Antioxidant response and quality of sunburnt Beurré D'Anjou pears (*Pyrus communis* L.)

Nazarena Spera, Laura Inés Vita, Pedro Marcos Civello, Graciela María Colavita

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CONTRIBUTION

Authors: Nazarena Spera, Laura I. Vita, Pedro M. Civello, Graciela M. Colavita.

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Authors: Nazarena Spera[a,b*], Laura Inés Vita[a,b], Pedro Marcos Civello[c,d], Graciela María Colavita[a,b]

[a] CITAAC (CONICET-UNCo), Centro de Investigaciones en Toxicología ambiental y Agrobiotecnología del Comahue. Buenos Aires 1400, Neuquén (CP8300), Argentina.
[b] Facultad de Ciencias Agrarias. Universidad Nacional del Comahue. Km 12,5 Ruta 151, Cinco Saltos (CP8303), Argentina.
[c] INFIVE (CONICET-UNLP), Instituto de Fisiología Vegetal. Diagonal 113 Nº 495, La Plata (CPB1900), Argentina.
[d] Facultad de Ciencias Exactas. Universidad Nacional de La Plata. Calle 115 s/n, La Plata (CPB1900), Argentina.
*Corresponding author: nspera@gmail.com

HIGHLIGHTS

- Mild sun damage increased the amount of photoprotective pigments in Beurré D'Anjou pears, while moderate sun damage enhanced their degradation.
- Some quality parameters in sunburn pear tissues correlated with advanced ripening stage, but the flesh firmness remained higher.
- Sun exposure increased antioxidant response in pear fruit, though it was insufficient to avoid cell membrane oxidative damage.
- Pear fruits with mild sun damage, associated with a poor commercial quality, had indeed higher polyphenols levels that are beneficial for human health.

ABSTRACT

Sunburn is a physiological fruit disorder induced by exposure to excessive solar radiation. This disorder leads to significant losses in the yield of marketable fruits by negatively affecting quality parameters such as maturity and external color of the fruits. The purpose of this work was to characterize the physiological and biochemical aspects related to oxidative metabolism in Beurré D´Anjou pear fruit with different sunburn levels. Fruits were collected and classified into three sunburn levels at harvest: no sunburn (S0), mild sunburn (S1), and moderate sunburn (S2). On sunburned area, the maturity indices were measured on the fruit flesh, while external color, photosynthetic and photoprotective pigments, total phenols, electrolyte leakage, lipid peroxidation, antioxidant capacity and antioxidant enzymatic activities were

determined on fruit peel. The hue angle and saturation of peel color of pears with different sunburn levels showed significant reduction with increasing damage. These changes in peel color were associated with a reduction in chlorophyll content and variations in carotenoid and anthocyanin levels. Due to metabolic changes resulting from defense and adaptive responses to high solar radiation, sunburned tissues showed significantly increased firmness, soluble solids content, and starch degradation, and lower acidity compared to undamaged fruits. We observed also increased antioxidant capacity in the peel of S1 and S2 fruit, related to higher phenolic contents and increased SOD and APX activities. Consistent with previous reports in apple, our study demonstrates that sunburn affects pear fruit quality traits and maturity state by enhancing oxidative metabolism.

Keywords: antioxidants, fruit, maturity index, oxidative stress, pear, skin, sun damage.

1. Introduction

Abiotic stress resulting from high radiation and temperature affects plant functioning by inducing oxidative damage (Chen et al., 2008). In response, various defense mechanisms are activated to elicit physiological, biochemical, and morphological adaptive changes (Chelli-Chaabouni, 2014). When stress conditions overcome the plant's defense mechanisms, visual symptoms of sun damage (sunburn) develop in fruits, affecting their commercial quality (Colavita, 2022). Pear fruits showing this symptomatology are discarded, causing important economic losses that range from 24% to 33%, respectively, in the varieties 'Beurre D'Anjou' and 'Packham's Triumph' (Colavita et al., 2011).

Detailed studies have described the environmental causes of sunburn in apple fruits and the physiological alterations underlying this disorder (Morales-Quintana et al., 2020; Munné-Bosch and Vincent, 2019; Racskó and Schrader, 2012). Three types of sunburn, namely photo-oxidative, necrotic, and browning sunburn, were characterized in apple fruit. Photo-oxidative sunburn is usually caused by sudden exposure to solar radiation and evidenced by development of white spots on the surface of the fruit that ultimately lead to necrosis (Felicetti and Schrader, 2008). Necrotic sunburn is the most easily identifiable type of sun damage, consisting of black or brown spots on the surface exposed to solar radiation. It occurs when the

surface temperature of the fruit reaches 52 ± 1°C for at least 10 min and can develop through sun exposure or experimentally in the darkness upon exposure to high temperatures (Racskó and Schrader, 2012). The third type of sunburn, commonly known as browning, is the most common one and occurs in fruit exposed to solar radiation when the surface temperature of the fruit reaches 40 to 50 °C for approximately 60 min (Felicetti and Schrader, 2009; Racskó and Schrader, 2012). This type of sunburn is characterized by the development of yellow, brown, or bronze colored spots on the parts of the fruit exposed to solar radiation. Recent work on apple has focused on understanding the effects of sunburn in relation to changes in oxidative metabolism and the antioxidant response (Colavita, 2022; Vita, 2018), morphological, biochemical, and genetic modifications in the cell wall (Torres et al., 2020), and alterations in water dynamics and osmoregulation in affected tissues (Torres et al., 2013). However, information is scarce on the molecular bases of the damage produced by different sunburn intensity levels in pear fruit. In red pears, it was determined that the development of browning-type sunburn starts with temperatures above 47 °C, and it may vary according to the environmental conditions of each season and the concentration of anthocyanins in the fruit peel (McClymont et al., 2016). In this regard, work on red pear clones of the D´Anjou variety showed that anthocyanins increase fruit tolerance to high radiation and temperature (Li and Cheng, 2009). Recent studies characterized and described sunburn and sunscald in pear var. Packham's Triumph in physiological, biochemical, and metabolic levels with the aim of developing tools to predict and distinguish these disorders (Torres and Mogollon, 2022; Yoo et al., 2023). Other authors described in turn the effects of different sunlight conditions on physiological and biochemical changes in the metabolism of pear fruits that did not develop sunburn (Rudell et al., 2017; Serra et al., 2018; Zhao et al., 2016).

Argentina is currently the main producer and exporter of fresh pears in the southern hemisphere, with recognized notoriety in terms of quality and production in counter season with respect to northern countries. However, the climatic characteristics of Argentina’s main production area, the Alto Valle de Río Negro and Neuquén, favor the development of sunburn in the fruit. The main pear varieties produced in this region are Bartlett, Packham's Triumph, and Beurré D'Anjou. The latter is exclusively destined for

commercial export and is also the most susceptible to sunburn (Benítez et al., 2005). In this crop, sunburn causes losses of 20%-30% of total fruit yield and thus represents one of the main determinants of exclusion in export markets. Of note, this problem appears to be progressively aggravated by climate change (Morales-Quintana et al., 2020; Parajuli et al., 2019). As this context poses important challenges for pear production, in-depth studies of the metabolic changes associated with sunburn are warranted to develop technological strategies to mitigate its effects. Hence, the goal of this work was to characterize physiological and biochemical aspects related to oxidative metabolism in Beurré D'Anjou pear fruit with different levels of sunburn.

2. Materials and methods

2.1. Plant materials

The Beurré D'Anjou pear fruit used for this work were collected in an orchard located in the Rio Negro Upper Valley, Northern Patagonia, Argentina, during the commercial harvest on the 2018/2019 growing season. Fruits of medium weight (145 $\pm$ 30 g) were harvested and classified into sunburn damage categories (Spera et al., 2023): S0 (healthy, green peel with no sunburn damage), S1 (mild sunburn browning: discolored light yellow spots on the sun-exposed area of the peel), and S2 (moderate sunburn browning: discolored dark yellow browning on the sun-exposed area of the peel) (Fig. 1). Twenty fruits from each damage category were pooled to constitute a composite sample (biological replicate). Fives replicate of each category were used in the study. Before assays, the fruits were cleaned and disinfected with 5% neutral detergent and 70% v/v ethyl alcohol and rinsed with distilled water. All determinations were done within 12 h of harvest.

Fig. 1. Sunburn classification in Beurré D´Anjou pears. S0: no sunburn; S1: mild sunburn; S2: moderate sunburn.

2.2. Peel color and maturity indices

For the determination of peel color and maturity indices, were analyzed and quantified over S0, S1 and S2 tissues. Each biological replicate corresponds to a replicate composed of 20 fruits.

2.2.1. Peel color

Peel color was analyzed averaging 3 measurements from healthy (S0) and sunburn-affected (S1 and S2) areas of each fruit. A CR-300 colorimeter (Minolta, Japan) was used to measure the CIELAB coordinates (L*, lightness; a*, and b*), from which matrix or hue angle, saturation (CHROMA), and lightness (L) were calculated (Mesa Juliani, 2015).

2.2.2. Maturity indices

Flesh firmness was determined using an Effegi penetrometer (FT 327, Alfonsine, Italy) equipped with an 8 mm tip. The epidermis was removed and a measurement was taken on each fruit (Mitcham et al., 1996). The results are expressed in N. Fruits were cut along the equatorial axis and treated with a solution of Lugol (0.33 % w/w I_2 + 0.66 % w/w KI) for 1 min. Staining results were compared against the INTA's starch degradation test chart, where 0 % indicates maximum starch content and 100 % indicates absence of starch (Candan and Calvo, 2015).

For determinations of soluble solids and titratable acidity, a 5-mm-thick piece of pulp immediately under the sun-damaged area was extracted from each fruit, excluding the peel. Juice extraction from flesh was carried out using a PE-EJ306 juicer (Peabody, China). The concentration of soluble solids was measured using an Atago 0-32% self-compensating digital refractometer (Tokyo, Japan), and results are expressed in degrees Brix (°Brix). To determine titratable acidity, the juice was diluted 1/10 with distilled water and titrated to pH 8.20 with NaOH 0.1 N, measured with a digital pH meter (Arcano PHS-3E). The results are expressed as % malic acid equivalent (% mal. acid eq.) (Mitcham et. al. 1996).

2.3. Biochemical analyses

All determinations were performed on 0.5-mm-thick peel discs extracted from S0, S1 and S2 areas of the fruits. Replicates were homogenized in liquid nitrogen and kept at -80 °C until analysis. Fresh material was used for the determination of electrolyte leakage.

2.3.1. Electrolyte leakage

Twenty peel discs per replicate were immersed into 20 ml of distilled water in a Falcon tube and shaken at 130 rpm at 20°C for 24 h. Initial electrical conductivity (ECi) was measured using a manual conductometer (LF 92 OHAUS, model ST10-C-B; USA). Then, the replicates were boiled for 1 h in a water bath and frozen at -20°C for 24 h. The replicates were then thawed at 20°C using a shaker at 130 rpm (Campos et al., 2003), after which final electrical conductivity (ECf) was measured. Electrolyte leakage is expressed as (ECi/ECf) × 100.

2.3.2. Lipidic peroxidation analysis

Malondialdehyde (MDA) content in pear peel was determined by measuring thiobarbituric acid reactive substances (TBARs) through the 2-thiobarbituric acid (TBA) reaction (Hodges et al., 1999). Peel tissue (0.25 g fresh weight; FW) was ground with 2 ml trichloroacetic acid (TCA) 0.1 % w/v and centrifuged at 10,000g for 5 min. An aliquot (0.5 ml) of the supernatant was added to a test tube with 2 ml of either (i) -

TBA solution (20.0 % w/v TCA), or (ii) +TBA solution (20.0 % w/v TCA plus 0.1 % w/v TBA). All replicates were heated at 95°C for 30 min and then quickly cooled in an ice bath and centrifuging at 10,000g for 10 min. The absorbance was read at 440 nm, 532 nm, and 600 nm. Data are expressed as nmol MDA g^{-1} FW. MDA equivalents were calculated with the following equation:

$$MDA\ (nmol\ ml^{-1}) = \left[\frac{(A_{+TBA532} - A_{+TBA600}) - (A_{-TBA532} - A_{-TBA600}) - (A_{+TBA400} - A_{+TBA600})\ x\ e}{C}\right] x\ 10^6$$

C: 157,000 nM^{-1} cm^{-1} (molar extinction coefficient).

e: 0.057142857 (ratio of molar absorbance of sucrose, 532 nm and 440 nm).

2.3.3. Methanolic extract profiling

Fruit peel replicates (0.35 g) were homogenized with 2 ml of methanol/HCl 32% v/v-bidistilled water (159/1/40, v/v/v), shaken at 150 rpm for 2 h at room temperature, and centrifuged at 15,000g for 20 min. The supernatant was transferred and stored at -20°C until use. The extract was used for determinations of antioxidant capacity, total polyphenols, and total monomeric anthocyanins.

2.3.3.1. Free radical scavenging (DPPH) assay

Antioxidant capacity (free radical scavenging activity) was determined using the 2,2,-diphenyl-2-picryl-hydrazyl (DPPH) method as described by Vita et. at. (2019). Methanolic extracts were diluted 4-fold with bidistilled water. An aliquot of 20 µL of this dilution was mixed to 780 µL of methanolic DPPH. For blank sample, we used 20 µL of bi-distilled water in replacement of the sample extract. All samples were incubated for 30 min in the dark at 4ºC, and the absorbance was read at 515 nm. The antioxidant capacity was expressed as percentage inhibition of DPPH, were calculated according to the formula:

$$DPPH\ percentage\ inhibition = [(A_A - A_B)/A_A]\ x\ 100$$

A_A: absorbance value of the blank sample

A_B: absorbance value of the sample

2.3.3.2. Total polyphenols

Total polyphenol content was determined according to the protocol described by Emmons et al. (1999), with modifications. Methanolic extracts were diluted 3-fold with bi-distilled water, and the reaction was performed by mixing 10 µl of diluted sample with 780 µl of bi-distilled water and 50 µl of Folin-Ciocalteu reagent (Anedra) and resting 8 min in the dark. Then 150 µl of sodium carbonate 20% w/v (Anedra) were added and it was homogenized and incubated for 2 h at room temperature in the dark. The absorbance was measured at 760 nm. A calibration curve was performed with a standard solution of gallic acid 5 g l^{-1} (Biopack). Each determination was carried out in duplicate, and total polyphenol content was expressed as mg gallic acid per 100 g FW.

2.3.3.3. Total monomeric anthocyanins

Anthocyanin contents were determined by the differential pH method (Gorriti et al., 2009). Two reactions were performed in parallel varying the reaction medium (0.025 M KCl solution pH 1.0 and 0.4 M sodium acetate buffer (pH 4.5). The acid buffer/methanolic extract ratio was 1:3. The reaction was allowed to stand for 15 min and the solution was then centrifuged at 15,000g for 15 min at 15°C. The absorbance was measured at 510 nm and 700 nm. Each determination was performed in duplicate, and total monomeric anthocyanin content is expressed as mg of cyanidin-3-glucoside (cyn-3-glu) in 100 g FW. The extinction coefficient used was 26,900 M^{-1} cm^{-1}.

2.3.4. Quantification of photosynthetic pigments

Chlorophylls a, b and carotenoid contents in fruit peel were measured following the protocol described by Lichtenthaler and Buschmann (2001), with modifications. Fresh peel tissue (0.1 g FW) was placed into 2 ml of 95% v/v ethanol. Samples were heated in a thermostatic bath for 2 h at 65°C, cooled on an ice bed for 15 min, and centrifuged at 500g for 10 min at 5 °C. During the whole procedure, the samples were kept in the dark to avoid pigment degradation. Absorbance was measured at 470, 648.6, and 664.1 nm in a UV-Vis DU 800 spectrophotometer (Beckman Coulter, Germany) and results are expressed as mg of each pigment per g FW.

2.3.5. Antioxidant enzyme analyses

Antioxidant enzyme activity was evaluated on non-denatured protein extracts from fruit peel replicates. The activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) were measured along with total protein content. Each determination was performed in triplicate.

2.3.5.1. Enzyme extraction

SOD and CAT extracts were prepared by homogenizing frozen peel (0.3 g FW) with 3 mL extraction buffer consisting of 0.1 mM potassium phosphate buffer (pH 7.5), 0.01 M EDTA, and 0.05% w/v polyvinylpolypyrrolidone (PVPP). APX extracts were obtained using a buffer consisting of 0.1 mM potassium phosphate buffer (pH 7.5), 0.01 M EDTA, 0.04% w/v PVPP, and freshly-prepared 0.0175 % w/v ascorbate. Extracts were then centrifuged at 20,000g for 20 min at 4°C and the supernatants stored at -80 °C until use. All enzyme activities were assessed spectrophotometrically at 25 °C on a UV-Vis DU 800 instrument.

2.3.5.2. SOD activity

SOD activity was determined based on the inhibition of NADH oxidation by superoxide radicals in the presence of the extract, according to the technique described by Paoletti et al. (1986) with modifications. The reaction medium consisted of 800 µl of 100 mM triethanolamine-diethanolamine (TEA-DEA) buffer (pH 7.4), 100 µl of 100 mM EDTA solution plus 50 mM $MnCl_2$ (pH 7.0), and 40 µl of 7.5 mM NADH. After addition of different volumes (5 to 100 µl) of enzyme extract and 100 µl of 10 mM 2-mercaptoethanol, the preparation was mixed and allowed to react 10 min at 20 °C. The decrease in absorbance was measured at 340 nm for 5 min. One unit of SOD was defined as the amount of enzyme that halves the rate of NADH oxidation, and results are expressed as SOD units (USOD) per mg FW.

2.3.5.3. CAT activity

CAT activity was determined by measuring the decomposition of hydrogen peroxide (H_2O_2) in the reaction medium at 240 nm for 240 s (Ma and Cheng, 2003). The reaction medium consisted of 10 µl H_2O_2 30 % v/v, 2.94 ml potassium phosphate buffer 100 mM, pH 7, and 50 µl of enzyme extract. Results are expressed as µmol H_2O_2 consumed per min per g FW.

2.3.5.4. APX activity

APX activity was determined in reaction medium containing 2 ml buffer of 50 mM potassium phosphate, pH 7, 0.1 mM sodium EDTA, to which 150 µl of 10 mM ascorbic acid and 100 µl of extract was added. The reaction was initiated by adding 15 µl H_2O_2 30 % v/v. APX activity was determined by recording H_2O_2-dependent ascorbate decomposition at 290 nm for 60 s (Mishra et al., 1993), and results are expressed as µmol of ascorbic acid consumed per min per g FW. The extinction coefficient used for these calculations was 2.8 mM^{-1} cm^{-1}.

2.3.5.5. Protein content quantification

Total protein content was measured on enzymatic extracts by the Bradford (1976) technique with modifications. Dilutions (1/8) of the extracts were made and absorbance was measured at 595 nm. A calibration curve was constructed by serial dilution of a bovine serum albumin solution (0.5 mg ml^{-1}). Total protein content is expressed as mg protein per g FW.

2.4. Statistical analysis

All statistical analyses were performed using Infostat software version 2018 (Di Rienzo et al., 2018). Statistical evaluation of the data was performed by analysis of variance, with multiple comparisons between means assessed by Tukey's test. Differences in means were considered significant at the $p<0.05$ level. The analyses were performed with n= 5, for replicates composed of 20 fruits each.

3. Results

255 *3.1. Fruit color and maturity indices*

256 Sunburn affected peel color in pear fruit (Table 1). By comparison, the peel of sunburned fruit showed a
257 significant reduction in the hue angle, ranging from ~14 % in S1 to ~26 % in S2. Color saturation was also
258 reduced with increasing sunburn, from ~10 % in S1 to ~14 % in S2, compared to S0. These alterations are
259 evidenced as semi-saturated yellowish-green tones in S1 and yellow coloration in S2. No significant
260 difference in color lightness (p= 0.5499) between different sunburn levels were found.

261

Table 1. Color parameters in Beurré D´Anjou pear peel with different sunburn levels at harvest

Sunburn level	Hue	Chroma	Lightness
S0	123.9 ± 0.7 a	46.9 ± 0.6 a	73.2 ± 1.2 a
S1	106.8 ± 2.9 b	42.3 ± 0.7 b	74.2 ± 0.9 a
S2	95.5 ± 4.1 c	40.4 ± 2.0 b	73.9 ± 1.8 a

Values are mean ± standard deviation (n=5). Different letters within each parameter (column) indicate significant differences (Tukey; p ≤ 0.05).

262

263 The maturity indices evaluated were also affected by sunburn (Table 2), with variance analysis showing
264 significant differences in flesh firmness, soluble solids content, titratable acidity, and starch degradation
265 ($p<0.0001$ for all).

266 Pulp firmness increased with sunburn intensity, from 7.7 % in S1 to 13.7 % in S2 with respect to S0.
267 Similarly, we detected a gradual increase in soluble solids contents in sunburned peel, associated with
268 greater starch degradation in S1 and S2 relative to S0. The titratable acidity was 0.37 % malic acid eq. in
269 S0, and this value was significantly reduced (by ~38 % in S1 and by ~57 % in S2) in sunburned tissues.

270

Table 2. Maturity indices in Beurré D´Anjou pear with different levels of sunburn at harvest

Sunburn level	Firmness (N)	Soluble solids (°Brix)	Titratable acidity (%)	Starch degradation (%)
S0	61.7 ± 2.0 b	12.0 ± 0.3 c	0.37 ± 0.01 a	26.0 ± 9.0 c
S1	67.4 ± 0.8 a	14.1 ± 0.2 b	0.23 ± 0.03 b	56.0 ± 9.0 b

S2	69.7 ± 2.4 a	15.4 ± 0.3 a	0.16 ± 0.01 c	96.0 ± 9.0 a

Values are mean ± standard deviation (n=5). Different letters within each parameter (column) indicate significant differences (Tukey; $p \leq 0.05$).

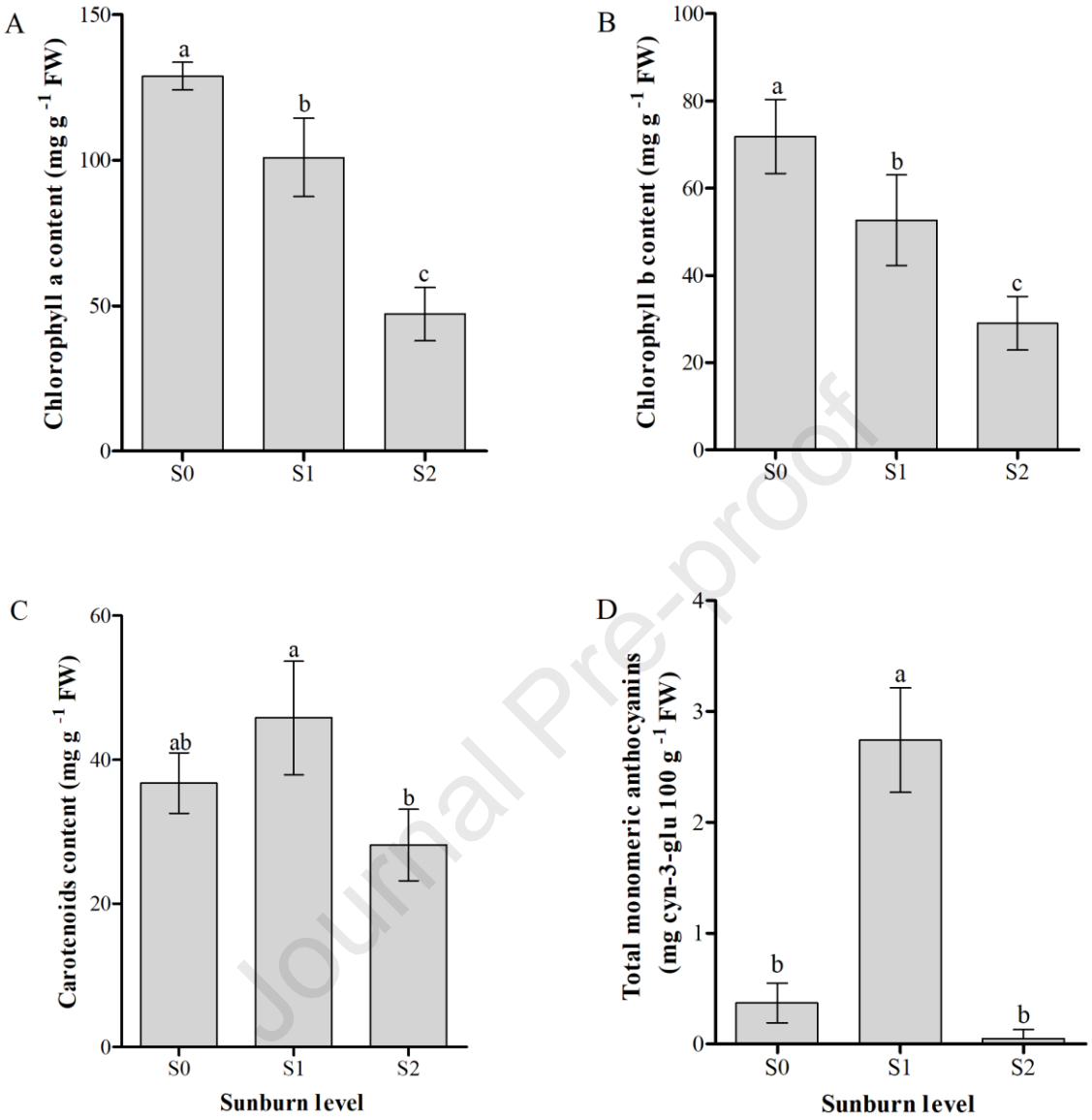
271

272 *3.2. Peel pigments*

273 Chlorophyll a and b contents in S0 tissue were 129.0 and 71.9 mg g^{-1} FW, respectively (Fig. 2A-B). A
274 significant decrease in chlorophyll a and b contents, accentuated by the severity of the damage, was
275 recorded in the peel of sunburned fruits. Thus, relative to S0, chlorophyll a content decreased by ~22 % in
276 S1 and by ~63 % in S2, while chlorophyll b content was similarly reduced, by ~27 % in S1 and by ~60 %
277 in S2.

278 Compared to S0, carotenoid content was higher (by ~25%) in S1 but dropped below S0 levels (a ~23%
279 reduction) in S2. A significant difference in carotenoid content (~39%; p= 0.0019) was observed between
280 S1 and S2 (Fig. 2C).

281 Anthocyanin content in S0 tissue was 0.39 mg cyn-3-glu 100 g^{-1} FW. This value was markedly increased
282 in S1 tissues (~697%), but reverted to near S0 levels in S2 (Fig. 2D).



283

284 **Fig. 2.** Pigments content variation in Beurré D´Anjou pear peel with different levels of sunburn at harvest.
285 (A) Chlorophyll a content; (B) Chlorophyll b content; (C) Carotenoids content; (D) Total monomeric
286 anthocyanins content (cyn-3-glu: cyanidin-3-glucoside). S0: no sunburn; S1: mild sunburn; S2: moderate
287 sunburn. Values are mean + standard deviation (n=5). Different letters indicate significant differences
288 (Tukey; $p < 0.05$).
289

290 *3.3. Oxidative metabolism*

Pear peel cell membrane properties were negatively affected by sunburn. Compared to S0, progressive increments in membrane permeability (of ~16% and ~37%, respectively) were detected in S1 and S2 fruit (Fig. 3A).

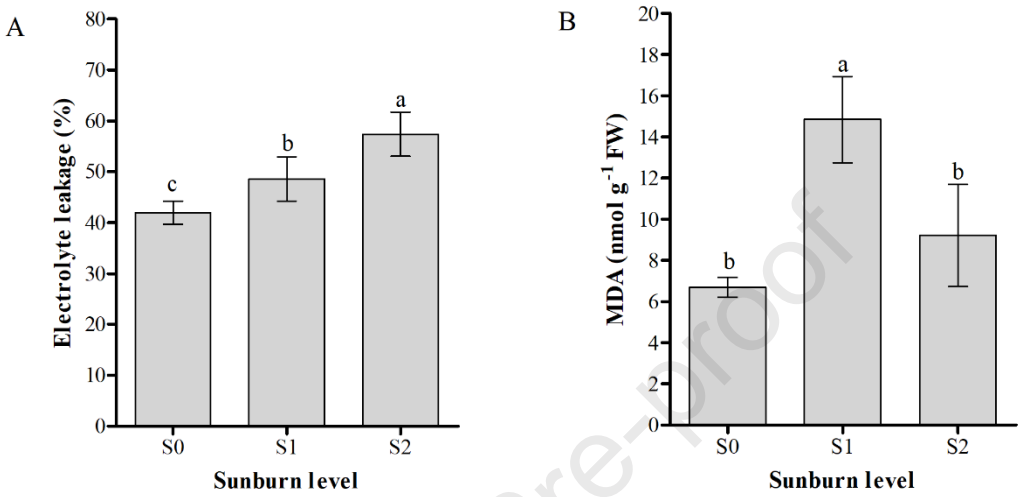
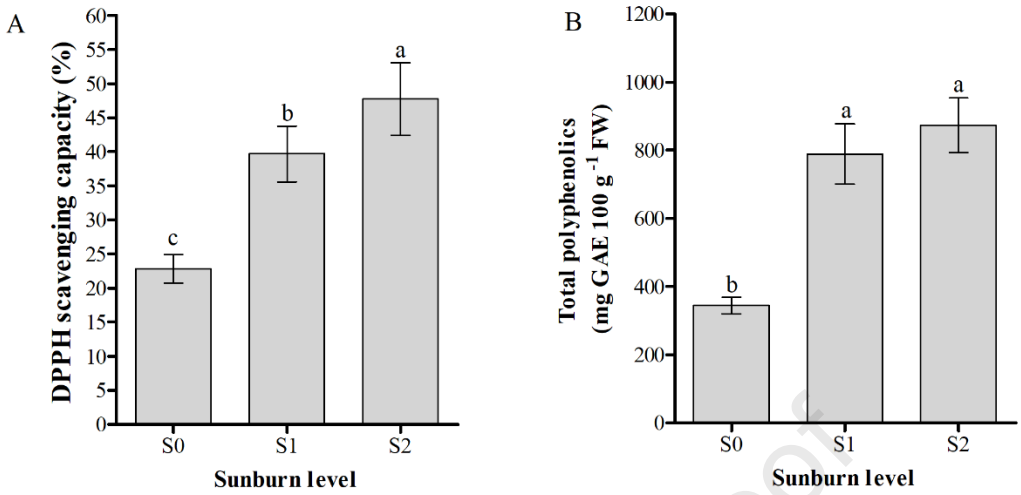


Fig. 3. Analysis of cell membrane properties in Beurré D´Anjou pear peel with different levels of sunburn at harvest. (A) Electrolyte leakage test results; (B) Lipid peroxidation assay results (MDA: malonaldehyde). S0: no sunburn; S1: mild sunburn; S2: moderate sunburn. Values are mean ± standard deviation (n= 5). Different letters indicate significant differences (Tukey; $p \leq 0.05$).

Compared to S0, lipid peroxidation levels were significantly increased (by ~122%) in S1 fruit peel, but reverted to near S0 levels in S2 tissue (Fig. 3B).

Antioxidant capacity also increased along with damage extent in the peel of sunburned fruit, with increments in DPPH scavenging activity of 74% and 109% being observed, respectively, in S1 and S2 tissues compared to S0 (Fig. 4A).



306
307 **Fig. 4**. Antioxidant response in Beurré D´Anjou pear peel with different levels of sunburn at harvest. (A)
308 Antioxidant capacity (DPPH assay). (B) Total polyphenol content (GAE: gallic acid equivalent). S0: no
309 sunburn; S1: mild sunburn; S2: moderate sunburn. Values are mean ± standard deviation (n=5). Different
310 letters indicate significant differences (Tukey; $p \leq 0.05$).
311

312 Total polyphenol content in S0 was 344.0 mg GAE 100 g^{-1} FW, and increased significantly (by ~129%
313 and 154%, respectively; $p < 0.0001$) in S1 and S2 fruit peel (Fig. 4B).

314 Antioxidant enzyme activities in pear peel varied also depending on sunburn severity (Fig. 5). CAT
315 activity decreased significantly with increasing sunburn level, from 151.3 to 122.7 to 48 µmol min^{-1} g^{-1}
316 FW in S0, S1, and S2, respectively. Contrary to the reduction observed in CAT activity, the enzymatic
317 activities of both APX and SOD increased with sunburn. APX activity increased by ~417% in S1 and by
318 ~621% in S2, compared to S0 (309.5 µmol min^{-1} g^{-1} FW) (Fig. 5B). In turn, SOD activity was estimated as
319 1.7 USOD g^{-1} FW in S0, and reached 13.8 USOD g^{-1} FW in S1 and 23.1 USOD g^{-1} FW in S2 (Fig. 5C).
320

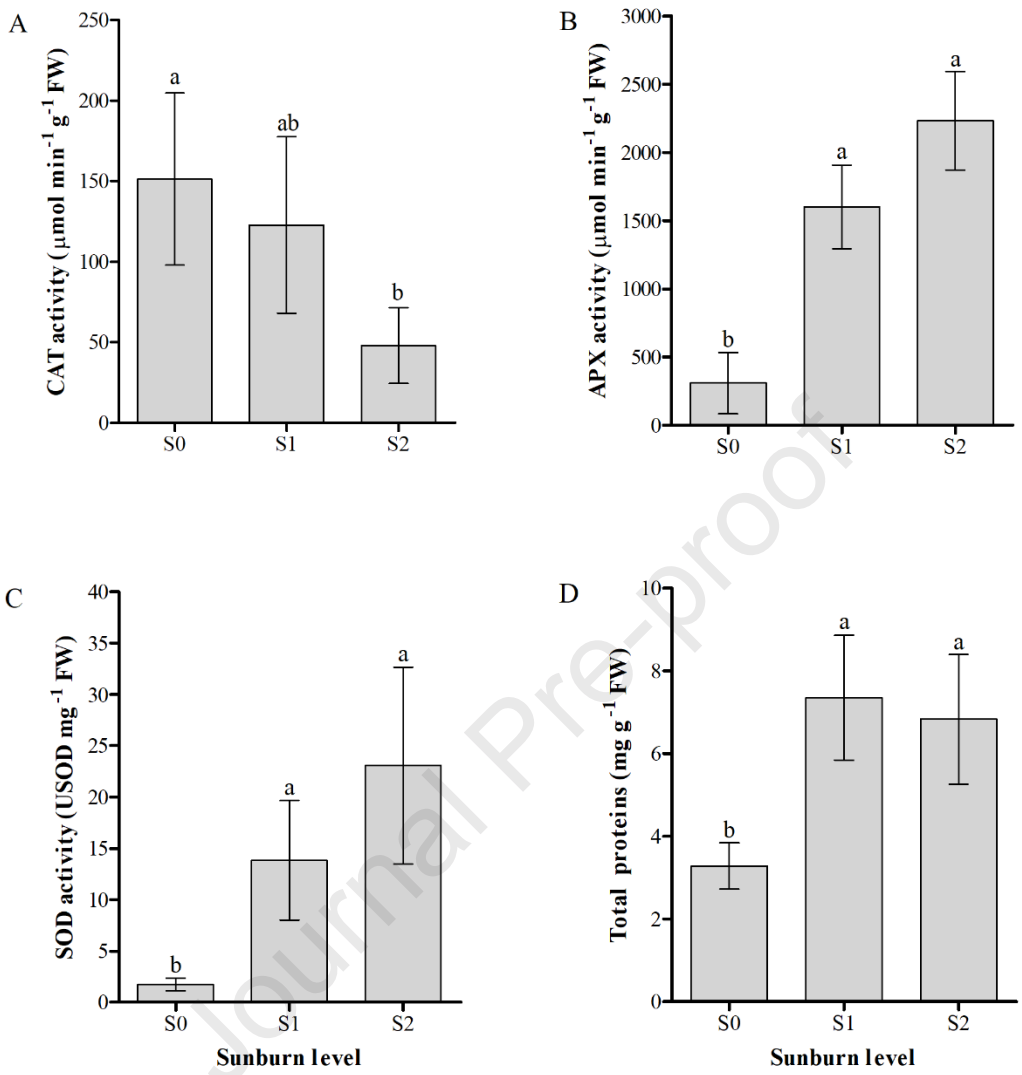


Fig. 5. Changes in antioxidant enzyme activity and soluble protein content in Beurré D´Anjou pear peel with different levels of sunburn at harvest. (A) Catalase (CAT) activity; (B) Ascorbate peroxidase (APX) activity; (C) Superoxide dismutase (SOD) activity; (D) Total soluble protein content. S0: no sunburn; S1: mild sunburn; S2: moderate sunburn. Values are mean ± standard deviation (n=5). Different letters indicate significant differences (Tukey; $p \leq 0.05$).

Total soluble protein content in the enzymatic extract was significantly higher in sunburned tissues, with increments of ~124 % in S1 and ~108 % in S2 with respect to S0 (Fig. 5D).

4. Discussion

4.1. Sunburn alters pigment composition and affects peel color in Beurré D´Anjou pear fruit

The solar radiation conditions under which fruits develop affect peel color, impacting both the classification of fruits into commercial categories as well consumers' choice (Munné-Bosch and Vincent, 2019).

The recorded values of the color-defining parameters hue, chroma, and lightness determined in healthy fruits (Table 1), characterized by light green shades, agree with previous descriptions in the peel of fruits of var. Beurré D'Anjou (Rudell et al., 2017). In pears var. Beurré D'Anjou and Bartlett, fruits developing under the leaf canopy presented higher hue and chroma values compared to those exposed to solar radiation (Rudell et al., 2017; Serra et al., 2018, Raffo et al., 2011). Similarly, in the present study sunburn tissues exhibited changes in color parameters that became accentuated with the severity of the damage (Table 1). Thus, progressively decreasing hue angle values indicate transition from green tones in healthy fruits to yellowish-green tones in S1, and to yellow tones in S2. Likewise, there was a progressive sunburn-related loss in color saturation, reflected by a reduction in chroma index. Similar changes, denoted by transition to yellow and brown tones with increasing damage severity, were reported in the peel of sunburned apple fruit irrespective of variety color (Felicetti and Schrader, 2009; Zupan et al., 2014) and were identified as a major cause of consumer rejection (Munné-Bosch and Vincent, 2019).

Fruit color is associated with the composition and concentration of different peel pigments. Three families of pigments, i.e. chlorophylls, carotenoids, and anthocyanins, predominate in the peel of pear fruits (Ngo, 2007). The characteristic green color of pear fruits var. Beurré D'Anjou is related to the high content of chlorophylls relative to other pigments such as carotenoids or anthocyanins, which are in turn associated with yellow, orange, and reddish tones. The chlorophyll, carotenoid, and anthocyanin contents (Fig. 2) recorded in S0 tissues in our study are similar with those reported for this variety in healthy fruit (Li et al., 2008; Li and Cheng, 2009).

Color changes in the peel of sunburned fruits have been associated with variations in the content of photosynthetic and photoprotective pigments present in the cells of the epidermal and hypodermal tissues (Morales-Quintana et al., 2020). In apple fruits, green and red tones, associated with chlorophylls and anthocyanins, respectively, are degraded under high solar radiation conditions; in contrast, yellow and

brown tones, related to the content of carotenoids and different phenolic compounds, are instead maintained or increased (Felicetti and Schrader, 2009).

In Anjou pear fruit peel, S1 and S2 tissues showed a marked, sunburn-dependent, reduction in chlorophyll content compared to healthy ones (Fig. 2A-B). This effect was described also in sunburn-affected green and yellow apple varieties (Hernandez et al., 2014; Vita, 2018). Chlorophyll loss is a consequence and a general symptom of oxidative stress promoted by high solar radiation incident on the fruit surface, resulting in the breakdown of chlorophyll mediated by reactive oxygen species (ROS) (Merzlyak et al., 1998).

Carotenoids share with other pigments photosynthetic and photoprotective properties. In the present study, no differences in carotenoid contents were detected between S1, S2, and healthy (S0) tissues; however, a higher carotenoid content was recorded in S1 tissue with respect to S2 (Fig. 2C). In turn, anthocyanin content was greatly increased in S1, but exhibited near baseline values in S2 (Fig. 2D). Increased levels of both compounds in peel tissue with mild sunburn (S1) suggest the activation of protective mechanisms against high solar radiation stress. In this regard, the protective effect of xanthophylls and their accumulation under high radiation conditions have been previously reported in pear fruits (Rudell et al., 2017).

Anthocyanins participate in the mitigation of photo-inhibition damage under stress conditions by buffering damage mediated by UV radiation, visible light, and high and low temperatures, acting also as antioxidant agents (Carmona Córdova, 2016; Li and Cheng, 2009). Red Beurré D'Anjou clones show higher photoprotective capacity against light stress compared to green fruit clones due to a higher anthocyanin content in the fruit peel (Li et al., 2008). Anthocyanin biosynthesis and degradation are related to light (intensity and quality) and temperature (Chen et al., 2019; Feng et al., 2013), and its final concentration in tissues will depend on the combination of these two factors during fruit development. Along with the above environmental conditions, anthocyanin concentration is influenced by genetic factors specific of species and cultivar. In pears, anthocyanins develop during fruit growth and show a reduction towards harvest (Zhang et al., 2011). Beurré D'Anjou pear fruits developing under intense light conditions tend to

develop a reddish hue, associated with increased anthocyanin content, on the sun-exposed area (Serra et al., 2019). The higher content of photoprotective pigments in S1 with respect to S2 would indicate that fruits with mild damage (S1) developed under light and thermal conditions that promoted the synthesis of these compounds, whereas in fruits with moderate damage (S2), the exposure conditions enhanced instead their degradation. Under high radiation stress conditions, the concentration of anthocyanins in plant tissues increases, which attenuates damage to chloroplasts (Zheng et al., 2021). However, depending on the intensity and duration of the stressful event, anthocyanin content can be drastically reduced by chemical or enzymatic degradation processes (peroxidases, β-glucanases) induced by oxidative imbalance. Although the degradation of anthocyanins by light and temperature has been demonstrated, the mechanisms underlying this process have not yet been clearly established (Thomson et al., 2018). We speculate that the higher chlorophyll content in S1 compared to S2 fruits could result from the protective effect of anthocyanins on chloroplasts (Merzlyak et al., 2002; Zheng et al., 2021).

4.2. Sunburn alters the internal quality of pear fruit

The internal quality of fruit is defined by different attributes, characterized through various indices such as firmness, and sugars and organic acids composition. In pears, these indices determine the optimal harvest time, as well as conservation management and commercial destination of the fruit (Mesa Juliani, 2015). Coinciding with previous reports on apple fruits (Racskó and Schrader, 2012; Torres et al., 2013; Vita et al., 2019), in this study pear fruits showed an increase in flesh firmness below the areas with symptoms of solar radiation stress (Table 2). The increased firmness of sunburned tissues correlates with anatomical and morphological changes that promote greater stiffness. Microscopic observations of apple and pear tissues with sunburn revealed a reduction in cell size, an increase in the number of cells per unit area, and an increase in cell wall thickness (Felicetti and Schrader, 2008; Gambetta et al., 2021; Spera et al., 2023). It is well known that high solar radiation stress triggers changes in the structure and composition of the cell wall (Raffo et al., 2011; Spera et al., 2023; Torres et al., 2020), which modify its biomechanical properties and promote changes in pulp texture. Likewise, the water status of sunburned tissues

411 contributes to alterations in tissue firmness (Racskó and Schrader, 2012). Therefore, the greater firmness
412 observed in S1 and S2 fruit pulp may be related to loss of water in the sector affected by excessive solar
413 radiation.

414 Additional parameters affected by sunburn included soluble solids and starch content. Pears with sunburn
415 showed higher soluble solids content and higher starch degradation values, with the differences becoming
416 more pronounced with the severity of the damage (Table 2). Similar results have been reported for apple
417 fruit (Tartachnyk et al., 2012; Vita et al., 2019). While past research attributed these changes to
418 accelerated ripening (Schrader et al., 2009), current reports relate starch metabolism and changes in sugar
419 concentration to tolerance mechanisms in stress situations. These variations in sugar content affect various
420 physiological processes involved in the regulation of cell turgor, the protection of cell membranes and
421 proteins, and the availability of energy intermediates necessary for the biosynthesis of protective
422 compounds, chemical messengers, and proteins (Dong and Beckles, 2019; Thalmann and Santelia, 2017;
423 Zhao et al., 2019).

424 Acidity in sunburned pear fruit was lower than in healthy fruit, a behavior described in different apple
425 varieties with different sunburn levels (Schrader et al., 2009). The decrease in the content of malic acid, a
426 compound utilized as a carbon source for different metabolic processes in fruits, is associated with
427 ripening progression (Rudell et al., 2017). However, although it is clear that high radiation stress decreases
428 acidity in the affected tissues, it has not been yet corroborated whether this is evidence of an accelerated
429 maturity state or, as indicated for sugars, results from activation of metabolic pathways associated with a
430 stress response (McTavish et al., 2020). Recent studies highlighted the NADP-malic enzyme as a key
431 factor in plant tolerance to abiotic stress. This enzyme catalyzes the oxidative decarboxylation of malic
432 acid, leading to increased NADPH content. NADPH acts as a reducing agent in cellular reactions,
433 participates in the synthesis of defense substances, and contributes directly to the metabolism of ROS, thus
434 reducing cell damage caused by oxidative stress (Blanch et al., 2013; Li et al., 2022; Sun et al., 2019).

435 Therefore, starch degradation, increased soluble solids, and reduced malic acid content in sunburn fruit
436 may not simply indicate a more advanced maturity stage, but reflect instead a series of mechanisms

437 activated in response to stress. New scientific approaches related to plant behavior under abiotic stress
438 (Dong and Beckles, 2019; Le Gall et al., 2015; Sun et al., 2019), allow us to suggest that the observed
439 changes in maturity parameters in sunburned fruits may result from activation of different metabolic
440 pathways associated with defense mechanisms, acquisition of tolerance, and survival.
441
442 *4.3. Sunburn affects oxidative metabolism in pear fruit peel*
443 Membrane permeability and lipid peroxidation status are indicators of oxidative damage in plant tissues
444 exposed to stress (Sharma et al., 2012). In this study, evidence of sunburn-induced oxidative damage is
445 reflected by progressive electrolyte loss and increased MDA contents in S1 and S1 fruit peel (Fig. 3B).
446 These results are in agreement with evaluations carried out on apple, which revealed increased lipid
447 peroxidation in parts of the fruit affected by sunburn (Chen et al., 2008; Colavita, 2022; Munné-Bosch and
448 Vincent, 2019; Vita, 2018). According to these studies, increased lipid peroxidation in sunburn peel
449 indicates that the endogenous antioxidant system cannot effectively counteract the photooxidative process
450 triggered by high solar radiation and temperature. A prime consequence of unmitigated ROS production is
451 damage to biomolecules. Excess ROS triggers lipid peroxidation in both cell and organelle membranes,
452 ultimately decreasing membrane fluidity, increasing membrane permeability, and inducing secondary
453 damage to membrane proteins, all of which hinder cell function (Halliwell, 2006).
454 Also in agreement with results reported in apple (Colavita, 2022 and Vita, 2018), a higher antioxidant
455 capacity was recorded in sunburned peel from pear fruit. However, as discussed in preceding sections, the
456 parallel increase in the lipid peroxidation and the drop in chlorophyll content observed in sunburned
457 tissues indicate that stimulation of the antioxidant response was not sufficient to counteract sunburn-
458 related damage in pear peel.
459 Phenols play prominent roles in plant physiology, contributing importantly to defense against biotic and
460 abiotic stresses (Carmona Córdova, 2016). Phenolic compounds sustain oxidative stability and their levels
461 correlate positively with the antioxidant capacity of fruits (Vieira et al., 2009). The increase in
462 polyphenols observed in the peel of S1 and S2 pear fruit (Fig. 4) agrees well with previous research in

Braeburn, Golden Delicious, Granny Smith, and Red Delicious apples, in which in addition to increased polyphenols levels, an enhanced antioxidant capacity was observed in fruit peel with sunburn symptoms (Colavita, 2022; Yuri et al., 2010; Zupan et al., 2014). We thus conclude that the increase in polyphenols recorded in S1 and S2 contributed to increased antioxidant capacity in sunburn tissues. Indeed, increases in both polyphenols and antioxidant capacity were reported by previous studies on pear fruit tissues exposed to direct sunlight but without concurrent development of sunburn symptoms (Serra et al., 2018; Zhao et al., 2016). Such increase in polyphenol levels, although visually unappealing due to its association with development of yellow and brown tones, may represent a commercial advantage for these fruits given the beneficial effects of these compounds on human health (Yuri et al., 2010).

Paralleling the increase in polyphenol contents, other defense mechanisms, notably the enzymatic antioxidant system, are activated in plant tissues facing stressful conditions. Main enzymes comprising the antioxidant machinery in plants include CAT, APX, GR, and SOD. These enzymes have been proposed to play a more important role than phenolic compounds in preserving fruit quality (Zhao et al., 2016).

In pear var. Beurré D'Anjou fruits exposed to sunlight but without sunburn development, significant increases in the activity of SOD, APX, and GR were recorded compared to fruits kept in the shade (Li et al., 2008; Zhao et al., 2016). In this study, we also detected a significant increase in the activity of SOD and APX in the peel of fruits with sunburn symptoms (Fig. 5). Likewise, previous studies documented that sunburn increases the activity of these enzymes in apple fruit (Chen et al., 2013; Colavita, 2022; Racskó and Schrader, 2012). SOD is considered the first line of defense against elevated ROS production (Gill and Tuteja, 2010), whereas APX participates in the elimination of H_2O_2 in the glutathione-ascorbate cycle (Suzuki et al., 2012). The increments in both SOD and APX activities in sunburn affected tissues suggest the direct participation of both enzymes in detoxification processes against oxidative damage caused by high radiation and temperature conditions in Beurré D'Anjou pear.

Although in previous studies in green- and red-skinned varieties of Anjou pear CAT activity was not affected by sunlight exposure (Li et al., 2008; Zhao et al., 2016), in the present evaluation a decrease in CAT activity was recorded in S1 and S2 samples (Fig. 5A). These results are in line with studies on

sunburn effects in several apple varieties, where a marked loss of CAT activity was correlated with increased damage (Colavita, 2022; Ma and Cheng, 2003; Tsantili et al., 2007). CAT activation contributes to the elimination of H_2O_2 generated in peroxisomes upon β-oxidation of fatty acids, photo-respiration, and purine catabolism, and under various abiotic stress conditions (Sharma and Ahmad, 2014). However, our findings indicate that sunburn negatively impacts CAT activity in the peel of Beurré D'Anjou pear, thus negating its contribution to ROS detoxification.

The increase in soluble protein content observed in enzyme extracts from sunburned tissues may be related to upregulated synthesis of antioxidant enzymes, as evidenced by the higher activity of SOD and APX in S1 and S2 samples (Fig. 5). However, it should be considered that the referred increase in soluble protein content may be also due to augmented synthesis of other compounds not analyzed in this study.

Overall, our findings indicate that in Beurré D'Anjou pear fruit with mild (S1) and moderate (S2) sunburn damage both enzymatic and non-enzymatic antioxidant responses became activated. However, these responses were insufficient to prevent oxidative damage and development of sunburn symptoms.

5. Conclusions

Sunburn tissues in Beurré D´Anjou pear presented physiological and biochemical changes associated with quality decline. Surface color transition from green to yellow sunburn was associated with and increased in photoprotective pigments content, while in moderate damage was lost this defense response against stressful conditions. Besides modification of external color, other aspects impacting fruit quality, marketing, and storage potential are altered by sunburn. Increased flesh firmness and soluble solids content, as well as decreased acidity, are some of the metabolic changes induced in response to high solar radiation and elevated temperature. Excessive solar radiation increased oxidative metabolism in the peel of Beurré D'Anjou pear fruits. Accordingly, accumulation of metabolites involved in tolerance to oxidative damage, such as polyphenols, in parallel with increased antioxidant activity, are hereby reported in sunburn-affected tissues. However, these defense systems are not entirely capable of preventing

sunburn symptoms caused by natural exposure to high solar radiation and temperature in the fruit examined.

Contributions

Author Contributions: conceptualization, N.S., L.I.V., P.M.C. and G.M.C.; methodology, N.S., L.I.V. and G.M.C.; validation, N.S.; formal analysis, N.S; investigation, N.S.; data curation, N.S.; resources, N.S., L.I.V., P.M.C. and G.M.C.; writing – original draft, N.S. and L.I.V; writing – review & editing, N.S., L.I.V., P.M.C. and G.M.C.; funding acquisition, L.I.V and G.M.C; project administration, G.M.C; supervision, P.M.C and G.M.C.

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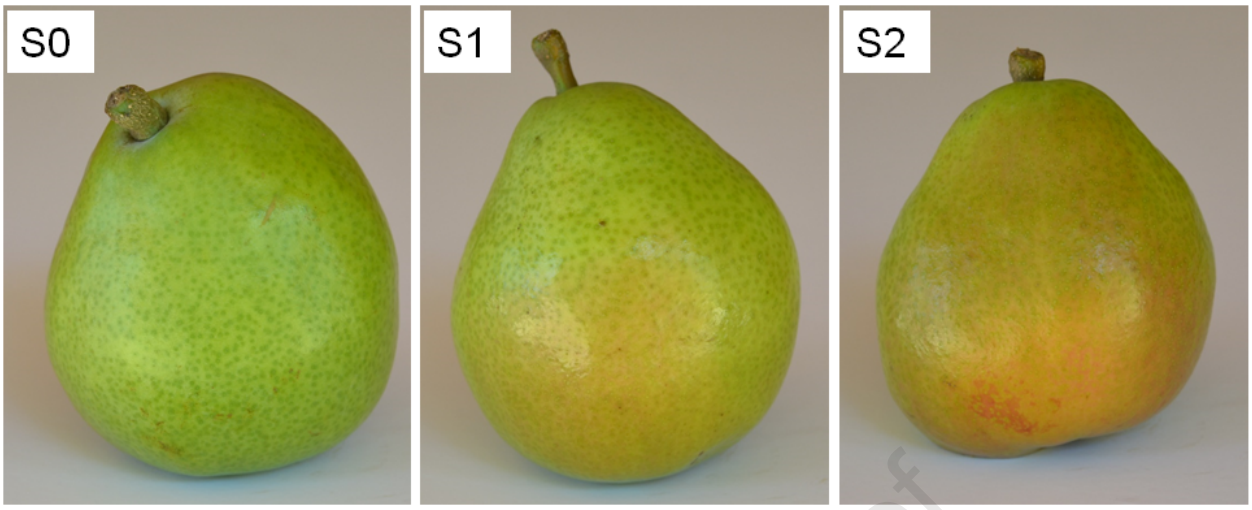

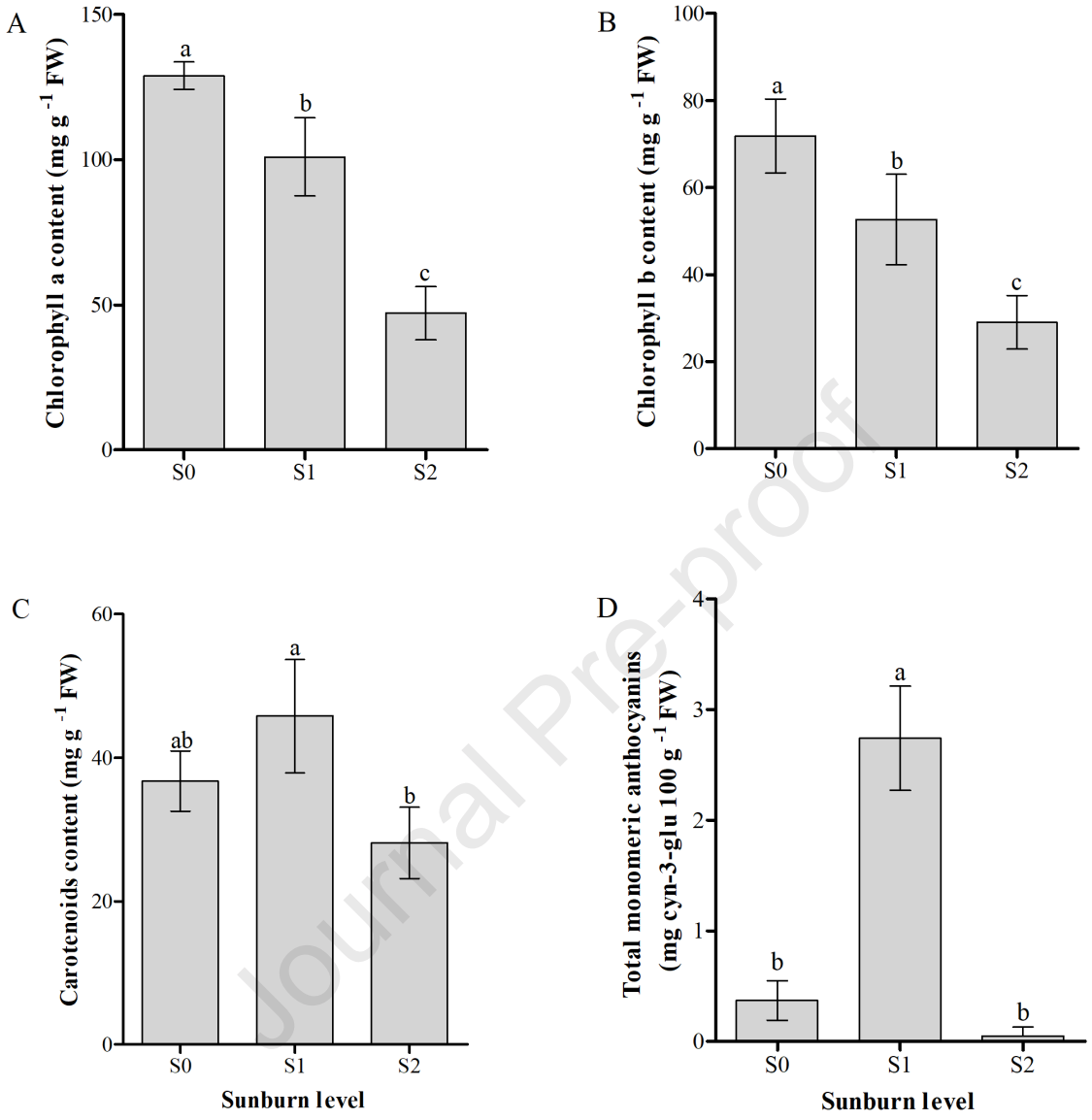

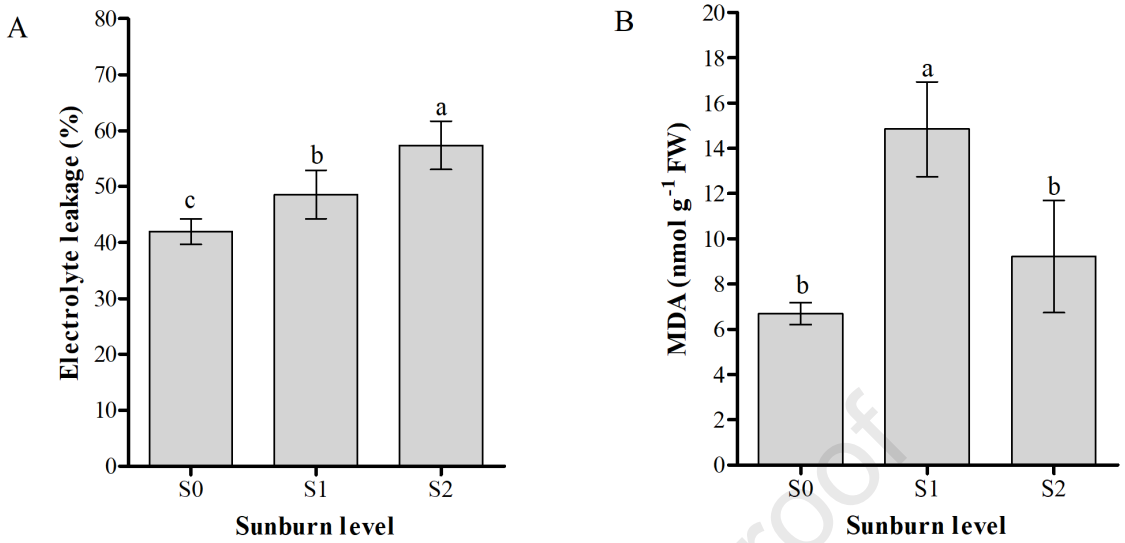

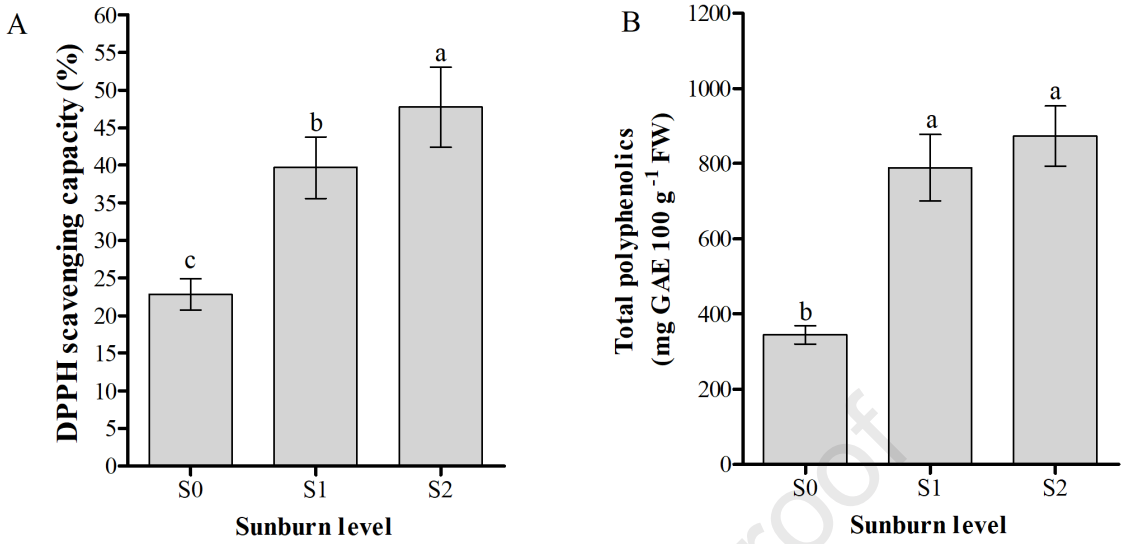

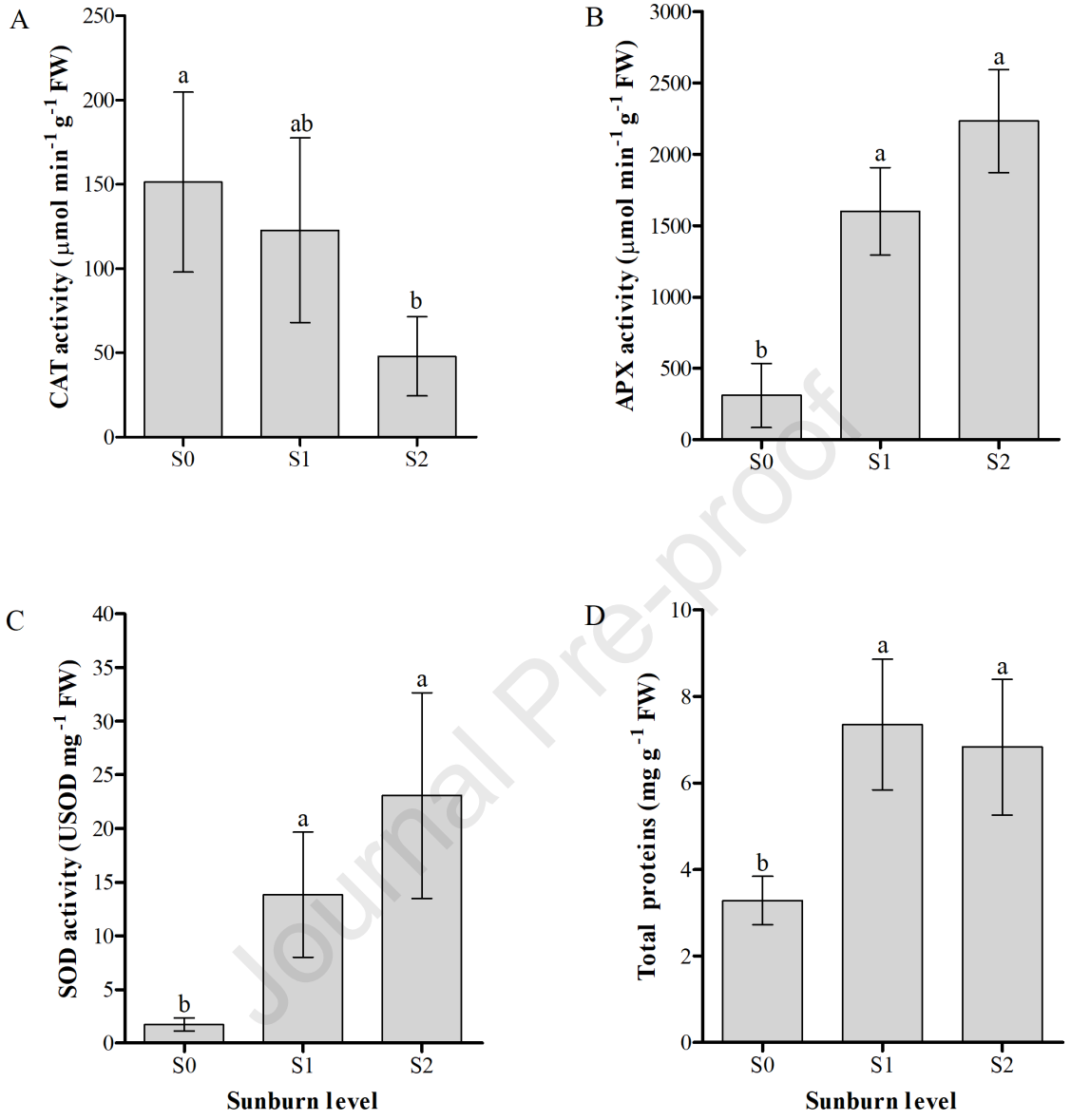

HIGHLIGHTS

- Mild sun damage increased the amount of photoprotective pigments in Beurré D'Anjou pears, while moderate sun damage enhanced their degradation.
- Some quality parameters in sunburn pear tissues correlated with advanced ripening stage, but the flesh firmness remained higher.
- Sun exposure increased antioxidant response in pear fruit, though it was insufficient to avoid cell membrane oxidative damage.
- Pear fruits with mild sun damage, associated with a poor commercial quality, had indeed higher polyphenols levels that are beneficial for human health.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: