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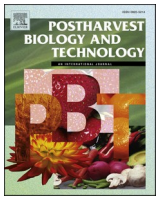

Use of riboflavin to reduce decay and extend the shelf-life of fresh-cut sweet pepper

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ABSTRACT

Riboflavin (vitamin B_2) generally is recognized as a safe (GRAS) additive. It is used in food to fortify products with low natural content or compensate for the possible vitamin losses during processing. In this work, we studied the effect of pre-storage riboflavin dips on the quality maintenance of fresh-cut pepper (*Capsicum annum* L). We initially tested the efficacy of different riboflavin concentrations (0, control; 1; 10 and 100 μmol L^{-1}) to reduce spoilage in fresh-cut green sweet pepper sticks (5 × 1 cm). Treatments with 100 μmol L^{-1} riboflavin effectively controlled pepper spoilage and were therefore selected for further evaluation along with refrigerated storage. Riboflavin dips reduced soft-rot decay incidence and severity and prevented the increase in electrolyte leakage, and respiration during storage. Riboflavin treatments preserved textural properties by preventing tissue hardness, stiffness, and resistance to bend losses. Treated peppers also presented lower yeasts and molds and aerobic mesophilic bacteria counts. Finally, riboflavin-dips improved antioxidant and phenolics retention without alterations in color, weight loss, sugar, or organic acids. Taken together, results show, for the first time, that riboflavin may be used as a postharvest preservative agent to extend the shelf-life of fresh-cut vegetables.

1. Introduction

The segment of fresh-cut fruits and vegetables has rapidly expanded in the last decades in line with socio-cultural changes increasingly demanding ready-to-eat foods (Gil et al., 2009). Fresh-cut vegetables have also been a choice for consumers looking for healthy foods (Cisneros-Zevallos, 2021), being at the same time easy to use and not generating high wastes at the point of consumption (Velderrain-Rodríguez et al., 2019). However, these products are much more perishable than the corresponding unprocessed commodities and thus require a tightly adjusted postharvest management (Ansah et al., 2018). Refrigerated storage is necessary to minimize the microbiological risks and slow down the most relevant deteriorative reactions in fresh-cut vegetables (Artés-Hernández et al., 2017). Even when combining cold storage with other available technologies (i.e., modified atmosphere packaging), the postharvest life of fresh-cut products rarely exceeds 10–14 days (Rodoni et al., 2015, Ma et al., 2017). Consequently, there is a great interest in finding novel strategies to improve the postharvest life of fresh-cut products (Batiha et al., 2021). With regards to chemical treatments, a relatively small number of compounds have advanced from the laboratory scale and reached the market. Water disinfectants (i.e., chlorinated compounds, peracetic acid) and antioxidants (i.e., citric and ascorbic acid) are among the most commonly used to prevent contamination or when browning may become problematic, respectively (Capotorto et al., 2017; da Silva Borges et al., 2019). In some cases, calcium salts as firming agents were successfully applied (Aguayo et al., 2008). The search for new food substances and uncovering novel uses for known compounds are not easy endeavors, especially in a context in which, given their potential environmental externalities and adverse effects on human health, all chemicals incorporated into food production systems are under increasing scrutiny (Perito et al., 2020).

Riboflavin (*Rib*) is one of the thirteen essential vitamins for human

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health (vitamin B_2) (Bates, 2013). Its recommended daily intake is relatively low (0.6–1.6 mg). The body needs constantly incorporate *Rib* through the diet since it cannot synthesize this molecule (EFSA Panel on Dietetic Products, Nutrition and Allergies NDA et al., 2017). Natural good sources of *Rib* include green vegetables, meat, and dairy products (Bates, 2013). One of the well-studied biological functions of the *Rib* is its role in redox reactions. The flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) contain *Rib* in their molecules. Both FMN and FAD are basic coenzymes of the Krebs cycle, protein, fat, and steroid metabolism (Bosch, 2019).

Rib plays, in addition to such central metabolic functions, a role in the interaction between plants and microorganisms (Jaiswal et al., 2021). Working in *Arabidopsis* and tobacco, Dong and Beer (2000) found that *Rib* treatment elicited systemic acquired resistance (SAR) against *Peronospora parasitica, Pseudomonas syringae*, tobacco mosaic virus, and *Alternaria alternata*. Subsequent studies in rice and sugar beet by Taheri and Tarighi (2010, 2011) and grapevine by Boubakri et al. (2013) identified the phenylpropanoid as one of the biosynthetic pathways strongly triggered by *Rib* treatments. However, not all species have shown such kinds of defensive responses to *Rib* (Azami-Sardooei et al., 2010). So far, almost all studies assessing the efficacy of *Rib* to control pathogens have focused on preharvest diseases. Only one report evaluated *Rib* in the postharvest environment showing positive effects in the control of *Alternaria* rot (Li et al., 2012).

Ought to its natural occurrence whiting food products, essentiality, and long-term safe human use and consumption, *Rib* is an accepted GRAS food additive (Suwannasom et al., 2020). In this context, it has been used almost exclusively for fortification purposes in products with

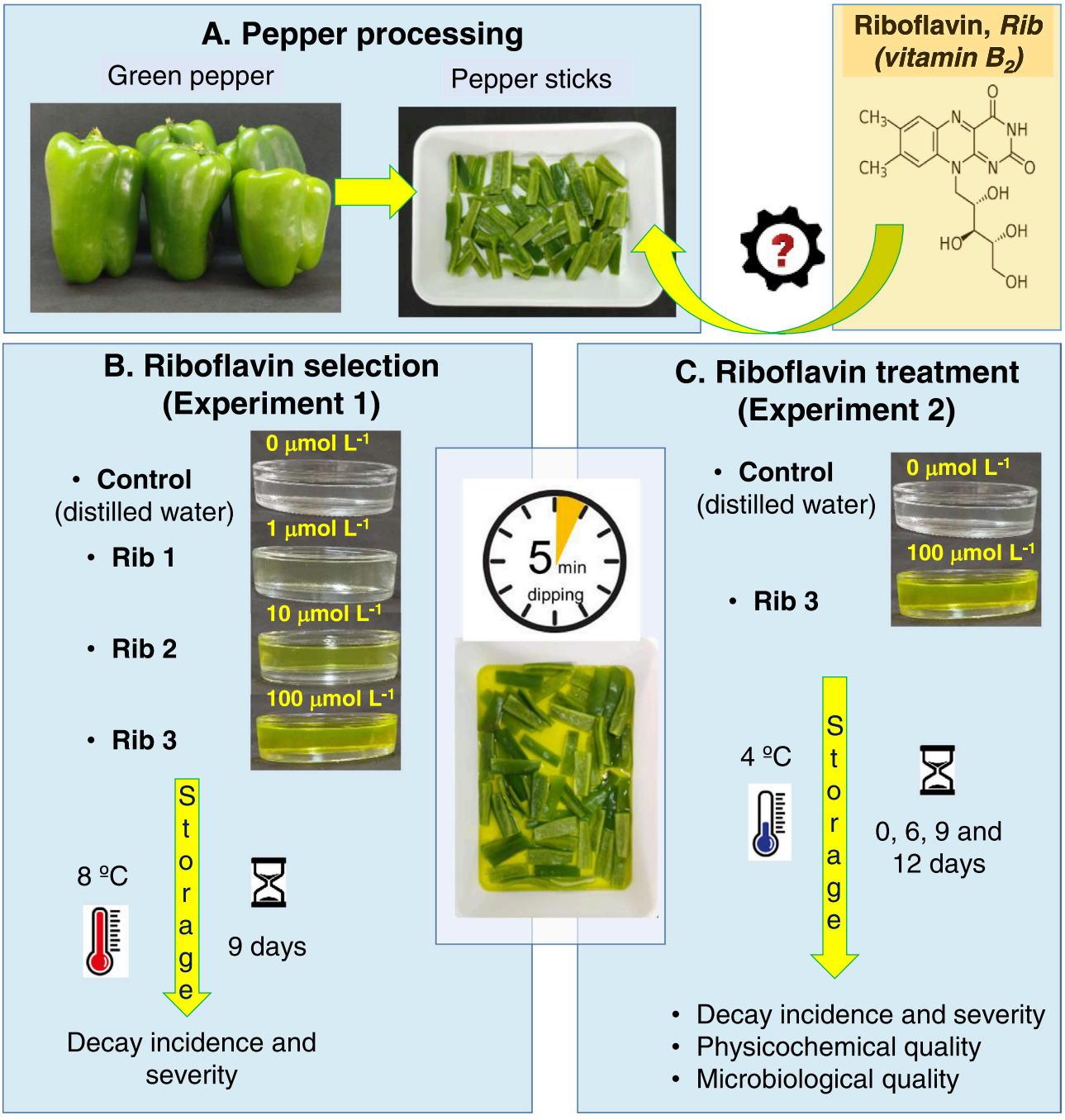

Fig. 1. Schematic representation of the experimental design of the study.

low natural contents or when the processing causes a loss of this vitamin. As far as we know, no works have evaluated the efficacy of *Rib* as a preservative agent in fresh-cut produce. In this work, we selected a proper *Rib* treatment for fresh-cut pepper and determined its effects on product physical, chemical, and microbiological quality.

2. Materials and methods

2.1. Selection of riboflavin concentration for pepper decay control

Green sweet pepper (*Capsicum annum* L.) cv. Margarita was purchased at the Central Market in La Plata, Argentina, and immediately transported to the laboratory. The fruit was washed with chlorinated water (sodium hypochlorite, 100 mg L^{-1} pH 6.5 for 1 min), the peduncles, placenta and seeds were removed, and the pericarp was longitudinally cut into sticks (5 × 1 cm) (Fig. 1A). Groups of 20 pepper sticks prepared were dipped in water (control) or solutions containing 1, 10, or 100 μmol L^{-1} of *Rib* at 20 °C for 5 min (Fig. 1B). The *Rib* solutions were prepared placing the required mass of riboflavin (Parafarm®) in a 2 L volumetric flask. After adding 750 mL of distilled water, the dispersion was hand-stirred for 2 min to ensure dissolution and brought to 2 L with distilled water. All *Rib* solutions were prepared immediately before use. After the treatment, samples were drained, and packed polyethylene terephthalate plastic trays covered with perforated polyvinylchloride (PVC) (four 1-mm diameter holes per cm^2) to prevent CO_2 accumulation and were stored at 8 °C (temperature abuse) and 94% relative humidity (RH) for 9 days. After storage samples were taken and the individual sticks were assessed for decay. Decay incidence was determined by evaluating the number of pepper sticks showing disease symptoms and expressed in percentage. Decay severity was evaluated by using a hedonic four-level (0−3) intensity scale (0 = healthy; 1 = low rot incidence with incipient macerated areas; 2 = moderate rot incidence with clear macerated areas showing exudates, and 3 = high rot incidence with extended macerated area or visible mycelia growth). For each treatment group, the frequency of sticks in each level was recorded and the median was calculated.

2.2. Effect of the selected riboflavin treatment on fresh-cut green pepper physical, chemical, and microbiological properties during refrigerated storage

Green pepper sticks prepared as described in Section 2.1 were dipped in water (control) or 100 μmol L^{-1} *Rib* at 20 °C for 5 min (Fig. 1C). The samples packed in plastic trays and covered with perforated PVC were stored at 4 °C and 94% RH for 0, 6, 9 or 12 days. At each storage time, samples were taken and used to assess quality. Decay incidence and severity, ion leakage, respiration, textural properties (hardness, stiffness, and bend resistance), microbiological counts (yeasts and molds and mesophilic aerobic bacteria), weight loss and color were evaluated immediately after sampling. For sugar, organic acids (citric, malic and quinic acid) antioxidant capacity, total phenolics, ascorbic acid, analyses samples were frozen in liquid nitrogen and stored at − 80 °C until use. Ten trays containing 25 pepper sticks each were evaluated for each treatment.

2.3. Quality assessment

2.3.1. Decay and electrolyte leakage

Decay incidence was evaluated by determining the number of rotten pepper sticks per tray. Decay severity was assessed based on a hedonic four-level intensity scale as described in Section 2.1. For each tray a decay severity index (DSI) was subsequently calculated as DSI = ∑ Ni Di/Nt being:

Ni. Number of sticks at the i-damage level.

Di. Damage level.

Nt. The total number of sticks.

Five biological replicates consisting of 25 pepper sticks each were used per treatment.

For electrolyte leakage evaluation, fruit sticks weighing approximately 10 g were taken and incubated in plastic tubes containing distilled water for 5 min at 20 °C with gentle stirring. The tissue was removed, and the conductivity of the solution was measured with a conductimeter (Oakton Model 510, IL USA). To evaluate the total electrolytes in the tissue, the pepper sticks were placed back into the plastic tubes and ground with an Omnimixer (Sorvall Inc., CT, USA). The suspension was then centrifuged at 10,000 × *g* for 10 min and the total conductivity of the supernatant was measured at 20 °C as described above. Results were expressed as the percentage of electrolytes that leaked out of the tissues during the incubation period. Three measurements were done for each treatment and storage time.

2.3.2. Respiration rate

Green pepper samples weighing approximately 80 g, were put into a 500 mL glass flask, sealed, and incubated at 20 °C for 20 min. The CO_2 concentration in the headspace was monitored with an infrared sensor (Compu-flow, Model 8650, Alnor, USA). Fruit respiration was calculated, and results were expressed in mmol of CO_2 produced per kilogram of fresh weight in an hour. Three measurements were done per treatment and storage time.

2.3.3. Microbiological counts

Twenty-five grams of pepper sticks were stirred in 225 mL of 0.1% w/v peptone for 15 min. Serial dilutions from the resulting suspensions, dilutions from (10^{-1} to 10^{-5}) were prepared and 100 mL of appropriate dilutions was poured into Petri plates containing plate count agar (PCA) and chloramphenicol yeast glucose agar media (YGC) for mesophilic aerobic bacteria and yeast and molds, respectively. Plates for mesophilic aerobic bacteria were incubated at 30 °C for 3 days and yeast and molds at 20 °C for 5 days. Results were expressed as log of colony-forming units per gram of fresh weight (log CFU g^{-1}). Three replicates were done for each treatment and storage time.

2.3.4. Textural properties

A Texture Analyzer (TA.XT2, Stable Micro Systems Texture Technologies, NY, USA) was used. For hardness and stiffness assessment samples were punctured in the equatorial zone (inner pericarp side) with a 3 mm diameter flat probe at a speed of 1 mm s^{-1}. The maximum force (hardness) and the initial slope of the force vs distance curve (stiffness) were recorded in Newton (N) and N mm^{-1} respectively. Fifty measurements were done for each treatment and storage time. To evaluate the bend resistance, the individual sweet pepper sticks were suspended with the epidermic side down in a metal platform having two contact points 3 cm far from each other. A normal compression force was applied on the center of the suspended section with a dented rectangular probe (Section 5 × 2 mm) at a speed of 1 mm s^{-1}. The force required to bend the sticks was recorded and expressed as N mm^{-1}. Fifty independent measurements were done for each texture assessment test, treatment, and storage time.

2.3.5. Weight loss

The trays containing the pepper samples were individually weighed at the beginning of the experiment and along with storage. Weight loss was calculated as WL = 100 × Wi -Wf)/Wi, being Wi the initial sample weight and Wf the weight at the sampling date. Results were expressed in percentage. Ten measurements were done for treatment and storage time.

2.3.6. Color

The surface color was measured on the epidermic side of the sticks with a colorimeter (Model CR-400, Minolta, Osaka, Japan) to obtain L^*, a^* and b^* values. Thirty measurements were done for each treatment and storage time.

2.3.7. Sugars

Approximately 25 g of frozen fruit was ground in a mill and 0.6 g of the resulting sample were homogenized in 5 mL of ethanol and vortexed for 1 min. The suspension was centrifuged at 5,000 × *g* for 10 min at 4 °C. Sugars were measured with the anthrone reagent (Andersson et al., 2006). Glucose was used as a standard and results were expressed as glucose equivalents in gram per kilogram on a fresh weight basis. Three independent extractions were analyzed for each treatment and storage time.

2.3.8. Organic acids

Approximately 500 mg of the frozen tissue was ground in a mill and mixed with 1 mL of cold trifluoroacetic acid (TCA, 3% v/v) in a plastic tube and centrifuged at 16,000 × *g* for 10 min at 4 °C. The pellet was separated and re-extracted with TCA. Both supernatants were combined and maintained in an ice bath for the measurement. Samples were eluted in C18 cartridges (Sep-Pak® Vac 3 cc, 500 mg, Waters, Ireland). Organic acids contents were measured in an HPLC equipped with an LC-10 AT pump (Shimadzu®, Japan) at a flow rate of 0.5 mL min^{-1} of 100 mmol L^{-1} potassium phosphate buffer adjusted at pH 3.0 and using a UV-VIS detector (Model SPD-10AV, Shimadzu®, Japan) at 265 nm. Citric, malic and quinic acids were used as authentic standards and results were expressed in gram per kilogram on a fresh weight basis. Three measurements were done for each treatment and storage time.

2.3.9. Antioxidant capacity

Samples weighing 25 g of pepper sticks were ground in a mill (Model A11, IKA Works Inc., SP, Brazil) and 1 g of the resulting powder was vortexed for 1 min in 10 mL of cold ethanol and centrifuged at 15000 × *g* for 10 min at 4 °C. The supernatant was brought to 100 mL with distilled water. The $DPPH^{\bullet}$ assay was done according to Brand--Williams et al. (1995), with minor modifications. Two milliliter aliquots of the ethanolic extracts were brought to 10 mL with ethanol and used to evaluate $DPPH^{\bullet}$-scavenging capacity. Test tubes containing 0, 50, 75, 125, 175 and 225 μL of sample and ethanol to a final volume of 500 μL were prepared. After that, 800 μL of a 60 mg L^{-1} solution of the radical $DPPH^{\bullet}$ in ethanol was added. Samples were vortexed and incubated at 20 °C for 60 min. The absorbance at 515 nm was measured and the equivalent mass of fruit tissue required to consume 50% of the initial $DPPH^{\bullet}$ absorbance was calculated (EC50). The antioxidant capacity was defined as $EC50^{-1}$ (kg^{-1}). Three measurements were done for each treatment and storage time.

2.3.10. Total phenolics

Fruit samples (5 g) were frozen in liquid N_2 and ground in a mill (Peabody, PE-MC9100, China). The resulting powder was suspended in 25 mL of ethanol and vortexed for 3 min. The extraction procedure was repeated three times. Samples were centrifuged at 13,000 × *g* for 10 min at 4 °C (Sorvall ST 16 R, United States). The pooled supernatants were used to determine total phenolics spectrophotometrically at 760 nm according to Singleton et al. (1999) by using the Folin-Ciocalteu reagent. Gallic acid was used as a standard. Samples were evaluated in triplicate. Results were expressed as chlorogenic acid equivalents in milligrams per kilogram on a fresh weight basis.

2.3.11. Ascorbic acid

Samples were frozen in liquid nitrogen, processed in a mill and approximately 1 g of the resulting powder was homogenized with 5 mL of 2.5% m/v *m*-phosphoric acid. The mixture was vortexed for 1 min and then centrifuged at 12,000 × *g* for 10 min at 4 °C. The supernatant was saved and filtered through 0.2 μm RC membrane. Ascorbic acid (AsA) determination was done by using a high-performance liquid chromatograph (Model 1525, Waters Corp., USA), fitted with a photodiode array detector and a C18 column (5 mm particle size, L × I.D. 25 cm × 4.6 mm. Waters Corp., USA). The mobile phase consisted of 0.5% m/v *m*-phosphoric acid/ acetonitrile (93/7) and was run isocratically at a flow rate of 1.0 mL min^{-1}. Samples were detected at 254 nm and AsA identification and quantitation were done by using a calibration curve with an authentic standard. Results were expressed in milligram per kilogram on a fresh weight basis. Three measurements were done for each treatment and storage time.

2.4. Statistical analysis

For selecting the most effective riboflavin concentration, a fully randomized design was used. Pepper decay incidence and severity data were analyzed by Chi-squared test and Mann-Whitney test, respectively ($P < 0.05$, N = 80). For riboflavin effect on pepper quality evaluation, a 2 × 4 factorial design (treatment × storage time) was used. Data were analyzed with ANOVA general linear model test. When differences among treatments were significant, the mean values were compared by the least significant difference Fisher test ($P < 0.05$). The Info Stat S.A software was used (Di Rienzo, et al., 2012).

3. Results

3.1. Effect of riboflavin concentration on pepper decay control

After 9 days, 45% of control fruit sticks presented soft-rot symptoms (Fig. 2A). Decay incidence was lower in riboflavin (*Rib*) treated peppers with greater efficacy as the concentration of the dipping solution increased in the range 1–100 μmol L^{-1}. Indeed, sticks treated with the highest *Rib* concentration only had incipient decay after 9 days of

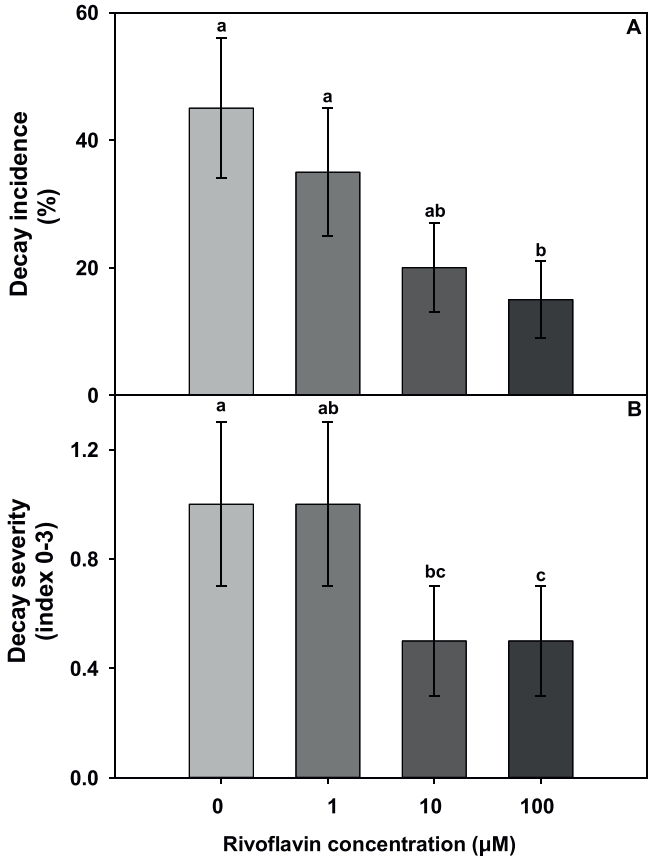

Fig. 2. A. Decay incidence and B. severity of green sweet pepper sticks dipped in 0 (control), 1; 10 or 100 μM riboflavin for 5 min and stored at 8 °C for 9 days. Each column value and bar represent the median and standard error, respectively. For decay incidence and decay severity letters indicate significance differences according to Chi-squared test and Mann-Whitney test ($P < 0.05$), respectively.

storage. The *Rib* treatment with 10 and 100 μmol L^{-1} also markedly reduced disease severity (Fig. 2B). Based on such results, to further investigate the effects on quality along with refrigerated storage, 100 μmol L^{-1} of *Rib* was selected.

3.2. Effect of 100 μmol L^{-1} riboflavin treatment on fresh-cut green pepper physical, chemical, and microbiological properties during refrigerated storage

3.2.1. Decay incidence and severity

The pepper sticks stored at 4 ºC showed limited decay until day 9. Subsequently, the incidence of soft rots markedly increased in control sticks (Fig. 3A). After 12 days of storage, 33% of control sticks showed decay symptoms as opposed to only 6% of *Rib*-treated fruit. Decay severity was also low during the first 9 days of cold storage without differences between treatments. However, at the last sampling date, it dramatically increased in the control fruit with values threefold higher than those found in *Rib* treated fruit (Fig. 3B).

3.2.2. Respiration and electrolyte leakage

To further characterize the effect of *Rib* on fruit metabolism and tissue integrity, we determined the changes in respiration rate and electrolyte leakage. Immediately after the dipping treatment, the fruit respiration rate was *c.a.* 0.4 mmol CO_2 kg^{-1} h^{-1}, with no differences from control (Fig. 4A). Subsequently, the respiration rate of control pepper showed an increasing trend, reaching 0.95 mmol CO_2 kg^{-1} h^{-1} at day 12. In contrast, *Rib*-treated fruit showed no significant changes in respiration along the storage period.

Electrolyte leakage (EL) showed no variation in control fruit until day 6 but increased rapidly afterward. On day 9 treated pepper already presented significantly lower EL than the control. The differences were even higher after 12 days of storage when EL values were four-fold higher in the control than in *Rib*-treated fruit (Fig. 4B).

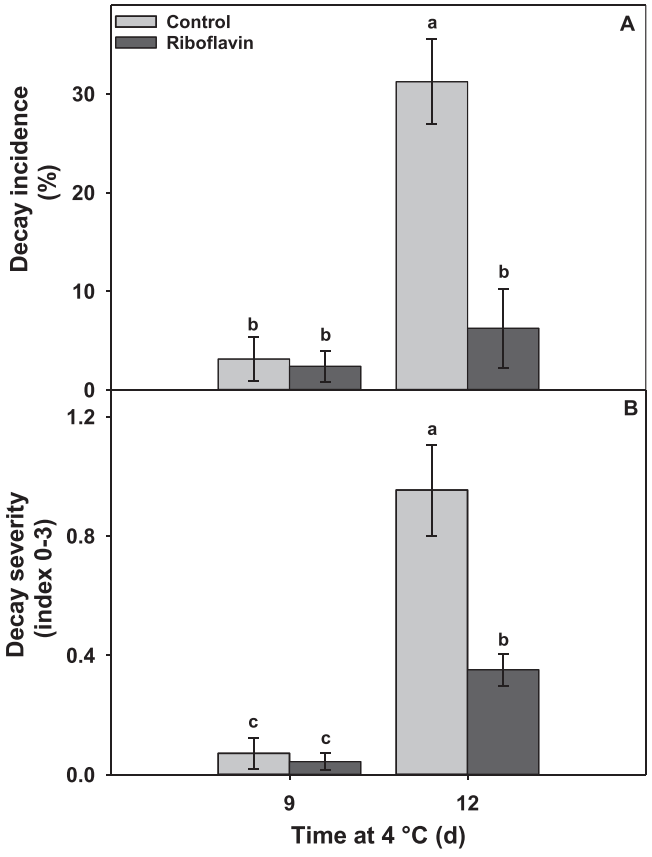

Fig. 3. A. Decay incidence and B. decay severity index in control and riboflavin treated green sweet pepper sticks during storage at 4 °C for 0, 6 9 and 12 days. Each column value and bar represent the mean and standard error, respectively. Letters indicate significant differences based on a Fisher test at $P < 0.05$.

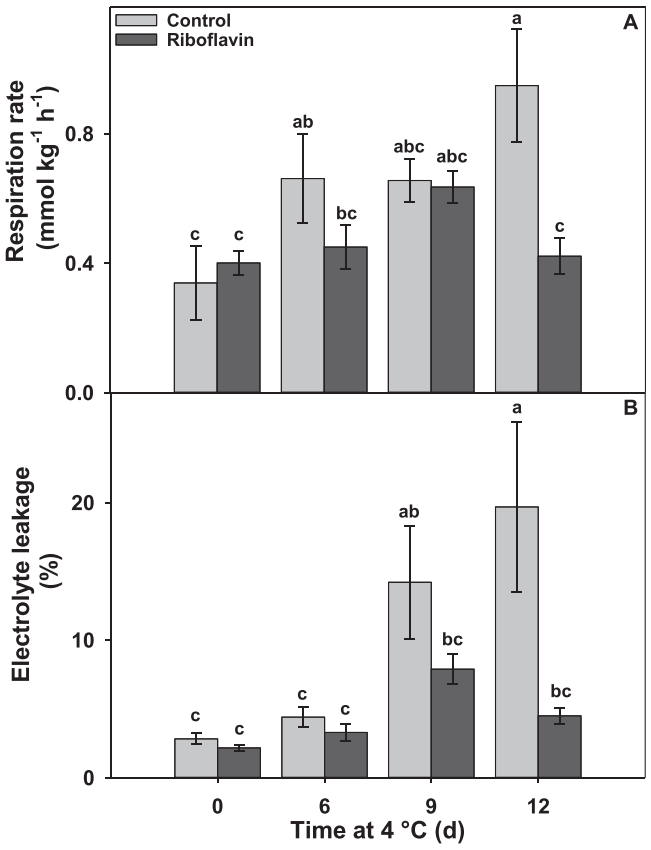

Fig. 4. A. Respiration rate and B. electrolyte leakage in control and riboflavin treated green sweet pepper sticks during storage at 4 °C for 0, 6, 9 and 12 days. Each column value and bar represent the mean and standard error, respectively. Letters indicate significant differences based on a Fisher test at $P < 0.05$.

3.2.3. Microbial counts

The counts of mesophilic aerobic bacteria and yeasts and molds were before storage 3.5 and 4.0 log CFU g^{-1}, respectively (Fig. 5). No changes in bacteria or yeasts and mold counts were found immediately after *Rib* treatment. Along with storage, the microbial counts of control pepper increased rapidly, reaching 7.3 and 8.5 log CFU g^{-1} for bacteria and yeasts and molds, respectively, after 12 days. *Rib*-treated pepper presented after both 6 and 9 days of cold storage significantly lower yeasts and molds counts (~1 log) than the control (Fig. 5A). Also, lower counts for mesophilic aerobic bacteria (1.4 log) were found in *Rib*-treated fruit at the last sampling date (Fig. 5B).

3.2.4. Texture

Besides the beneficial effect induced in decay control, *Rib* treatment also delayed the postharvest changes in fruit textural properties. Pepper sticks hardness was before cold storage ca. 8 N. Remarkably, hardness dropped rapidly in the control as storage time progressed but remained unchanged in *Rib*-treated fruit. In turn, treated peppers maintained firmer than control after both 9 and 12 days of storage (Fig. 6A). Control fruit also showed a reduction in stiffness during cold storage. Alike observed in hardness, the stiffness of *Rib*-treated fruit remained much more stable during storage. Consequently, after 12 days of refrigeration, *Rib*-treated pepper showed significantly higher (35%) stiffness values than the control (Fig. 6B). We also determine the resistance to bend (BR) of the pepper sticks as a textural quality indication. The BR showed a

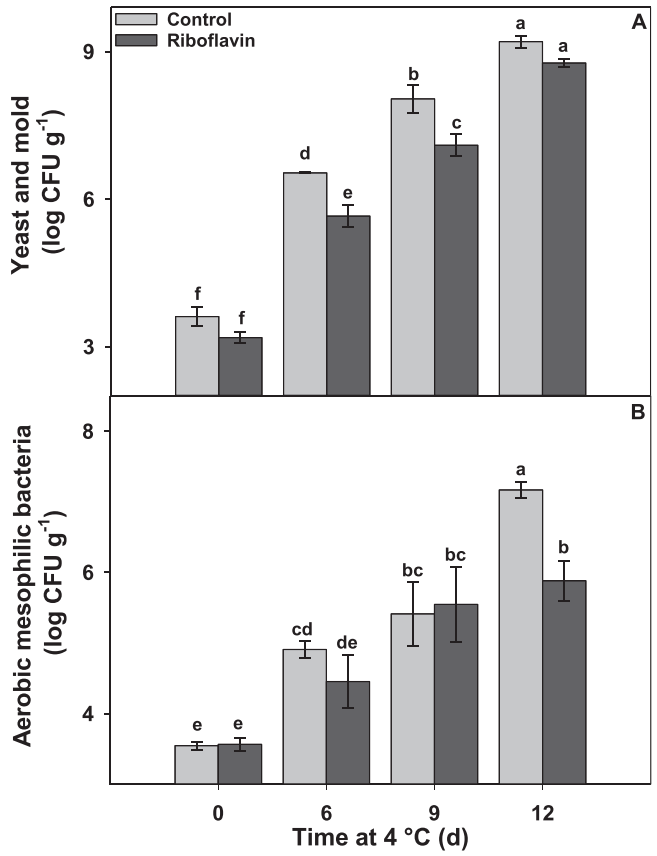

Fig. 5. A. Yeasts and molds and B. aerobic mesophilic bacteria counts in control and riboflavin treated pepper sticks during storage at 4 °C for 0, 6, 9 and 12 days. Each column value and bar represent the mean and standard error, respectively. Letters indicate significant differences based on a Fisher test at $P < 0.05$.

substantial reduction in the control during storage (40% drop in 12 days). In line with the results observed for tissue hardness and stiffness, the *Rib* treatments prevented such loss of BR (Fig. 6C). Overall, results show that pre-storage *Rib* dips effectively delayed texture changes in refrigerated fresh-cut pepper.

3.2.5. Weight loss, color, sugars, and organic acids

Weight loss was ca. 2% during the 12-day storage period without differences between control and *Rib*-treated fruit (Table 1). Regarding color, treatment or storage did not change lightness (L*). The a* color values increased just one unit in 12 days, indicating a very lowly loss of green color. The b* color component showed a mild increase during the initial 6 days of storage, followed by a final drop. All color changes were restricted and without differences between treatments (Table 1). Sugar content ranged between 23 and 29 g kg^{-1} and showed no variation during storage or in response to *Rib* treatments (Table 1). Citric and quinic acids accounted for 75% of total organic acids, followed by malic acid (Table 1). Titratable acidity presented a decreasing trend during cold storage but also without differences between control and treated fruit. The decrease in acidity resulted mainly from a drop in citric acid. Other than that, there were no alterations in acid profiles in response to *Rib* treatments. Altogether, results indicate that *Rib* dips caused no changes in pepper fruit susceptibility to dehydration, color, or chemical components relevant for flavor.

3.2.6. Ascorbic acid, total phenolics and antioxidant capacity

Ascorbic acid showed a decreasing trend during storage without differences between treatments (Fig. 7A). Total phenolics of control sticks showed a steady decrease during storage. Also, some losses were

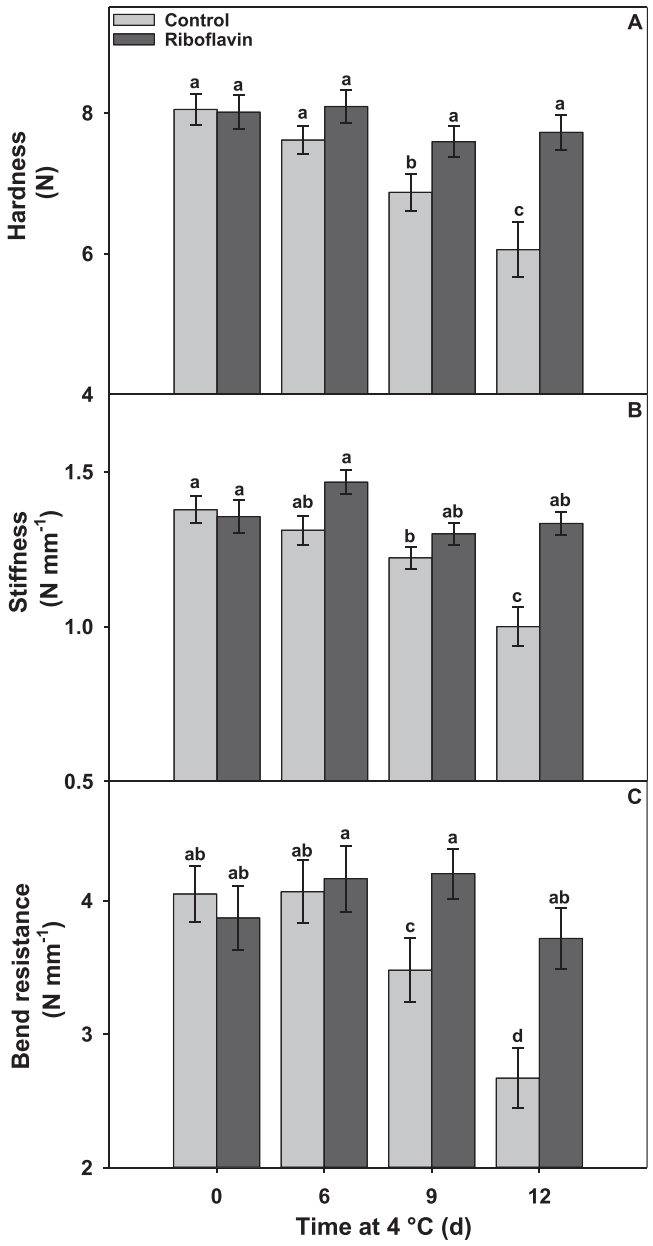

Fig. 6. A. Hardness, B. stiffness and C. bend resistance in control and riboflavin treated green sweet pepper sticks during storage at 4 °C for 0, 6, 9 and 12 days. Each column value and bar represent the mean and standard error, respectively. Letters indicate significant differences based on a Fisher test at $P < 0.05$.

found in *Rib*-treated pepper, though they were more limited. Therefore, *Rib*-treated pepper retained higher phenolics levels after 9 and 12 days at 4 °C (Fig. 7B). Fruit $DPPH^{\bullet}$ radical-scavenging capacity was unaffected immediately after the treatments and until 9 days of storage. At the last sampling date, antioxidant capacity dropped in the control but was stable in *Rib*-treated fruit (Fig. 7C).

4. Discussion

Riboflavin (*Rib*) is a precursor of central metabolic metabolites in bacteria, plants, and animals (Serer et al., 2019). Its derivatives FMN and FAD play crucial roles as cofactors for enzyme-catalyzed reactions. *Rib* depletion is known to impair mitochondrial function (Mosegaard et al., 2020). Flavoenzymes participate in the fatty acid desaturation and oxidative polypeptide folding within the endoplasmic reticulum (Lienhart et al., 2013; Kodali and Thorpe, 2010). Thus, protein and lipid

Table 1
Color (L* a*, b*), sugars, total acidity, citric, malic and quinic acid in control and riboflavin treated green sweet pepper sticks during storage at 4 °C for 0, 6, 9 and 12 days. Mean ± standard error are shown. Letters indicate significant differences based on a Fisher test at ($P < 0.05$).

		Time at 4 °C (d)			
		0	6	9	12
L*	*Control*	38.3±*0.5* c	39.8±*0.6* ab	41.3±*0.9* a	39.2±0.6 bc
	Riboflavin	38.2±*0.5* c	*39.2*±*0.7* bc	39.3±*0.6* abc	39.3±*0.6* bc
a*	*Control*	-16.3±*0.2* b	-16.4±*0.4* b	-15.0±*0.4* a	-15.1±*0.2* a
	Riboflavin	-16.2±*0.2* b	-16.0±*0.5* ab	-16.1±*0.3* ab	-15.2±*0.4* a
b*	*Control*	23.0±*0.5* b	25.9±*0.6* a	20.4±*0.7* c	21.2±*0.5* bc
	Riboflavin	22.8±*0.5* b	24.9±*1.1* a	22.6±0.6 bc	21.0±*0.9* bc
Weight loss (%)	*Control*	–	0.9±*0.1* c	1.3±*0.2* b	2.0±*0.2* a
	Riboflavin	–	0.8±*0.1* c	1.2 ±*0.2* bc	1.9±*0.2* a
Sugars (g kg^{-1})	*Control*	22.7± *0.3* a	28.7±*0.2* a	25.1 ±*0.2* a	23.4±*0.2* a
	Riboflavin	23.9± *0.2* a	26.4±*0.2* a	29.2±*0.1* a	27.4 ±*0.5* a
Total acidity (g kg^{-1})	*Control*	3.1±*3*×10^{-3} a	2.0 ± *2* × 10^{-3} c	2.3 ± *1* × 10^{-2} c	2.5 ± *3* × 10^{-2} c
	Riboflavin	3.0±*1*× 10^{-2} ab	2.0± *2* × 10^{-2} c	2.1 ± *9* × 10^{-3} c	2.5 ± *1* × 10^{-3} c
Citric acid (g kg^{-1})	*Control*	1.0±*2*× 10^{-2} a	0.2± *3* × 10^{-3} b	0.3 ± *2* × 10^{-3} b	0.4± *6* × 10^{-3} b
	Riboflavin	1.2±*4*× 10^{-3} a	0.3± *5* × 10^{-3} b	0.3 ± *4* × 10^{-3} b	0.4± *4* × 10^{-3} b
Quinic acid (g kg^{-1})	*Control*	1.3±*1*× 10^{-3} a	1.1± *2* × 10^{-3} ab	1.2 ± *6* × 10^{-3} ab	1.3± *2* × 10^{-2} ab
	Riboflavin	1.1±*3*× 10^{-3} ab	1.1± *1* × 10^{-2} b	1.0 ± *2* × 10^{-3} b	1.2 ± *5* × 10^{-3} ab
Malic acid (g kg^{-1})	*Control*	0.8±*3*× 10^{-3} ab	0.6± *1* × 10^{-3} b	0.6 ± *3* × 10^{-3} b	0.8 ± *9* × 10^{-3} ab
	Riboflavin	0.7±*3*× 10^{-3} ab	0.6± *7* × 10^{-3} b	0.7± *3* × 10^{-3} ab	0.8 ± *1* × 10^{-3} ab

metabolism also depend on riboflavin availability. In the food industry, *Rib* is considered a GRAS additive. So far, the *Rib* usage was for fortification purposes (Averianova et al., 2020). Although its deficiency is not very common, its levels markedly can be reduced during processing in some cases. That is the case for cereal flours, in which *Rib* is frequently added together with other B-group vitamins and minerals such as Fe (Suwannasom et al., 2020). The use of *Rib* in fruits and vegetable-derived foods is infrequent. Based on a few previous reports indicating the role of *Rib* in preventing diseases in field crops (Dong and Beer, 2000) and stress acclimation (Guhr et al., 2017), we decided to test its efficacy as a preservative agent in fresh-cut vegetables. Interestingly, we found that a 5 min pre-storage-dip in 100 μM *Rib* can significantly reduce postharvest spoilage (Fig. 2). Such *Rib* concentration is comparable with that used by Azami-Sardooei et al. (2010) and Guhr et al. (2017) but much lower (5–100-fold) than the applied in most other studies under field conditions (Dong and Beer, 2000; Zhang et al., 2009). High *Rib* intake is not generally considered an issue. However, some urine alternations may occur (Kenefick et al., 2015). Given the concentration of the used *Rib* solution (100 μmol L^{-1}) and the volume of the dipping solution absorbed (22 mL) per kilogram of fresh pepper tissue and assuming 100% stability for riboflavin (conservative given that some light exposure and degradation would be likely unavoidable during treatment and packing) the fruit will increase its *Rib* content by *c.a.* 1 mg kg^{-1}. This level is close to that generally found in peppers (FoodData Central, U.S Department of Agriculture, 2022) and comparable to several *Rib*-rich foods content (Price and Welch, 2013). Besides this estimation, it would be necessary to measure riboflavin in the fruit tissues performing a complete study regarding the fate of the applied *Rib* in the postharvest environment.

After selecting a proper condition for *Rib* postharvest treatment (100 μmol L^{-1} dip for 5 min), we studied the effect of *Rib* on the fruit́s physical, chemical, and microbiological properties at refrigeration conditions commonly found during commercial distribution (4 °C). The treatments caused a significant reduction in soft-rot incidence and severity (Fig. 3). Consistent with a low tissue disruption, *Rib* treated pepper maintained at long storage times lower respiration rate and electrolyte leakage than the corresponding control (Fig. 4). There are opposing data in the literature regarding any direct antimicrobial properties of *Rib*. Dong and Beer (2000) found no antimicrobial activity in vitro test against fungi or bacteria. Contrarily, other authors have observed that *Rib* can reduce fungal spore germination (Li et al., 2012) and bacterial growth (Ahgilan et al., 2016). The counts of mesophilic bacteria, yeasts and molds were not significantly affected immediately after *Rib* dips. Thus, at least under the concentration tested, a direct germicide action cannot be assigned to the disease control observed. The fact that *Rib* limited disease incidence, severity, and microbial counts at long storage times, points more to a germstatic effect, host induced defense responses, or their combination (Fig. 5). *Rib* has been reported to down-regulate critical genes involved in bacterial replication (Kwon et al., 2020), indicating that direct effects against pathogenic organisms are a possible mode of action. Systemic acquired resistance against some fungal, bacterial, and viral pathogens reported in plants treated with *Rib* under field conditions suggests that a potential role for induced host defenses may be relevant as well (Dong and Beer, 2000). In line with that, *Rib* and other B-group vitamins induced protection against pathogens in plant-microorganism symbiosis models (Dakora et al., 2015; Boubakri et al., 2016). Although, there are some hints about the underlying signaling pathways behind *Rib*-induced defense responses, and the overall mechanism is far from being understood. Some studies have suggested that the resistance induced by *Rib* is salicylic acid independent (Dong and Beer, 2000; Zhang et al., 2009). Participation of protein kinases, MPK3 and MPK6, reactive oxygen species, and Ca^{+2} in riboflavin defense activation cascades have been reported (Nie and Xu, 2016). However, the relative relevance of the mentioned *Rib* mechanisms seems to depend on the plant-pathogen system considered (Azami-Sardooei et al., 2010). It is important to note that all this progress comes from observations made in whole plants developing under field conditions. With regards to harvested organs, so far, only one study has reported improved disease control in response to *Rib* treatments (Li et al., 2012). The authors conducted a dipping treatment with 0–4 mM *Rib* to control *Alternaria* rots in room temperature stored Asian pear. The present work shows that *Rib* pre-storage dips may reduce spoilage in fresh-cut and refrigerated vegetables.

The *Rib* was also beneficial to maintain fruit textural properties during storage (Fig. 6). The treatments prevented the drop in tissue hardness and stiffness and reduced the bending resistance of the individual sticks. In fruit showing no melting softening properties such as pepper, texture modification frequently was linked to tissue water loss (Ilić et al., 2017). However, this did not seem to be the cause in this work since the beneficial effects of *Rib* dips in texture maintenance occurred with no differences in postharvest dehydration between control and treated fruit. Since *Rib* is an effective crosslinker when exposed to ultraviolet A light, it may be acting as a wall reinforcing agent (Uemura et al., 2019). Biopolymeric materials cross-link induced by *Rib* it is thought mediated by reactive oxygen species (ROS), which are already known to be active players in the polymerization of lignin and the

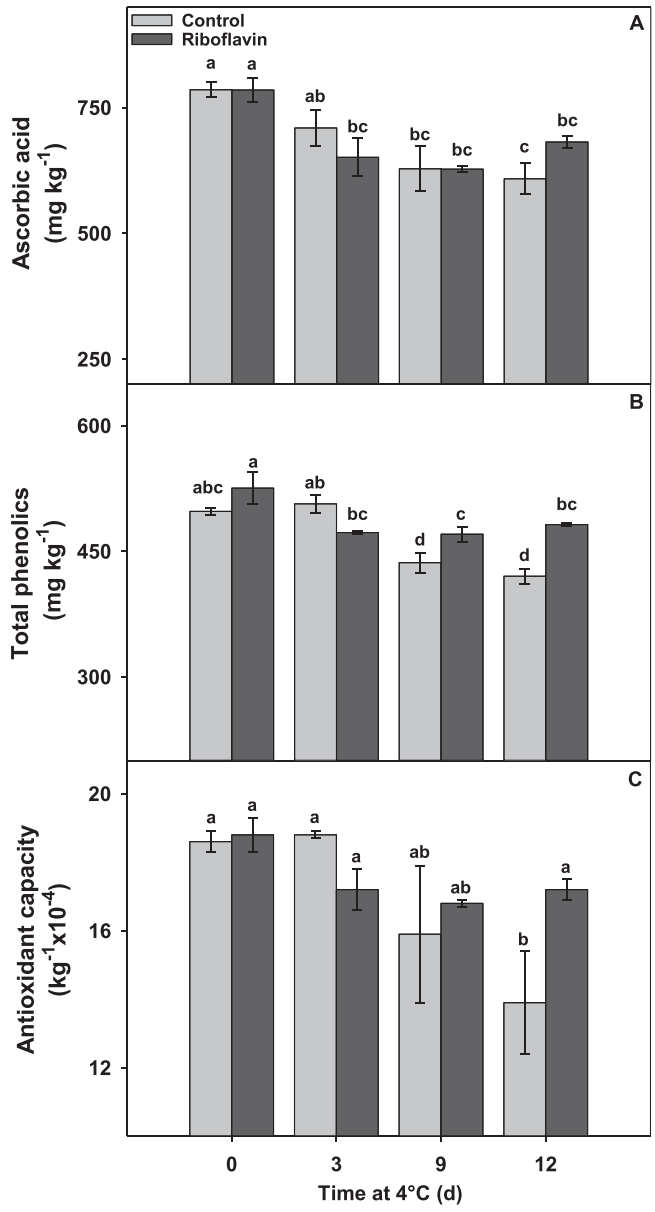

Fig. 7. A. Ascorbic acid, B. total phenolics and C. Antioxidant capacity against DPPH• radical in control and riboflavin treated pepper sticks during storage at 4 °C for 0, 6, 9 and 12 days. Each column value and bar represent the mean and standard error, respectively. Letters indicate significant differences based on a Fisher test at $P < 0.05$.

entanglement of proteins or other structural wall components (Liyama et al., 1994). The possible role of *Rib* as a crosslinker from cell wall polymers facilitating the healing of cut areas is attractive given that this may contribute to explaining changes in tissue rigidity, strength, and susceptibility for pathogen penetration (Liyama et al., 1994). Also, *Rib*-treated plants accumulate healing polysaccharides such as callose (Boubakri et al., 2013) and showed delayed senescence (Xiao et al., 2004). *Rib* is a yellow compound and fluorescent under blue light exposure. Previous works reported that *Rib* treatment leaves residual yellow color (Dong and Beer, 2000). However, the concentration used herein did not alter tissue color (Table 1). Soluble sugars, citric, malic, or quinic acids were unaffected by the treatment as well (Table 1). Altogether, these results show that *Rib* dips do not alter pepper color or taste-related compounds.

Regarding antioxidant compounds, the treatments also proved beneficial (Fig. 7). Such effect could be a consequence of improved maintenance of overall tissue integrity as depicted from electrolyte leakage results (Fig. 4B). Phenolics are potent free radical scavengers in fruits and vegetables (Cisneros-Zevallos, 2021). A higher level of total phenolics seems to explain the lower loss of antioxidant activity in *Rib*-treated fruit (Fig. 7B). Also, *Rib*-treated pear showing improved tolerance to fungal decay, presented greater levels of phenolic compounds (Li et al., 2012). In line with that, *Rib* upregulates genes in the phenylpropanoid pathway (Taheri et al., 2011). Based on that, it is tempting to link the higher retention of phenolics in *Rib*-treated tissues with the reduced incidence of soft rots (Fig. 3). Addressing whether there is such causal association would require further work. Ascorbic acid, one of the most abundant antioxidants in pepper fruit, is considered the most labile vitamin, easily altered by high temperature, light exposure, pH changes, and metal ions (Njus et al., 2020). However, in the present study, the loss of ascorbic acid through storage was relatively small and without differences between treatments (Fig. 7A). This is particularly relevant since, in some food matrices, *Rib* quickly degraded ascorbic acid in the presence of oxygen and light (Choe et al., 2005; Alvarado et al., 2022). Further work is needed to fully characterize the effect of *Rib* on fruit non-enzymatic and enzymatic antioxidant metabolism. Ashoori and Saedisomeolia (2014) reported that *Rib* could alter the activity of key-flavoenzymes such as glutathione reductase that modulate the levels of master redox regulators such as glutathione.

Altogether results show that *Rib* dips could reduce decay, prevent textural and nutrient deterioration, and extend the shelf-life of fresh-cut green peppers. With regards to the potential mechanisms involved, several interesting queries arise. One is whether there is a nexus between *Rib* responses and flavoenzymes involved in epigenetic control such as lysine-specific demethylase (Hino et al., 2012). Additional experiments should explore whether such mechanisms contribute to the preservative postharvest effects of *Rib* reported herein. In any case, the presented results clearly show for the first time that *Rib* may be a suitable preservative agent for fresh-cut vegetables.

5. Conclusions

In this work, we selected a suitable riboflavin concentration for preservative purposes in fresh-cut vegetables. Short (5 min) dips in 100 μmol L^{-1} reduced pepper decay incidence and severity. The treatment maintained tissue integrity delayed the increase in respiration and electrolyte leakage and prevented postharvest textural modifications. Furthermore, riboflavin treatment reduced the counts of yeasts and molds and aerobic mesophilic bacteria during storage. The riboflavin caused no alterations in color, sugars, acids, or weight loss and improved antioxidant stability during cold storage. Altogether, the results show for the first time that riboflavin may be a suitable GRAS preservative agent for fresh-cut products.

CRediT authorship contribution statement

Pintos Federico Martín: Investigation, Formal analysis, Writing – original draft. **Lemoine María Laura:** Conceptualization, Investigation, Review & editing. **Gergoff Grozeff Gustavo:** Investigation. **Hasperué Héctor Joaquín:** Review & editing. **Vicente Ariel Roberto:** Funding acquisition, Resources, Conceptualization, Writing – review & editing. **Rodoni Luis María:** Conceptualization, Investigation, Supervision, Formal analysis, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

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.

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