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Overall quality and antioxidant enzymes of ready-to-eat 'Purple Queen' pomegranate arils during cold storage

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ABSTRACT

This study evaluated the effectiveness of two different packaging systems, using micro-perforated (MPP) and semipermeable (SP) films, on the physico-chemical and nutraceutical traits, microbial quality, and antioxidant enzymatic system of ready-to-eat pomegranate (*Punica granatum* L.) arils (cultivar 'Purple Queen') stored at 5 °C for 16 d. Statistically significant differences in gas composition and arils qualitative traits such as pH and titratable acidity between the two packaging systems were found. Arils packaged in the SP system had higher polyphenols and anthocyanins contents, followed by a high antioxidant activity, with a positive correlation (r = 0.610 and 0.940, respectively) among them. An increase in the activities of antioxidant enzymes, such as superoxide dismutase, catalase, and ascorbate peroxidase, were registered in the arils in the SP system, with a decrease in polyphenol oxidase and peroxidase activity involved in arils-browning. Overall, SP packaging could be a valid system to preserve ready-to-eat arils within food chains that maintain high qualitative and nutraceutical features up to 16 d of storage.

1. Introduction

Pomegranates (*Punica granatum* L.) are considered a functional food due to the high content of nutraceutical and antioxidant compounds and has been used since ancient times for medical benefits (Ismail et al., 2012). Several compounds such as phenols, anthocyanins, flavonoids, vitamins, minerals, and tannins have been identified in peels and arils (Abid et al., 2017; Tuppo et al., 2017; Tzulker et al., 2007). Epidemiological studies have focused on the antioxidant, anticancer, and anti-inflammatory properties of pomegranate fruit consumption. The health-promoting effects could be due to synergistic interactions between several bioactive phytochemicals in the arils (Jurenka, 2008). Arils are the edible parts of pomegranates, approximately 60% of total fruit weight, but their consumption is still limited due to the difficulties of their extraction from the fruit (Al-Maiman and Ahmad, 2002). Minimally ready-to-eat arils could be a valid alternative that could increase the consumption of this fruit, and as a commercial strategy to use with externally damaged pomegranates that are unacceptable for fresh marketing (Lopez-Rubira et al., 2005).

Minimally processed fruit are highly susceptible to biochemical, enzymatic, and microbial reactions that reduce the shelf-life. Several treatments have been proposed to extend the storage and shelf-life of pomegranate arils such as cold storage (Palma et al., 2015), edible coating (Özdemir and Gökmen, 2017; Yousuf and Srivastava, 2017), γ-irradiations (Ashtari et al., 2019), active and passive modified atmospheres (Adiletta et al., 2017; Belay et al., 2017, 2018; Pareek et al., 2015), and perforated films (Hussein et al., 2015a,b).

Packaging of the pomegranate arils is the most important step to have a longer shelf-life by maintaining the quality of arils (Kahramanoglu and Usanmaz, 2018). Several studies have demonstrated that the shelf-life of packed arils varied from 7 to 18 d at temperatures between 0 and 5 °C under different packaging conditions (Artes et al., 2000; Ayhan and Eştürk, 2009; Gil et al., 1996; Lopez-Rubira et al., 2005; Sepulveda et al., 2000).

Passive modified atmosphere packaging (MAP) are created using semipermeable films with different permeabilities to CO_2 and O_2, and are cheaper and easier to apply than active MAP of minimally processed fruit (De Reuck et al., 2010; Caleb et al., 2013; Barrios et al., 2014). In passive-MAP storage conditions, the desired gas composition, inside a package, can be achieved by relying on the gas exchange through the semipermeable film and the fruit respiration rate (Caleb et al., 2018), although low respiration rate of pomegranate arils was registered at

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low temperatures (Caleb et al., 2013; O' Grady et al., 2014a). Garcia et al. (2000) evaluated the ready-to eat 'Mollar' arils packaged in semipermeable and an impermeable plastic bag stored at 4 °C for 10 d, demonstrating that semipermeable plastic package and refrigerated storage prolonged the shelf-life (10 d) and maintained high quality pomegranate arils. Sepulveda et al. (2000) tested different semipermeable films and antioxidant solutions to study the storage of minimally processed arils from pomegranate cultivar 'Wonderful' and found that the use of semipermeable film, with or without an antioxidant solution, preserved the physical and microbiological properties up to 14 d at 4 °C. Antioxidant compounds showed a low browning-inhibition due to a reduction of spoilage microorganisms. In another study, the overall quality of ready-to-eat arils (cultivar 'Mollar de Elche') were maintained by sanitisation with UV-C, and packaging under passive atmosphere with bi-oriented polypropylene film up to 13 or 15 d at 5 °C (Lopez-Rubira et al., 2005).

Semipermeable films are valid tools in passive modified atmosphere packaging in the food supply chain, especially in view of a globalized market which includes long-distance distribution systems (Pant and Thielmann, 2018).

Soft-seeded, in pomegranate fruit, is a desirable agronomic trait, that improves the market expansion of ready-to-eat arils. Soft-seed pomegranates are pleasant and acceptable for the consumers, although other traits such as taste, and colour are taken into account (Zarei et al., 2015). Soft-seed genotypes are restricted to a few narrow ecological regions, although in the last years, new soft-seed pomegranate cultivars are developed by breeding programs (Sarkhosh et al., 2011).

The objective of this study was to evaluate the changes in physico-chemical and microbial traits of soft-seeded pomegranate arils 'Purple Queen' packaged with passive modified atmosphere during cold storage. In addition, changes in the bioactive compounds content and in the antioxidant enzymatic system in pomegranate arils were also investigated during the storage time.

2. Material and methods

2.1. Fruit sample preparation and packaging

In this study, we used a new soft-seeded pomegranate cultivar, 'Purple Queen'®, which grows ready-to-eat arils. The ripening stage of this cultivar is during an extra-early period between mid-August and late-September in the Northern hemisphere when the fruit's outer skin shows an exceptionally uniform red purple colour. Pomological traits were examined on a sample of 30 fruit, which were used to evaluate pomegranate weight (PW; g), arils weight (AW; g), seed weight (SW; g), aril length and width (AL; AWI; mm), seed length and width (SL; SWI; mm), and juiciness (J; %) according to International Union for Protection of new Varieties of plants (UPOV, 2012) (Table 1).

Pomegranate fruit were randomly harvested from trees grown under organic practices in the commercial orchard owned by Comercial Gallo srl, located in Massafra (Taranto, Italy; 40° 32′ N, 17° 04′ E). Fruit were picked up during the commercial ripening stage of depth-red skins and arils in mid-September and cold transported to the Food Technology Laboratory of the University of Salerno and stored at 4 °C before processing.

Table 1
Mean values and standard deviations of pomological traits of 'Purple Queen' pomegranate (n = 30; α = 95%).

Pomological traits	'Purple Queen'
PW (g)	338.04 ± 18.30
AW (g)	0.22 ± 0.02
AL (mm)	9.17 ± 0.58
AWI (mm)	6.03 ± 0.37
SW (g)	0.05 ± 0.01
SL (mm)	7.15 ± 0.47
SWI (mm)	3.04 ± .36
J (%)	47.67 ± 5.25

(Pomegranate weight (PW), arils weight (AW), seed weight (SW), aril length and width (AL; AWI), seed length and width (SL; SWI) and juiciness (J)).

Selected fruit were without sunburns, blemishes or any visible damage, and were sanitised with a sodium hypochlorite solution (0.2 g L^{-1}) (Hussein et al., 2015a). Afterwards, the fruit were manually and aseptically processed for aril extraction. The arils were mixed to obtain a homogeneous initial sample that had qualitative and enzymatic analyses (described below) carried out before packaging.

Approximately 100 g of arils were placed inside sanitised cellulosic trays (30 × 10 × 3 cm) and wrapped with two films with different properties: a semipermeable film (SP) (Cryovac, Elmwood Park, New Jersey, USA) (polyolefines; 15 µm thickness; CO_2 transmission rate 41,000 $cm^3\,m^{-2}\,day^{-1}$; O_2 transmission rate 10,000 $cm^3\,m^{-2}\,day^{-1}$ at 23 °C and 0% RH; moisture transmission vapour 25 g $m^{-2}\,day^{-1}$ at 38 °C and 100% RH), and a micro-perforated polymeric film (MPP) (Cryovac, Elmwood Park, New Jersey, USA) (polyethylene/polypropylene; 15 µm thickness; O_2 transmission rate > 500 $cm^3\,m^{-2}\,day^{-1}$ at 23 °C and 0% RH; moisture transmission vapour 44 g $m^{-2}\,day^{-1}$ at 38 °C and 100% RH; vent holes diameter 0.5 − 0.7 mm; approximately four vent holes cm^{-2}).

Two biological replicates were prepared, each made of 30 packages (15 for each film) that were prepared and stored for 16 d at 5 °C and a RH of 90 ± 2%. Analyses were conducted every four days, from three technical replicates. The chosen time of storage adopted in this study emerged from our preliminary analysis (data not shown) verifying that 16 d avoided the appearance of qualitative decay (aerobic mesophilic bacteria and yeasts and moulds counts) in the analyzed pomegranate arils.

2.2. Headspace gas composition

Headspace O_2 and CO_2 composition (%) in the packages was measured with a O_2/CO_2 gas analyser (Checkmate3, PBI Dansensor, Ringsted, Denmark) with the accuracy of 0.1%. Inside the packages, gas composition was measured on three technical replicates for each film monitored every four days.

2.3. Weight loss

The weight of each arils package was determined with an analytical balance (mod. Gibertini E42, Italy) at the beginning of the experiment and every four days during storage. Using these values, the weight loss (%) was calculated as follows:

$$\text{Weight loss (\%)} = [(W_0 - W_t)/W_0] \times 100 \quad (1)$$

where W_0 is the initial aril weight (g) and W_t is the weight (g) at the opening day of the arils in the package.

2.4. Physico-chemical traits

The arils from each package were used to obtain juice with a juice extractor (mod. HR1869/80, Philips) under aseptic conditions, which was used for determining physico-chemical and microbiological traits. The aril juice pH was measured with a digital pH meter (Model 2001, Crison, Barcelona, Spain), and titratable acidity (TA) (citric acid g L^{-1}) was measured by titration with NaOH 0.1 N up to a pH of 8.2. Total soluble solids (TSS; %) were evaluated by hand refractometer (Model N-10; Atago, Tokyo, Japan).

2.5. Bioactive compound analysis

The aril juice was centrifuged at 5000 × *g* for 10 min before the evaluation of total phenols (TP), total anthocyanins content (TAC), and antioxidant activity (EC_{50}). TP were determined using a Folin-Ciocalteu assay as described by Adiletta et al. (2018). The results were expressed as mg of gallic acid equivalents per L of juice (mg L^{-1} GAE). Total anthocyanins content (TAC) was estimated by the pH differential method using a potassium chloride (pH 1.0, 0.025 M) and sodium acetate (pH 4.5, 0.4 M) buffer. The assay mixture was prepared as described by Adiletta et al. (2018) and TAC was calculated according to the following equation:

$$C_3gE\ L^{-1} = (A \times MW \times DF)/(\varepsilon \times L) \quad (2)$$

Where $A = (A_{520}–A_{700})_{pH1.0} –(A_{520}–A_{700})_{pH4.5}$ absorbance (nm); MW (molecular weight) = 449.2 g mol^{-1} for cyanidin-3-glucoside; DF = dilution factor; L = cell path length (1 cm); and ε = 26.900 M absorptive coefficient for cyanidin-3-glucoside. The results were expressed as cyanidin-3-glucoside equivalents per L of juice (mg L^{-1} C_3g).

DPPH radical scavenging activity was used to determine the total antioxidant activity (AA) in aril juice (Adiletta et al., 2018). The assay reaction was prepared with (A_{sample}) and without (A_{blank}) the arils juice, with a spectrophotometer reading being recorded at 517 nm using a Perkin–Elmer lambda-Bio 40 (PerkinElmer Inc., Waltham, MA, USA) spectrophotometer at 25 °C. AA was calculated according the following equation:

$$\%\ \text{inhibition of DPPH} = [(A_{blank} - A_{sample})/A_{blank}] \times 100 \quad (3)$$

Where A_{blank} is the absorbance of the control at t = 0 min and A_{sample} is the absorbance of sample at t = 30 min. The results of antioxidant activity were expressed in terms of the EC_{50} value, which is the volume (mL) required to reduce 50% of the initial DPPH radical activity.

2.6. Microbial analysis

The total plate count method was used to determine total aerobic bacteria (TAB) using standard plate count agar (CM 0463, Oxoid). Yeasts and moulds (YM) were determined by oxytetracycline-glucose yeast extract agar (CM 0545, Oxoid). Serial dilutions of juice (1:10 v/v) were prepared and each dilution (1.0 mL) was pour-plated and incubated for TAB and YM at 37 °C for two days and at 25 °C for 3–5 d, respectively. Plates with 15–300 colonies were counted. The results were reported as logarithms of the number of colony forming units per mL of juice (log CFU mL^{-1}).

2.7. Enzyme extraction and activity assays

Powder arils (1 *g*), obtained using liquid nitrogen, were ground with an ice-cold phosphate buffer (5 ml) containing 100 mM potassium phosphate buffer (pH 7.8), 2 mM DTT, 1 mM sodium EDTA (pH 7), and 1 mM PMSF, 0.2% Triton X-100, 5% (w/v) PVPP. Ascorbic acid (5 mM) was added to the extraction buffer only for the ascorbate peroxidase enzyme extraction. Homogenate was centrifuged at 18,000 *g* for 10 min at 4 °C and supernatant was used for catalase and ascorbate peroxidase activity determinations. Total protein content was determined by Bradford assay (Bradford, 1976) using bovine serum albumin as a standard.

Catalase (EC 1.11.1.6) (CAT) and ascorbate peroxidase (EC 1.11.1.11) (APX) activities were evaluated as described by Pasquariello et al. (2015). CAT assay contained 50 mM potassium phosphate buffer (pH 7), 20 mM H_2O_2 and 100 µL of crude enzyme extract in a final volume of 1.5 mL. APX assay consisted of 100 mM potassium phosphate buffer (pH 7), 0.35 mM H_2O_2, 0.33 mM ascorbic acid, 0.66 mM sodium EDTA (pH 7) and 20 µL of crude enzyme extract in a final volume of 1.5 mL. The decrease in absorbance were measured for CAT and APX activity at 240 nm and 290 nm, respectively. The CAT and APX activities were expressed as millimoles per second on a protein content basis (mmol s^{-1} kg^{-1}).

Guaiacol peroxidase (EC 1.11.1.7) (GPX) and superoxide dismutase (EC 1.15.1.1) (SOD) activities were measured using the extraction method reported by Adiletta et al. (2018). Arils powder (1 *g*) was homogenized in ice-cold phosphate buffer (5 ml) containing 50 mM potassium phosphate buffer pH 7.8, 1 mM sodium EDTA pH 7, 2% (w/v) PVPP. The homogenate was centrifuged at 14,000 × g for 10 min at 4 °C and used for GPX and SOD activity determinations.

GPX activity was assayed evaluating the formation of tetraguaiacol according to Pasquariello et al. (2015). Assay mixture contained 100 mM potassium phosphate buffer pH 7, 0.20 mM sodium-EDTA pH 7.0, 4.84 mM H_2O_2, 6.4 mM guaiacol and 500 µL of extract in a final volume of 1 mL. GPX activity was expressed as nanomoles per second on a protein content basis (nmol s^{-1} kg^{-1}). The photochemical reduction of nitro blue tetrazolium (NBT) method was used to measure SOD activity in an assay mixture (1.5 mL) contained 50 mM potassium phosphate buffer pH 7.8, 0.1 mM sodium EDTA, 13 mM methionine, 75 µM NBT, 2 µM riboflavin and 100 µL of crude enzyme extract. The results were expressed as unit per second on a protein content basis (U s^{-1} kg^{-1}), where one SOD unit is the amount of enzyme that inhibits the rate of NBT reduction by 50% using the assay conditions described above. Polyphenoloxidase activity (PPO) was determined following the method described by Adiletta et al. (2018) and was expressed as millimoles per second on a protein content basis (mmol s^{-1} kg^{-1}).

2.8. Statistical analysis

A factorial combination of two packaging films and four times of storage using randomized complete block design with three biological replicates were used. All results conducted on the three biological replicates and two technical replicates were expressed as the mean ± standard deviation. Statistically significant differences ($P < 0.05$) between the two packaging systems were analysed by one-way ANOVA and Duncan's test. Statistically different samples were indicated with different letters.

Principal components analysis (PCA) was used to identify the components contributing to most of the variation within the dataset, and for evaluating in the aril packages the qualitative, nutraceutical and enzymatic changes during storage. All analyses were carried out using the SPSS software package, Version 20.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Package headspace gas composition and qualitative traits of arils during cold storage

Several postharvest treatments had different influences to the quality and the storage time of minimally processed pomegranate arils (Venkataramudu et al., 2018; Caleb et al., 2015). Passive-MAP is a valid tool to extend the shelf-life of minimally processed arils, but an accurate selection of semipermeable films is required to prevent condensation and minimise moisture loss (Somboonkaew and Terry, 2010). In this study, we evaluated passive-MAP to prolong the storage time of soft-seeded arils 'Purple Queen' in packages with different films. During 16 d of cold storage, we observed changes in O_2 (Fig. 1A) and CO_2 (Fig. 1B) levels in tested packages. In SP film the O_2 percentage decreased slightly from 21.7 ± 0.03% at harvest to 19.7 ± 0.08% at the end of cold storage, while CO_2 increased reaching a value of 0.8 ± 0.05%. In MPP film, as expected, O_2 and CO_2 contents showed no significant differences during the whole experiment, this may be due to the vent holes diameter (0.5 − 0.7 mm) and their number, about four, per cm.

Several studies have demonstrated that the packaging films can influence in-package headspace gas composition by packed

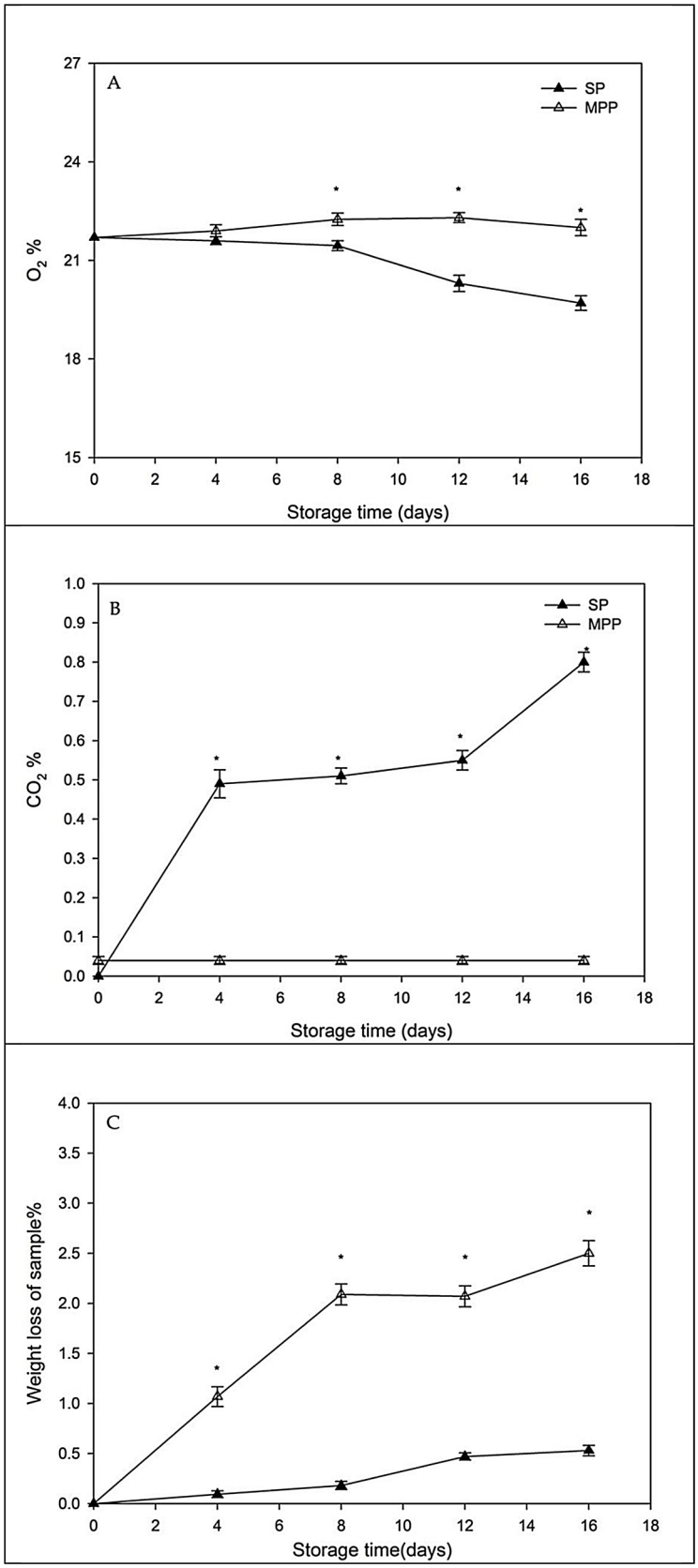

Fig. 1. Gas composition (O_2 percentage (A); CO_2 percentage (B)), and weight loss percentage (C) in pomegranate arils cultivar 'Purple Queen' packaged in semi-permeable (SP) and microperforated (MPP) films, stored at 5 °C for 16 d. Error bars indicate the standard deviation and * indicates statistical differences between samples for the same storage time.

Table 2
Effects of different packaging systems (SP: semipermeable film and MPP: microperforated film) on the pH, titratable acidity (TA, g citric acid per g L juice), soluble solid content (TSS, %), total anthocyanin content (TAC, mg L^{-1} C3g), total polyphenol content (TP, mg L^{-1} GAE), antioxidant activity (EC50, mL L^{-1}) of 'Purple Queen' pomegranate arils during 16 d of cold storage (5 °C). Means followed by the same letter do not differ significantly at P = 0.05 (Duncan Test).

Packaging System	Days	pH	TA	TSS	TAC	TP	EC50
SP	0	4.33 ± 0.04d	2.01 ± 0.09a	20.46 ± 0.11a	396.28 ± 5.29f	1770.55 ± 45.88c	22.29 ± 0.12a
	4	4.37 ± 0.02e	2.15 ± 0.01a	19.36 ± 0.20a	400.12 ± 2.38f	1518.50 ± 109.78b	29.08 ± 0.34b
	8	4.34 ± 0.02d	2.02 ± 0.07a	19.31 ± 0.20a	319.40 ± 10.24e	16.13.62 ± 121.64b	84.79 ± 0.22e
	12	4.34 ± 0.02d	2.09 ± 0.07a	19.28 ± 0.07a	244.46 ± 1.19d	1612.00 ± 47.44b	95.06 ± 0.34f
	16	4.29 ± 0.02c	2.40 ± 0.01b	19.34 ± 0.01a	237.52 ± 4.41d	1486.00 ± 251.92b	100.00 ± 0.32 g
MPP	0	4.33 ± 0.04d	2.01 ± 0.09a	19.46 ± 0.11a	396.28 ± 5.29f	1770.55 ± 45.88c	22.29 ± 0.12a
	4	4.25 ± 0.01b	2.06 ± 0.03a	19.25 ± 0.11a	324.13 ± 4.21e	1553.25 ± 34.65b	35.42 ± 0.33c
	8	4.38 ± 0.02e	2.12 ± 0.04a	19.34 ± 1.15a	214.14 ± 13.52c	1480.50 ± 71.47b	73.27 ± 0.37d
	12	4.28 ± 0.01c	2.16 ± 0.01a	19.25 ± 0.11a	151.90 ± 1.19b	1347.50 ± 39.12a	135.42 ± 1.09 h
	16	4.00 ± 0.01a	2.80 ± 0.38c	19.31 ± 0.06a	137.83 ± 1.57a	1284.50 ± 93.83a	145.00 ± 1.02i

pomegranate arils (Ayhan and Eştürk, 2009; Adiletta et al., 2017). Slow changes in headspace gas composition could be attributed to the low respiration rate of pomegranate arils at low temperatures (Caleb et al., 2013; O' Grady et al., 2014a).

Packaging systems, storage time, and packaging systems plus storage time interactions, have been found to influence qualitative traits of pomegranate arils (Banda et al., 2015; Belay et al., 2018). The results of qualitative traits of arils during cold storage are shown in Table 2. In SP and MPP films, after 16 d, a weight loss of 0.53 ± 0.05% and 2.50 ± 0.07%, respectively was observed (Fig. 1C).

Several authors have reported weight losses for pomegranate arils cultivars during storage (Caleb et al., 2013; Hussein et al., 2015a; Adiletta et al., 2017). 'Acco' and 'Wonderful' pomegranate arils packed with perforated film showed weight losses of 6.2% and 5.2% after 15 d at 5 °C, and 14 d at 4 °C, respectively (Sepulveda et al., 2000; Hussein et al., 2015a). In our previous study, using the same SP and MPP films, the greatest weight loss was observed for arils packed under MPP at the end of the storage period with losses up to 4.1% and 2.5% for 'Kingdom' and 'MR-100' pomegranate cultivars, respectively (Adiletta et al., 2017). This suggests that weight loss, in ready-to-eat arils, is significantly influenced by cultivar-dependent manner. Differences between our study and the literature reported data could be attributed to cultivar variations and storage condition.

The packaging conditions significantly influenced pH values during cold storage in different manner ($P < 0.05$), with TA values showing no significant changes until day 12, followed by an increase at day 16. Initial TSS displayed a good maturity index as recommend by Comercial Gallo for 'Purple Queen' pomegranate arils. At harvest, TSS value was 20.46 ± 0.11% and this trait did not show significant differences during cold storage for both systems.

In literature, different trends about the changes of TSS and TA were described in ready-to-eat arils. Hussein et al. (2015a) reported a decrease in TSS and TA of arils 'Acco' packaged with perforated film after 15 d of storage at 5 °C. In contrast, Banda et al. (2015) found a slight lowering in TSS without significant changes in TA in fresh processed 'Wonderful' pomegranate arils after 12 d of storage in passive-MAP. Ayhan and Eştürk (2009) demonstrated that passive-MAP had no significant effects on TSS until the storage time of 9 d in ready-to-eat arils 'Hicaznar' ($P > 0.05$), but the metabolic activities in pomegranate arils occur during storage, affecting pH and TA. O' Grady et al. (2014b) reported that TSS was quite stable after 14 d of storage at 1 or 4 °C for packaged ready-to-eat arils.

An increase in TA content was observed in 'MR-100' SP and MPP packaged arils after 11 d at 5 °C due to fermentation, which was confirmed by total aerobic bacteria, yeasts, and moulds growth (Adiletta et al., 2017). Similarly, an increase in TA was observed in the minimally processed arils cultivar 'Primosole', packed in perforated polypropylene film, stored at 5 °C for 10 d (Palma et al., 2009). Our findings are in agreement with Banda et al. (2015) that showed an increase of pH levels up to 9 d of cold storage and a decrease at day 12.

3.2. Bioactive compounds and antioxidant activity of arils during cold storage

Pomegranate is considered a 'super fruit' due its high nutraceutical value conferred by polyphenols, anthocyanins, flavonoids, and antioxidant capacity. Ready-to-eat arils are a more appealing product to consumers than whole fruit, and the selection of a proper packaging system is vital for the preservation of nutritional properties (Caleb et al., 2013; Dhinesh and Ramasamy, 2016).

Bioactive compounds and antioxidant activity in arils in SP and MPP packaging systems showed a decrease during storage (Table 2). At harvest, arils showed a high TP content of 1770.55 ± 45.88 mg L^{-1} GAE that significantly decreased during 4 d of storage in SP and MPP films. After this period no significant variation of TP values was observed in SP film, whereas a significant decrease was registered after 8 d in MPP film. TAC values ranged from 396.28 to 137.83 mg L^{-1}, and from 396 to 237.52 mg L^{-1} in MPP and SP systems, respectively. At the end of storage, TAC in arils showed a 65% reduction compared to the initial anthocyanins content in MPP film, whereas SP film had a significant decrease in TAC only during 4–8 d of storage.

TP content has been influenced by storage time in ready-to-eat arils (Palma et al., 2015), by the packaging system (Kulkarni and Aradhya, 2005; Palma et al., 2009) and by pomegranate cultivar (Adiletta et al., 2018; Pareek et al., 2015). In particular, 'Purple Queen' arils showed higher TAC values compared to 'Acco' (210 mg L^{-1}) and 'Herskawitz' (200 mg L^{-1}) arils at the beginning of storage (Caleb et al., 2013; Banda et al., 2015). Packaging and storage time influenced TAC content, a finding that was in agreement with Ayhan and Eştürk (2009) who reported a decrease from 311 to 279 mg L^{-1} C3gE in TAC of pomegranate arils cultivar 'Hicaznar' in passive-MAP. A significant decrease was also observed in 'Herskawitz' (0.20–0.12 g L^{-1}) packaged arils under passive-MAP at different temperatures (5, 10 and 15 °C) for 14 d (Banda et al., 2015). The stability of anthocyanins is influenced by several factors and their degradation occurs during processing and storage (Maghoumi et al., 2013; D'Abrosca et al., 2017).

Antioxidant activity showed a significant decrease (EC_{50} increase) during cold storage, whereas a different trend was found among packaging conditions. EC_{50} value was higher in MPP film compared to SP film at the end of storage (Table 2), which was also confirmed by a decrease in antioxidant compounds. Antioxidant activity is influenced by packaging system, and the SP system maintained a high bioactive compounds content in the arils during storage that correlated with lower EC_{50} ($r = -0.94$ for anthocyanin and -0.61 for phenol). These results agree with our previous study (Adiletta et al., 2017), where the antioxidant capacity in 'Kingdom' and 'MR-100' pomegranate arils packaged in SP system showed higher values compared to MPP system. An appropriate packaging system maintains a high nutraceutical value in ready-to-eat arils, although bioactive compounds depend on different pomegranate cultivars (Adiletta et al., 2018; Pareek et al., 2015).

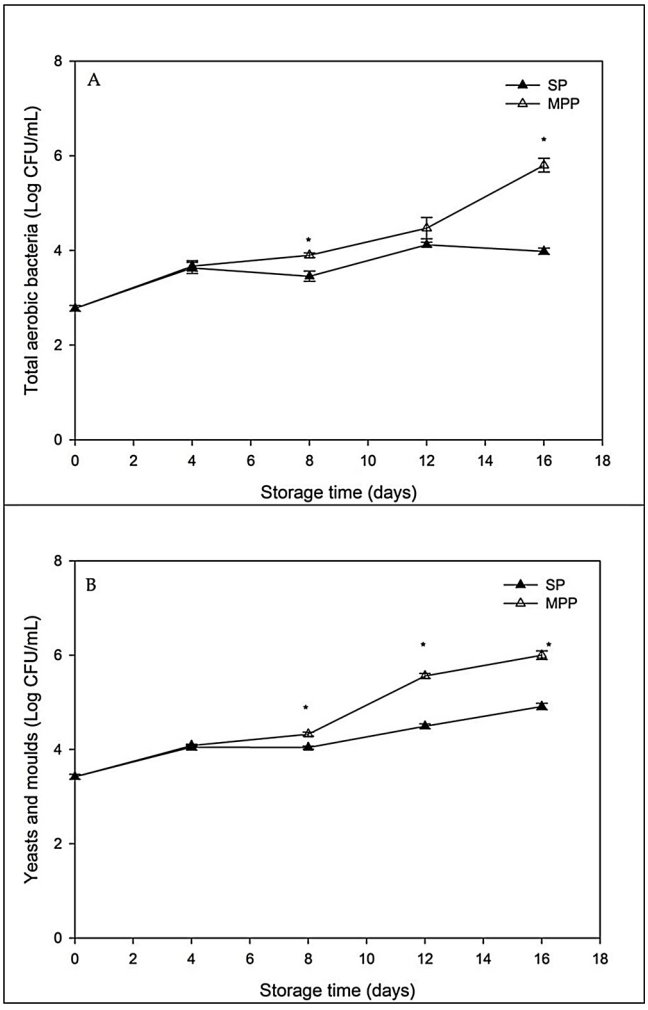

Fig. 2. Total aerobic bacteria (A), and yeasts and moulds (B) changes in pomegranate arils cultivar 'Purple Queen' packaged in semipermeable (SP) and microperforated (MPP) films, stored at 5 °C for 16 d. Error bars indicate the standard deviation and * indicates statistical differences between samples for the same storage time.

3.3. Microbial growth in the arils during cold storage

Microbial growth is the main cause of quality deterioration of ready-to-eat arils. This deterioration is especially facilitated by favourable conditions, such as a high-water vapour content inside the package during storage (Dhinesh and Ramasamy, 2016). Furthermore, a high water vapour condensation on the surface of packaging is responsible for off-flavours and promotes optimum conditions for microorganisms (Song et al., 2002).

Several studies on packaged arils have tested different polymeric films that created unfavourable conditions for microbial growth (Sepulveda et al., 2000; Banda et al., 2015; Belay et al., 2018). The tested films significantly influenced microbial growth on minimally processed arils ($P < 0.05$). Total aerobic bacteria during storage increased by 2.78–5.80 log CFU mL^{-1} and 2.78–3.98 log CFU mL^{-1} in MPP and SP film, respectively (Fig. 2A). Yeasts and moulds counts showed an increase during storage with smaller values in SP film (3.42–4.91 log CFU mL^{-1}) compared to MPP film (3.42–6.00 log CFU mL^{-1}) (Fig. 2B). The values of aerobic mesophilic bacteria, and yeasts and moulds counts in arils packaged in MPP film were similar to those reported by Banda et al. (2015) for ready-to eat arils packed in passive-MAP stored at 5 °C for 12 d. Different microbial counts observed for ready-to-eat arils in SP and MPP film during cold storage, may be attributed to different changes in chemical traits such as TA and pH, that are important features that can influence microbial growth (Soliva-Fortuny and Martín-Belloso, 2003). Aerobic mesophilic bacteria and yeasts and moulds counts increased up to the 16th d, reaching counts lower than 6.0 log10 CFU mL^{-1} which is considered the upper acceptable limit as suggested by other studies (Patrignani et al., 2009; Varela-Santos et al., 2012).

3.4. Antioxidant enzymes of arils during cold storage

Packaging conditions influenced the oxidative antioxidant system in pomegranate arils during cold storage in several fruit as demonstrated by other authors (Chen et al., 2005; Yang et al., 2009). An altered balance in antioxidant enzymatic activities, such as SOD, CAT, and APX, caused an accumulation of ROS that reduced the storage quality of fruit (Hodges et al., 2004). SOD is the first ROS scavenging enzyme involved in the dismutation of radical superoxide to H_2O_2 and O_2, while CAT and APX are involved in the detoxification of H_2O_2 however, this uses ascorbate as an electron donor (Maghoumi et al., 2013).

An increase in antioxidant enzymes activity was detected during storage of arils in different packaging conditions. CAT activity showed a significantly increase by 40% and 50% after 12 and 16 d of storage in SP film. In contrast, at the end of storage there was an increase in MPP film of CAT activity that reached 25% of the initial value. APX activity values were higher in arils in SP film compared to MPP film at the end of storage, with values of 42.40 ± 1.72 mmol s^{-1} kg^{-1} and 19.91 ± 0.88 mmol s^{-1} kg^{-1}, respectively (Table 3). Significant increases in SOD activity ($P < 0.05$) were registered during storage time with higher values in SP film compared to MPP film. GPX activity revealed the same trend in arils in both packaging conditions, with higher values for the MPP film. After 12 d of storage, no significant differences in GPX activity in arils packaged for each tested film were observed.

Table 3
Effects of different packaging systems (SP: semipermeable film and MPP: microperforated film) on the antioxidant system in regards to catalase (CAT; mmol s^{-1} kg^{-1}), ascorbate peroxidase (APX; mmol s^{-1} kg^{-1}), guaiacol peroxidase (GPX; nmol s^{-1} kg^{-1}), superoxide dismutase (SOD; U 10^3 s^{-1} kg^{-1}), and polyphenol oxidase (PPO; mmol s^{-1} kg^{-1}) of 'Purple Queen' pomegranate arils during 16 d of cold storage (5 °C). Means followed by the same letter do not differ significantly at P = 0.05 (Duncan Test).

Packaging System	Days	CAT	APX	GPX	SOD	PPO
SP	0	1.41 ± 0.11a	15.50 ± 0.29a	11.33 ± 0.74a	13.64 ± 0.55a	52.71 ± 0.93a
	4	1.43 ± 0.06a	26.43 ± 0.62d	13.70 ± 1.76ab	38.58 ± 1.71d	68.02 ± 0.77bc
	8	1.59 ± 0.03b	30.38 ± 1.09e	15.35 ± 0.57ab	52.35 ± 2.33e	75.19 ± 0.14e
	12	1.93 ± 0.11d	34.29 ± 0.22f	17.55 ± 1.09b	68.21 ± 2.76f	80.38 ± 2.59 cd
	16	1.99 ± 0.10d	42.40 ± 1.72 g	17.27 ± 2.42b	84.54 ± 5.33 g	107.47 ± 10.04e
MPP	0	1.41 ± 0.11a	15.50 ± 0.29a	11.33 ± 0.74a	13.64 ± 0.55a	52.71 ± 0.93a
	4	1.37 ± 0.05a	15.94 ± 0.57a	24.12 ± 0.50c	22.14 ± 0.91b	78.32 ± 0.87 cd
	8	1.62 ± 0.06bc	17.87 ± 0.93b	31.83 ± 1.55d	32.70 ± 0.96c	86.81 ± 1.57d
	12	1.51 ± 0.05ab	21.49 ± 0.93d	39.48 ± 1.98e	35.67 ± 3.57 cd	107.47 ± 3.84e
	16	1.76 ± 0.12c	19.91 ± 0.88c	42.19 ± 5.91e	51.85 ± 4.49e	137.45 ± 12.67f

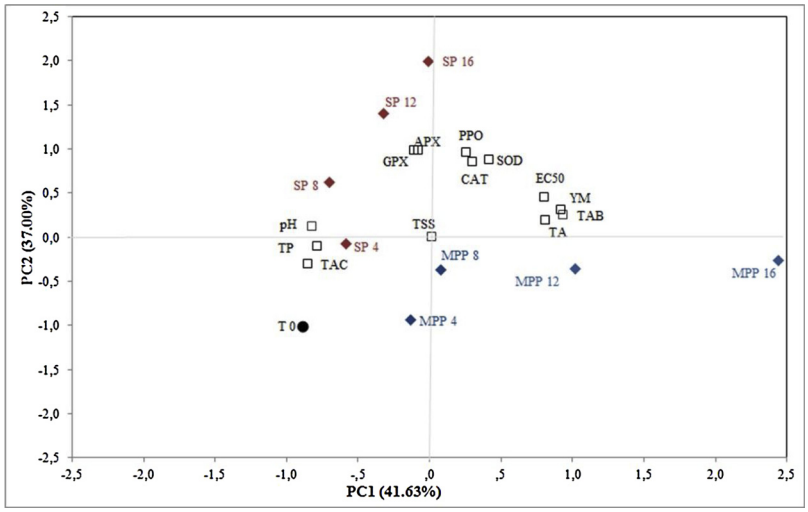

Fig. 3. Principal component analysis of the physicochemical, nutraceutical and enzymatic antioxidant system in pomegranate arils cultivar 'Purple Queen' packaged in semipermeable (SP) and microperforated (MPP) films, at harvest (0), and after 4 d (4), 8 d (8), 12 d (12) and 16 d (16) of storage at 5 °C. (TSS: total soluble solid content; TA: total titratable acidity; TAC: total anthocyanins; TP: total polyphenol content; EC_{50}: antioxidant activity; TAB: total aerobic bacteria; YM: yeasts and moulds; APX: ascorbate peroxidase; CAT: catalase; GPX: guaiacol peroxidase; PPO: polyphenol oxidase; SOD: superoxide dismutase).

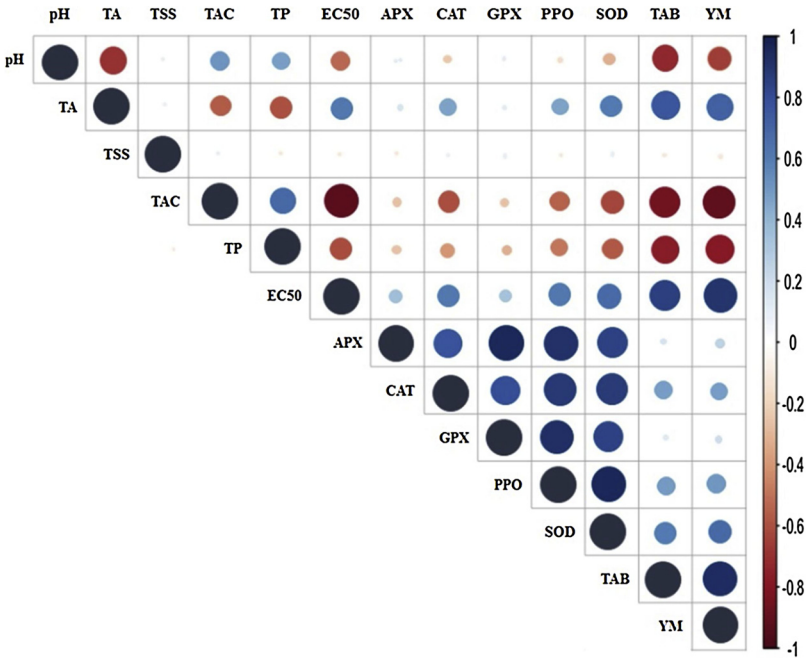

Fig. 4. Pearson's correlation matrix between pH, TSS (total soluble solid content), TA (total titratable acidity), TAC (total anthocyanins), TP (total polyphenol content), EC_{50} (antioxidant activity), TAB (total aerobic bacteria), YM (yeasts and moulds), APX (ascorbate peroxidase), CAT (catalase), GPX (guaiacol peroxidase), PPO (polyphenol oxidase), and SOD (superoxide dismutase).
The correlation coefficients are proportional to the circle size and colour intensity. Positive correlations are displayed in blue and negative correlations in red (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

PPO activity at the end of storage showed higher values in arils in MPP film, reaching an increase of 62% over SP film (Table 3).

The SP packaging system improved the SOD, CAT, and APX activities facilitating ROS scavenging in pomegranate arils during 16 d of cold storage. To the best of our knowledge, no previous studies have been undertaken to evaluate the antioxidant system in pomegranate arils packaged in passive-MAP. Other studies reported that pomegranate arils treated with hot salicylic acid delayed the decrease in CAT, APX, SOD, and GR activity during storage at 4 °C for 12 d (Dokhanieh et al., 2016).

PPO and GPX play an important role in fruit browning during storage due to the oxidation of phenolic compounds involved with fruit senescence processes (Tomás-Barberán and Espín, 2001). The MPP packaging system induced high PPO and GPX activities with the oxidation of phenolic compounds and anthocyanin degradation, causing browning and qualitative decay of fresh-cut arils. Our findings agree with the results of Maghoumi et al. (2013), who found that heat treatment, UV-C, and super-atmospheric oxygen packaging, delayed the activity of PPO and GPX, and maintained the concentration of antioxidant compounds within fresh-cut pomegranate arils.

3.5. Principal components analysis of arils during cold storage

PCA was applied to evaluate the changes of physico-chemical and nutraceutical traits, and antioxidant enzymatic system in ready-to-eat arils during cold storage (Fig. 3). Several studies have used this multivariate data analysis to highlight similarities and differences between variables and reduce large data matrices in samples tested during storage (Cano-Salazar et al., 2013; Pasquariello et al., 2015; Petriccione et al., 2015; Adiletta et al., 2018; Modesti et al., 2018; Petriccione et al., 2018).

2D-PCA plot showed that the first two principal components explain 78.63% of the total variance in the dataset, with 41.63% and 37.00% for the PC1 and PC2, respectively (Fig. 3). All loadings and scores are shown in the same PCA plot (Fig. 3). Physico-chemical traits such as TA, TSS, and EC_{50} were positively correlated with PC1 along with microbial parameters such as TAB and YM, whereas pH, TP, and TAC were negatively correlated with the same PC. All antioxidant enzymes tested in this study were positively correlated with PC2.

As shown in the PCA plot, the MPP system shifted scores from negative (T0) to positive (MPP16) values along PC1. The SP system moved

the score values in a different manner along PC2. The Pearson's correlation analysis showed positive and negative correlations among several traits (Fig. 4). TA was negatively correlated with pH ($r = -0.757$; $P \leq 0.01$), along with EC_{50} with TP and TAC ($r = -0.614$; $P \leq 0.01$; $r = -0.938$; $P \leq 0.01$, respectively). TAB and YM were positively correlated ($r = 0.946$; $P \leq 0.01$). APX activity was positively correlated with CAT and SOD activity ($r = 0.777$; $P \leq 0.01$; $r = 0.852$; $P \leq 0.01$, respectively). GPX and PPO activity were positively correlated ($r = 0.925$; $P \leq 0.01$) along with APX, CAT and SOD activity.

4. Conclusions

Ready-to-eat pomegranate arils 'Purple Queen' show changes in physico-chemical traits and bioactive compounds during cold storage at 5 °C for 16 d. SP packaging system is a valid tool to preserve qualitative and nutraceutical traits in ready-to-eat pomegranate arils compared to MPP system. Furthermore, SP packaging system reduces oxidative stress, which increases antioxidant enzyme activities such SOD, CAT, and APX. These enzyme activities are involved in the first line-of-defence against reactive oxygen species, detoxification, and the inhibition of PPO and GPX activity that are involved in aril-browning processes. SP packaging prevents microbial growth respect to MPP system and it allows the storage of packaged arils up to 16 d without exceeding the maximum-acceptable microbial limit. This packaging system for ready-to-eat arils could be used in food industry applications as a convenient alternative to fresh fruit consumption and may increase consumer demand for pomegranates.

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