may be highlight to good package permeability, whereas Caleb et al., (2013) [35] reported that headspace O_2 content significantly decreased over time inside packages at the different storage temperatures, on the other hand, CO_2 levels increased significantly during storage for all packaging conditions.

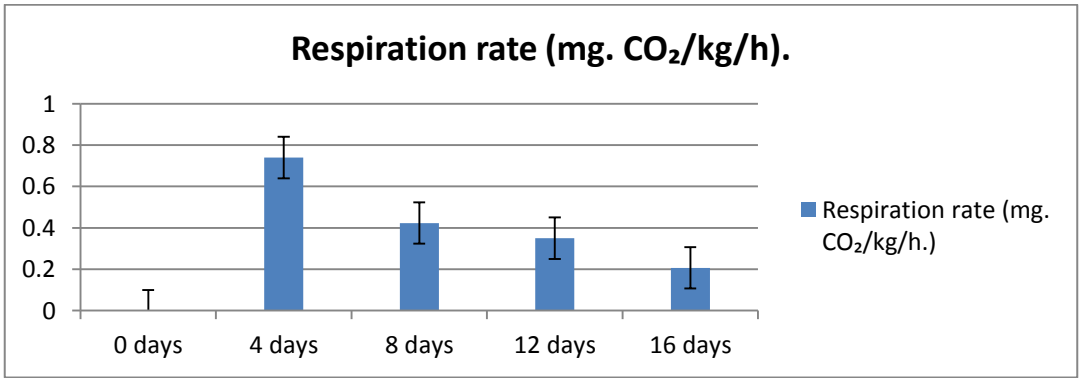

Fig. 7.The effect of package on the arils respiration rate without treatments.

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Microbial Load

Each value represents the mean of three plates; and the results were expressed as log of colony forming units only per gram of the sample (log10 CFU/g). The total bacterial count ranged from (1.65 to 3.6 log10 CFU/g) of ozone treatment for 1min. after one day and 10 min. after 19 days respectively. The total bacterial count was varied between treatments but, generally it increased with storage time.

The Total Fungal Count ranged from (1 to 3.4 log10 CFU/g) of ozone treatment for 5 min. after 19 storage days and after one storage day respectively. Generally the total fungal count of the ozone treatment for 10 min. was the least of the other ozone treatments as shown in (Fig. 8).

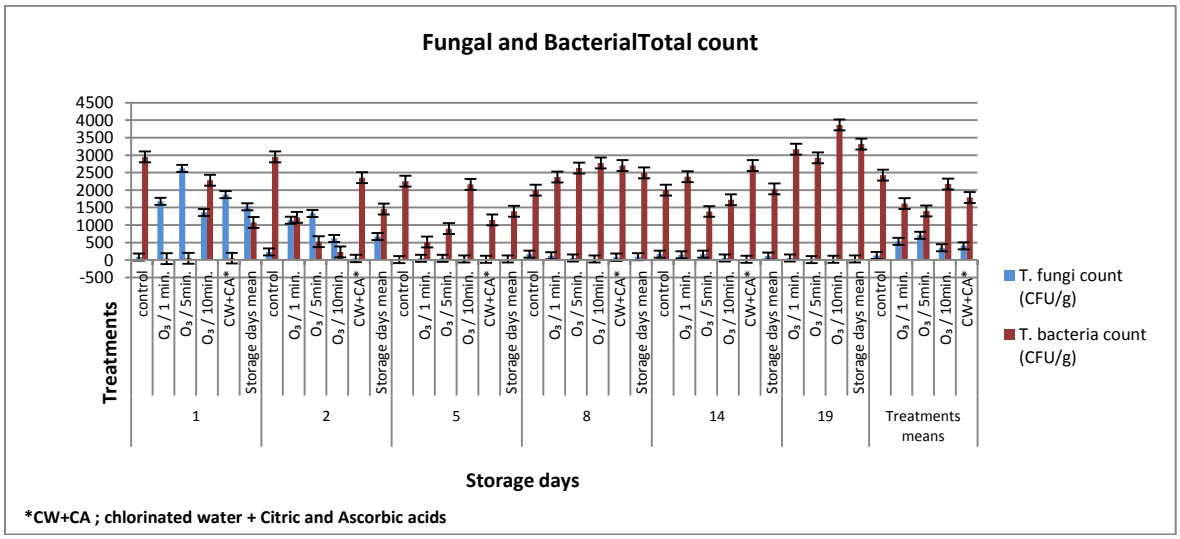

Fig.8.Fungal and Bacterial total count of antioxidants, sanitizing and ozone treatments.

The microbial visual defects were invisible due to the defects become visible from a microbiological count of 8 log CFU/g [36-39].

The results of the microbial load cleared that the population of bacteria and fungi were under the European limit as in reported [40], the Spanish legal limit for microbial populations on minimally fresh processed fruit for safe consumption are 7, 5, and 3 log10 CFU/g for aerobic bacteria, yeasts, and molds, respectively. Only ozone treatments reached 29 days storage but the control and (CW+CA) decayed at 14 days storage.

CONCLUSION

Generally, the best results for extending the storage life and maintaining the quality up to 19 days were obtained when pomegranates arils were treated with ozone and antioxidant. Our results showed that at 19 storage days ozone treatments had the least decay percentage. There was no weight loss during all storage periods of all treatments also; ozone treatments

reduced the total fungal count. The results proved that the smart package had an effect on gas permeability during storage, which the arils respiration rate decreased with the time storage.

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