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Isochoric Cold Storage for Postharvest Preservation of Green Grapes

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ABSTRACT

The study evaluated the effects of isochoric cold storage (ICS) on preserving green grapes (*Vitis vinifera*). Grapes were stored in an impregnation solution within isochoric chambers at -1.5°C and 16 MPa for 7 days. After this, they were stored either with or without the solution at 4°C for up to 3 weeks. A separate set of grapes was stored in the same solution in a refrigerator at 4°C for 7 days, and then also stored either with or without the solution at 4°C for up to 3 weeks. Fresh grapes served as a control group, with direct storage at 4°C for 4 weeks. The study found that after 4 weeks of storage, microbial counts in isochoric impregnated grapes were 1.5 log colony-forming unit (CFU) g^{-1} for those stored without the solution and 2.1 log CFU g^{-1} for those stored with the solution, indicating a lower incidence of decay. In contrast, the refrigerated grapes showed significantly higher microbial counts, reaching 3.2 log CFU g^{-1} without the solution and 4.4 log CFU g^{-1} with the solution. The refrigerated grapes experienced rapid weight loss, softening, color changes, and shifts in soluble solids to titratable acidity (TA) ratios, while grapes that underwent isochoric treatment and those refrigerated in solution maintained better quality. This study demonstrates that isochoric impregnation effectively preserves grape quality and ensures microbial safety, presenting a promising alternative to synthetic fungicides. The combination of isochoric impregnation and refrigerated storage in a solution can significantly extend the shelf life of grapes.

1 | Introduction

Table grapes are popular for their taste and nutritional value [1] and are among the most widely grown and consumed fruits globally [2]. However, they are highly susceptible to postharvest fungal decay, leading to quality deterioration such as color loss, shriveling, softening, and nutrient depletion [1, 3–5]. While these changes cannot be fully prevented, they can be managed through preharvest and postharvest practices [6].

Nicolosi et al. [7] reported that ascorbic acid and total phenolic compound content in table grapes decreased significantly after 7 days of storage at 4°C with 90% relative humidity, highlighting the influence of storage conditions on nutritional quality. While grapes store best at -1.0 – 0.0°C , such temperatures can cause structural damage and increase decay risk. Various methods, including cold storage and sulfur dioxide (SO_2) fumigation are common preservation methods [8, 9]. However, *Botrytis cinerea*, the main cause of gray mold, thrives in cold, humid conditions

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[3]. Though SO_2 effectively controls gray mold, caused by *B. cinerea*, excessive use can harm fruit and pose health risks [10, 11]. Thus, alternative strategies are needed to extend grape shelf life without compromising quality.

Cold storage slows metabolism and inhibits microorganisms, but traditional freezing causes ice damage, energy inefficiency, and weight loss. Isochoric cold storage (ICS) mitigates these issues by maintaining subfreezing temperatures without freezing the food, preserving quality [12]. During ICS, the food is immersed in an aqueous solution inside an isochoric (constant volume) chamber. When the chamber is cooled to subfreezing temperatures, the system forms into two phases, ice and solution as temperature and pressure follow the liquidus curve of the solution's phase diagram [13]. Le Chatelier's principle dictates that as water expands upon freezing, the resulting high pressure restricts further ice formation, leaving part of the solution unfrozen [14]. In ICS, the device is designed so that the food product is kept in the unfrozen part of the chamber experiencing, thereby, subfreezing temperatures without freezing. This design preserves the food at subfreezing temperatures without forming internal ice, thus preventing the quality degradation typical of conventional freezing [13]. ICS has been used to preserve organs for transplantation [15] and, more recently, has been shown to improve the quality of solid foods like sweet cherries [16], spinach leaves [17], blueberries and potatoes [18, 19], and liquid foods like carrot juice [20, 21] and orange juice [22]. Additionally, the high pressures that are passively generated during ICS can be used for isochoric impregnation, whereby substances in the surrounding solution are infused into the food product [23, 24]. ICS with calcium impregnation is a promising method to improve the shelf life of sensitive food products like berries due to calcium's ability to cross-link demethylated carboxyl moieties of pectin found in the fruit's cell walls. The cross-linked pectin will mechanically reinforce the cells, which can prevent structural degradation and moisture loss. Not only will the high pressures from ICS cause efficient impregnation of calcium into the grapes; high pressures are also posited to activate pectin methylesterase, leading to more available binding sites, and therefore, increased cross-linking by calcium [20, 21, 25].

The present study aimed to investigate the effects of ICS with calcium impregnation on the quality attributes of table grapes. Specifically, we examined changes in key physicochemical, nutritional, and microbial safety properties of grapes stored with and without a calcium impregnation solution for 3 weeks following 1 week of ICS in solution. These results were compared to those of refrigerated grapes stored with and without the solution for 3 weeks following 1 week of refrigeration in the solution.

2 | Materials and Methods

2.1 | Materials

Fresh green seedless table grapes were obtained from a local retail store; cultivar-level identification was not available from the supplier. The grapes were selected based on uniformity in size and color, excluding any blemished, damaged, or diseased grapes. Each grape, cut with its stalk, weighed approximately 9 ± 1.5 g, and grapes were placed in sterile bags with a fruit-to-solution ratio of 1:10 (w/w). The solution was made by dissolving sucrose

and 2% CaCl_2 in water. The concentration of CaCl_2 used in this study was selected based on prior research to avoid negative sensory effects. Moderate levels of CaCl_2 have been shown to maintain fruit firmness and quality without imparting a noticeable salty taste, as panelists rated calcium-treated fruit (including those infiltrated with 2% CaCl_2) as crisply firm and equally acceptable in flavor compared with untreated fruit (no off-flavors were detected) [26]. In our study, the sucrose concentration was selected to achieve osmotic balance between the solution and the grapes in each bag.

2.2 | Postharvest ICS

For ICS, chambers constructed from Aluminum-7075 and coated with type-II anodizing were utilized. Each chamber, sourced from BioChoric Inc. (Bozeman, MT, USA), had a total volume of 1.5 L and was designed to withstand pressures up to 210 MPa. To monitor internal pressure levels, a pressure gauge was affixed to each chamber. At the base of each unit, a steel nut was placed to act as an ice nucleating agent, directing ice formation away from the sample bags. A plastic spacer was then positioned inside, followed by the placement of three sample bags above the spacer.

Once assembled, the chambers were filled with water, securely sealed, and stored in chest freezers (Magic Chef 123, Model #HMC9W3, MC Appliance Corporation, Wood Dale, IL) at -1.5°C for 7 days. Due to natural temperature fluctuations in the freezer, the internal chamber pressure averaged 16 ± 4 MPa over the storage duration. These pressure variations are inherent to the isochoric system but remained well below the threshold (~ 30 MPa) reported to induce cellular or textural damage in plant tissues [16], and no loss of firmness or structural integrity was observed. After the ICS process, the chambers were transferred to a refrigerator maintained at 4°C and left overnight to allow the ice inside the chamber to fully melt before further analysis.

2.3 | Experimental Protocol

Four different methods were employed to preserve fresh grapes for up to 4 weeks: (1) refrigerated storage in the solution at 4°C for the first 7 days, followed by transferring the grapes to a container without the solution at the same temperature for an additional 3 weeks (referred to as RF), (2) refrigerated storage in the solution at 4°C for the entire 4 weeks (referred to as RF + s), (3) ICS in the impregnation solution at -1.5°C for the first 7 days, followed by refrigeration at 4°C without the solution for an additional 3 weeks (referred to as ICS), (4) ICS in an impregnation solution at -1.5°C for the first 7 days, followed by refrigeration at 4°C with the solution for an additional 3 weeks (referred to as ICS + s). Since ICS treatment inherently involves storage in a liquid medium, we aimed to maintain a comparable procedure by including a solution in all treatments. This approach allows for a more consistent comparison of storage conditions and their impact on grape quality. The processing temperature of -1.5°C and the resulting pressure of 16 MPa were selected based on preliminary studies and literature data to avoid exceeding pressures of 30 MPa, which could cause cellular damage [16]. Notably, this pressure was not externally imposed but naturally emerged from the inherent dynamics of the isochoric system. Unlike conventional freezing, which occurs under isobaric conditions (i.e., constant atmospheric pressure), isochoric freezing

constrains the sample within a rigid, sealed chamber, allowing pressure and temperature to vary simultaneously [12].

2.4 | Microbiological Analysis

Microbiological analysis of grapes was performed on each sampling day. A single grape was randomly chosen from each sample bag and placed in a sterile stomacher bag (Whirl-Pak, Nasco, Fort Atkinson, WI, USA), then blended with phosphate-buffered saline (PBS) at a 1:10 ratio (weight/volume). Homogenization was carried out using a Seward Stomacher 80 Biomaster Laboratory Blender (Seward Inc., Bohemia, NY, USA) at a standard speed for 60 s. A series of 10-fold serial dilutions were prepared from the homogenized sample, and 1 mL of each dilution was spread onto plate count agar (PCA; Sigma-Aldrich, St. Louis, MO, USA) to determine the total mesophilic aerobic bacteria (TMAB) count. The plates were incubated at 30°C for 48 h, and colonies within the range of 25–250 were enumerated and expressed as colony-forming units (CFU) per gram of fruit.

For yeast and mold (YM) analysis, 1 mL of the homogenized sample was plated onto potato dextrose agar (PDA; Difco, Detroit, MI, USA) and incubated at 25°C for 72 h. Colony counts were recorded and analyzed accordingly.

2.5 | Measurement of Physicochemical Parameters

2.5.1 | Weight Change, Moisture Content, Soluble Solids Content (SSC), pH, and Titratable Acidity (TA)

Weight change was calculated as a percentage relative to the initial sample weight. Moisture content was determined using a conventional drying oven (Fisher Scientific Isotemp Model 750 F, Pittsburgh, PA, USA) set at 105°C until a constant weight was achieved, following AOAC guidelines [27]. SSC, pH, and TA were evaluated using juice extracted from a pooled sample of three grapes per pouch. The SSC, expressed as a percentage, was measured with a digital refractometer (Maselli LR-01, Maselli Misure S.p.A., Parma, Italy). The pH was recorded using a calibrated pH meter (Hanna Instruments, USA). TA, quantified as citric acid percentage, was determined by titrating a 5 mL juice sample with 0.01 N NaOH until reaching the citric acid endpoint at pH 8.2. All measurements were performed in triplicate to ensure accuracy and reproducibility.

2.5.2 | Surface Color and Visual Appearance

To evaluate color changes, a digital colorimeter (CM-3500d, Konica-Minolta Inc., Ramsey, NJ, USA) with a 3 mm diameter CM-A195 target mask was employed. Eight grapes from each treatment group were randomly selected for measurement. The color attributes were analyzed within the L^* , a^* , and b^* color space, where L^* represents lightness (ranging from 0 = black to 100 = white), a^* spans from green (negative values) to red (positive values), and b^* extends from blue (negative values) to yellow (positive values).

Before conducting grape surface color assessments, the device was calibrated using standardized black and white reference plates. The recorded L^* , a^* , and b^* values were then used to calculate total color differences (ΔE), hue angles (h , in degrees), and chroma values (C) based on the following Equations (1)–(3),

respectively. Hue angle provides an indication of color shade (ranging from 0° for red–purple to 180° for bluish–green), while chroma reflects color intensity and saturation [28].

$$\Delta E = \sqrt{(L_t^* - L_0^*)^2 + (a_t^* - a_0^*)^2 + (b_t^* - b_0^*)^2}, \quad (1)$$

$$h = \arctan \frac{b^*}{a^*} \times \frac{180}{\pi} (\text{degrees}) + 180^\circ$$

when $a^* < 0$ and $b^* > 0$ or $b^* < 0$,

(2)

$$C = \sqrt{a^{*2} + b^{*2}}. \quad (3)$$

Total color difference, ΔE , was calculated with L_0^* , a_0^* , and b_0^* for fresh grape berries, and L_t^* , a_t^* , and b_t^* which are the L^* , a^* , and b^* values of grape berries after postharvest treatment and storage.

The visual appearance of the grapes was documented using a digital camera, capturing images of six grapes with stalks and one grape cut in half each week.

2.5.3 | Texture Profile Analysis (TPA)

To assess texture characteristics, eight grapes from each replicate were randomly selected and subjected to compression testing using a texture analyzer (TA.XT Plus, Stable Micro Systems, UK) equipped with a cylindrical probe (12 mm diameter). The analysis was performed under the following conditions: pre-test speed of 5.0 mm s^{−1}, test speed of 2.0 mm s^{−1}, and post-test speed of 2.0 mm s^{−1}.

Each grape was positioned horizontally on the analyzer's stationary platform, with compression applied at the equatorial region of the fruit. The testing procedure involved two consecutive compression cycles, where samples were compressed to 30% of their original height at a speed of 2 mm s^{−1}. A 5-s interval was maintained between the two cycles. Texture parameters, including hardness, springiness, gumminess, and cohesiveness, were extracted from the force–time profile generated during testing.

2.5.4 | Total Soluble Phenolics (TSP) Content

The TSP content in grape extracts was determined in triplicate following the methodology described by Bilbao-Sainz et al. [20]. Frozen grape samples, previously stored at −80°C, were flash-frozen in liquid nitrogen for 1 min and then manually crushed into small fragments using a hammer. A 1 g portion of the crushed grape material was placed into a centrifuge tube containing 20 mL of methanol. The tubes were then refrigerated at 5°C for 18–24 h before being centrifuged at 29,100 g for 15 min.

The resulting supernatant was carefully transferred into amber vials to minimize light exposure and stored at −20°C until further analysis. For quantification, 1 mL of the supernatant was combined with 8 mL of distilled water and 1 mL of 1 N Folin-Ciocalteu reagent in a test tube, followed by thorough mixing. After a 5-min incubation, 1 mL of 15% sodium carbonate solution was added and mixed well. The mixture was left at room temperature (~20°C) for 2 h, after which the absorbance was measured at 725 nm using a Shimadzu PharmaSpec UV-1700 spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD). The TSP concentration was determined using a gallic acid standard

curve and expressed as gallic acid equivalents (g L^{-1}) on a dry basis.

2.6 | Statistical Analysis

Statistical analyses were performed using Microsoft Excel. Analysis of variance (ANOVA) was used to determine significant differences among sample groups, followed by Tukey's post hoc test for pairwise comparisons at a 95% confidence level. Results were also visualized using interval plots with 95% confidence intervals. Non-overlapping confidence intervals were interpreted as indicating statistically significant differences between group means at the selected confidence level.

3 | Results and Discussion

3.1 | Microbiological Analysis

The TMAB and YM counts on fresh grapes were 2.0 ± 0.6 and $2.2 \pm 0.6 \log \text{CFU g}^{-1}$, respectively (Figure 1). When stored in refrigeration without any treatment (RF), the microbial levels increased by 1.5 log for TMAB and 1 log for YM over a 4-week period at 4°C . Guillén et al. [29] observed a similar trend in refrigerated grapes, where both TMAB and YM counts increased by almost 2 logs after 4 weeks at 1°C . Conversely, grapes stored in a solution (RF+s) exhibited a reduction in microbial counts during the first week, followed by a gradual rise to 4.3 ± 0.2 and $4.4 \pm 0.1 \log \text{CFU g}^{-1}$, respectively, at Week 4. Grapes subjected to ICS showed a slight reduction in microbial populations after 1 week, with TMAB and YM counts decreasing to approximately 1.8 ± 0.3 and $1.6 \pm 0.3 \log \text{CFU g}^{-1}$, respectively. The microbial counts of ICS-treated grapes remained relatively stable throughout storage, with a slight increase observed by week 4, reaching approximately 2.6 ± 0.1 and $1.3 \pm 0.2 \log \text{CFU g}^{-1}$ for

TMAB, and 2.1 ± 0.2 and $1.5 \pm 0.2 \log \text{CFU g}^{-1}$ for YM when stored with and without solution, respectively.

Achieving complete microbial inactivation using conventional methods such as modified atmosphere packaging (MAP) and edible coatings is challenging. The TMAB of grapes decreased from $4.7 \pm 0.2 \log$ to $3.3 \pm 0.1 \log \text{CFU g}^{-1}$, and the YM count declined from 4.9 ± 0.3 to $2.1 \pm 0.1 \log \text{CFU g}^{-1}$ after 35 days of storage at 1°C under MAP conditions [30]. After 21 days of storage at 1°C followed by an additional 4 days at 20°C , the microbial populations on edible-coated grapes were reduced to $2.6 \log \text{CFU g}^{-1}$ for TMAB and $1.5 \log \text{CFU g}^{-1}$ for YM [31].

In comparison, ICS has been shown to substantially reduce microbial loads in various fruits and juices. For instance, microbial loads in blueberries were reduced after 4 weeks of ICS at -2.5°C [20] and microbial counts dropped below the detection limit for pomegranate fruit after 30 days at -2.5°C under isochoric conditions [32], for carrot juice after 12 weeks of storage at 4°C [20], and for orange juice after 7 days of storage at 5°C [22].

3.2 | Weight Change

Weight loss is a critical factor affecting the postharvest quality and marketability of table grapes, with a 5% threshold generally considered the maximum acceptable limit before visible shriveling occurs, rendering the fruit unappealing to consumers [33, 34]. In this study, refrigerated grapes stored without solution (RF) experienced a weight loss over the storage period, starting at 3% in week 1 and reaching 25% by Week 4 (Figure 2). This substantial reduction in weight can be attributed to natural physiological processes, including increased metabolic activity and tissue senescence, which promote water loss through transpiration and xylem back-flow [35, 36]. The observed weight loss trends align with previous research on grape storage dynamics [37, 38].

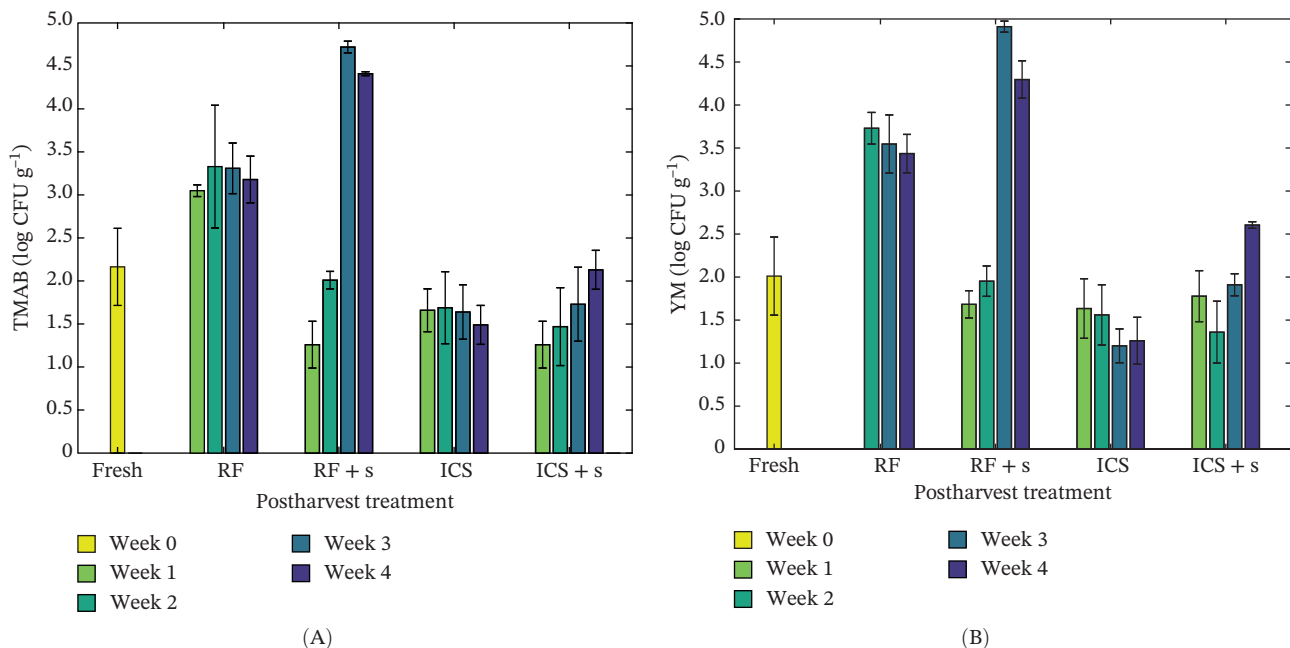


FIGURE 1 | (A) Total mesophilic aerobic bacteria (TMAB) and (B) yeast and mold (YM) of fresh and postharvest treated green grapes. ICS, isochoric cold storage with the solution (Week 1) then refrigerated without the solution (Week 2–4); ICS + s, isochoric cold storage with the solution (Week 1) then refrigerated with the solution (Week 2–4); RF, refrigerated with solution (Week 1) then refrigerated without the solution (Week 2–4); RF + s, refrigerated with solution. Data represents means and interval plots at 95% confidence interval.

In contrast, grapes subjected to ICS initially exhibited weight gain during the first week when stored in solution. However, when stored without a solution, weight loss became evident by the second week, increasing from 6% to 14% by Week 4. Despite this decline, ICS-treated grapes still retained more weight compared to their conventionally refrigerated counterparts (RF), suggesting that the preservation method, particularly when combined with calcium impregnation, helps mitigate dehydration. This improved retention of water content is likely due to the structural reinforcement of cellular tissues facilitated by calcium–pectin crosslinking [20, 21].

Refrigerated grapes stored with solution (RF + s) gained weight over the first 3 weeks, with no additional gain in Week 4, indicating the mitigating effect of the solution. However, isochoric-impregnated grapes stored with solution (ICS + s) continued to gain weight consistently throughout the 4-week storage period, likely influenced by both the impregnation solution and pressure-induced impregnation effects [35]. Similar benefits of ICS in minimizing weight loss have been reported in other perishable commodities, including cherries [16], grape tomatoes [13], apples, and sweet potatoes ([39], reinforcing the effectiveness of this storage approach in reducing moisture loss compared to conventional refrigeration.

3.3 | Moisture Content, SSC, pH, and TA

Low temperatures suppress aerobic respiration in fruits by slowing enzymatic and metabolic processes [40], while calcium chloride (CaCl₂) and sucrose-based osmotic treatments have been reported to further reduce oxygen consumption by stabilizing cell wall and

membrane structures and lowering free water activity, thereby delaying ripening and senescence during storage [41, 42].

Fresh grapes had an initial moisture content of 86.7% (Table 1). After 4 weeks of storage, a slight decline was observed in refrigerated grapes stored without a solution (RF), with moisture content decreasing to 84.1%. Similarly, isochoric impregnated grapes stored without a solution (ICS) experienced some moisture loss; however, no significant difference in moisture levels was detected when comparing ICS and RF grapes. On the other hand, grapes stored with a solution—both under refrigeration (RF + s) and isochoric conditions (ICS + s)—exhibited a slight increase in moisture content over the storage period, remaining comparable to fresh grapes.

The initial SSC of fresh grapes was 16.2% and remained unchanged after 1 week of storage in a solution. By week four, SSC had increased in refrigerated grapes stored without a solution (RF) and was significantly higher than in ICS grapes ($p < 0.05$), likely due to water loss concentrating the sugars in the fruit. Conversely, grapes refrigerated with a solution (RF + s) displayed a reduction in SSC. While ICS-treated grapes showed an increase in SSC over time, no notable differences were observed between ICS-stored grapes with (ICS + s) or without (ICS) a solution after 4 weeks.

The pH and TA of fresh grapes were recorded at 3.7% and 0.5%, respectively. As indicated in Table 1, while both pH and TA slightly declined in refrigerated grapes over storage, these changes were not substantial and followed a similar trend across treatments. This suggests that observed fluctuations were more likely due to natural fruit variability rather than the applied storage

TABLE 1 | Moisture content (%), pH, titratable acidity (TA, %), and soluble solids content (SSC, %) of fresh, RF: refrigerated with solution (Week 1) then refrigerated without the solution (Week 2–4), RF + s: refrigerated with solution, ICS: isochoric cold storage with the solution (Week 1) then refrigerated without the solution (Week 2–4), ICS + s: isochoric cold storage with the solution (Week 1) then refrigerated with the solution (Week 2–4).

Parameters	Fresh	Time (weeks)	RF	RF + s	ICS	ICS + s
Moisture (%)	86.72 ± 2.76 ^{abc}	1	85.55 ± 2.10 ^{bcd}	86.53 ± 0.76 ^{abc}	86.33 ± 0.52 ^{abc}	84.16 ± 1.74 ^{cde}
		2	82.91 ± 0.93 ^{de}	88.54 ± 0.47 ^a	86.03 ± 0.38 ^{abc}	84.41 ± 0.53 ^{cde}
		3	85.14 ± 2.75 ^{bcd}	87.47 ± 0.22 ^{abc}	84.35 ± 0.60 ^{cde}	85.51 ± 0.82 ^{bcd}
		4	84.11 ± 1.42 ^{cde}	88.49 ± 0.30 ^a	82.27 ± 2.19 ^e	85.62 ± 0.72 ^{bcd}
pH	3.65 ± 0.06 ^a	1	3.47 ± 0.14 ^{bc}	3.43 ± 0.04 ^{cd}	3.62 ± 0.08 ^{ab}	3.62 ± 0.06 ^{ab}
		2	3.57 ± 0.03 ^{ab}	3.57 ± 0.03 ^{ab}	3.67 ± 0.10 ^a	3.63 ± 0.03 ^{ab}
		3	3.66 ± 0.004 ^a	3.31 ± 0.09 ^{de}	3.61 ± 0.03 ^{ab}	3.54 ± 0.09 ^{bc}
		4	3.73 ± 0.09 ^a	3.31 ± 0.09 ^{de}	3.42 ± 0.11 ^{cd}	3.25 ± 0.02 ^e
TA (%)	0.45 ± 0.06 ^{def}	1	0.44 ± 0.03 ^f	0.53 ± 0.02 ^{abcdef}	0.55 ± 0.02 ^{abcde}	0.61 ± 0.03 ^{ab}
		2	0.51 ± 0.02 ^{cdef}	0.46 ± 0.05 ^{ef}	0.63 ± 0.03 ^a	0.52 ± 0.02 ^{bcd}
		3	0.55 ± 0.05 ^{abcde}	0.54 ± 0.09 ^{abcde}	0.53 ± 0.03 ^{bcd}	0.60 ± 0.07 ^{abc}
		4	0.56 ± 0.06 ^{abcd}	0.52 ± 0.08 ^{bcd}	0.59 ± 0.09 ^{abcd}	0.50 ± 0.03 ^{cdef}
SSC (%)	16.18 ± 1.91 ^{ef}	1	16.73 ± 0.25 ^{ef}	16.23 ± 0.25 ^{ef}	17.13 ± 0.75 ^{ef}	21.37 ± 1.50 ^{ab}
		2	20.40 ± 1.11 ^{abc}	15.97 ± 0.49 ^{fg}	19.60 ± 0.66 ^{bcd}	19.63 ± 1.34 ^{bcd}
		3	17.10 ± 1.95 ^{ef}	14.03 ± 1.46 ^{gh}	22.13 ± 1.12 ^a	19.13 ± 0.35 ^{cd}
		4	20.83 ± 2.82 ^{cd}	12.97 ± 0.68 ^h	18.13 ± 0.87 ^{de}	17.73 ± 0.76 ^{def}

Note: Values are presented as mean ± standard deviation. Different lowercase superscript letters within the same row indicate statistically significant differences ($p < 0.05$) based on ANOVA followed by Tukey's post hoc test.

conditions, as no clear treatment-dependent patterns emerged. The expected decline in acidity did not cause major pH shifts, likely due to the buffering effect of organic acids in grapes [36]. However, grapes stored with a solution (RF + s and ICS + s) exhibited a more pronounced reduction in pH and TA compared to those stored without a solution (RF and ICS) after 4 weeks. This phenomenon may be linked to the migration of potassium ions from the fruit into the solution. Since potassium plays a key role in regulating the sugar-to-acid ratio in grape juice, its movement out of the fruit can increase pH while reducing TA [43]. This ion exchange process likely contributed to the greater acidity loss observed in grapes stored with a solution.

3.4 | Surface Color and Visual Appearance

Table 2 presents the color parameters (L^* , a^* , b^* , C , h° , and ΔE) for fresh and stored grapes subjected to different postharvest

treatments, including refrigeration and ICS. A decline in L^* values was observed across all treatments over time, indicating progressive browning. While isochoric impregnation helped slow this browning effect, it did not fully prevent it, aligning with previous research findings [18, 19]. In fresh grapes, polyphenol oxidase (PPO) is naturally separated from its phenolic substrates due to the intact structure of cellular membranes [44]. However, during ICS, the application of pressure compromises membrane integrity. This disruption allows PPO to interact with its substrates, triggering enzymatic oxidation and initiating the browning reaction. Membrane stability is enhanced as calcium ions (Ca^{2+}) interact with the negatively charged phospholipid components and carboxyl groups of membrane proteins. This interaction strengthens the membrane, reducing solute leakage [44]. Consequently, the addition of Ca^{2+} to the impregnation solution can inhibit the browning reaction by maintaining cellular integrity and limiting the contact between PPO and its substrates.

TABLE 2 | Lightness (L^*), redness (a^*), yellowness (b^*), chroma (C^*), and hue angle (h°) of fresh, RF: refrigerated with solution (Week 1) then refrigerated without the solution (Week 2–4), RF + s: refrigerated with solution, ICS: isochoric cold storage with the solution (Week 1) then refrigerated without the solution (Week 2–4), ICS + s: isochoric cold storage with the solution (Week 1) then refrigerated with the solution (Week 2–4).

Color attribute	Fresh	Time (weeks)	RF	RF + s	ICS	ICS + s
L^*	31.23 ± 2.31^{ab}	1	33.50 ± 0.87^a	31.78 ± 1.22^{ab}	26.29 ± 1.30^d	27.80 ± 2.87^{cd}
		2	33.34 ± 1.40^a	32.23 ± 1.52^{ab}	25.87 ± 0.69^d	26.98 ± 0.65^{cd}
		3	33.65 ± 1.86^a	31.76 ± 1.51^{ab}	27.17 ± 1.13^{cd}	26.50 ± 1.23^{cd}
		4	29.83 ± 1.79^{bc}	31.31 ± 1.90^{ab}	27.37 ± 1.77^{cd}	27.01 ± 2.07^{cd}
a^*	-1.38 ± 0.20^{ab}	1	-1.72 ± 0.19^a	-1.33 ± 0.26^{abc}	-0.69 ± 0.18^d	-0.75 ± 0.38^d
		2	-1.48 ± 0.23^{ab}	-1.37 ± 0.27^{ab}	-0.67 ± 0.07^d	-0.66 ± 0.10^d
		3	-1.58 ± 0.38^a	-1.25 ± 0.23^{abc}	-0.74 ± 0.18^d	-0.87 ± 0.20^{cd}
		4	-1.46 ± 0.21^{ab}	-1.12 ± 0.27^{bcd}	-0.68 ± 0.13^d	-0.79 ± 0.18^d
b^*	3.41 ± 0.97^{ab}	1	3.98 ± 1.00^a	1.62 ± 1.40^{cd}	0.59 ± 0.52^e	0.09 ± 0.20^e
		2	1.56 ± 0.79^{bcd}	0.99 ± 0.51^d	0.59 ± 0.39^e	0.30 ± 0.13^e
		3	2.91 ± 1.24^{abc}	1.38 ± 0.74^{cd}	0.94 ± 0.34^d	0.30 ± 0.28^e
		4	2.51 ± 1.28^{abcd}	2.46 ± 1.14^{abcd}	1.34 ± 0.84^{cd}	0.56 ± 0.34^e
Chroma (C^*)	3.70 ± 0.85^{ab}	1	4.34 ± 0.96^a	2.18 ± 1.26^{cdef}	0.97 ± 0.41^{fg}	0.77 ± 0.40^g
		2	2.71 ± 0.60^{bcde}	1.77 ± 0.20^{defg}	0.95 ± 0.22^{fg}	0.73 ± 0.13^g
		3	3.44 ± 0.82^{abc}	1.94 ± 0.50^{defg}	1.24 ± 0.17^{fg}	0.95 ± 0.22^{fg}
		4	2.98 ± 1.09^{abcd}	2.76 ± 0.98^{bcde}	1.55 ± 0.75^{efg}	1.00 ± 0.28^{fg}
Hue (h°)	113.54 ± 5.09^e	1	114.17 ± 5.06^e	136.24 ± 16.59^{bcde}	146.85 ± 22.73^{abcd}	176.00 ± 12.18^a
		2	124.16 ± 12.41^{cde}	145.40 ± 18.77^{abcde}	143.12 ± 21.83^{bcde}	156.03 ± 8.30^{abc}
		3	122.76 ± 21.35^{de}	135.50 ± 17.94^{bcde}	130.12 ± 16.18^{cde}	162.08 ± 16.91^{ab}
		4	124.04 ± 14.45^{de}	117.98 ± 14.14^{de}	121.75 ± 16.06^{de}	146.40 ± 15.59^{abcd}
ΔE	—	1	2.52 ± 0.97^{cd}	2.49 ± 0.67^{cd}	5.77 ± 1.18^a	5.42 ± 1.16^a
		2	1.71 ± 1.06^d	2.91 ± 0.89^{bcd}	6.11 ± 0.71^a	5.32 ± 0.59^a
		3	2.69 ± 1.98^{bcd}	2.49 ± 0.92^{cd}	4.85 ± 0.92^{ab}	5.71 ± 1.14^a
		4	2.51 ± 0.92^{cd}	2.07 ± 1.10^d	4.56 ± 1.60^{abc}	5.26 ± 1.69^a

Note: Values are presented as mean $\pm$ standard deviation. Different lowercase superscript letters within the same row indicate statistically significant differences ($p < 0.05$) based on ANOVA followed by Tukey's post hoc test.

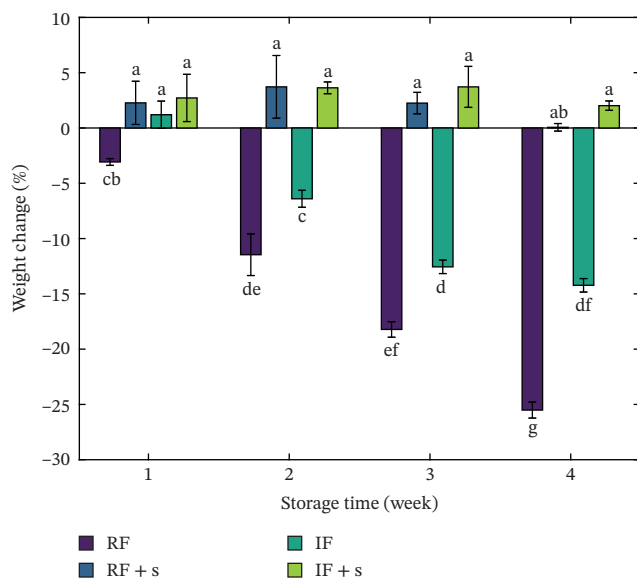


FIGURE 2 | Weight change (%) of postharvest treated green grape. ICS, isochoric cold storage with the solution (Week 1) then refrigerated without the solution (Week 2–4); ICS + s, isochoric cold storage with the solution (Week 1) then refrigerated with the solution (Week 2–4); RF, refrigerated with solution (Week 1) then refrigerated without the solution (Week 2–4); RF + s, refrigerated with solution. Data represents means and interval plots at 95% confidence interval.

The a^* and b^* values indicated lower greenness and yellowness in isochoric impregnated grapes (ICS and ICS + s) compared to refrigerated ones (RF and RF + s). Changes in chroma values were minimal over the storage period, though variations were evident between treatments. Isochoric impregnation resulted in slightly lower color saturation (reduced color purity), whereas refrigerated grapes maintained relatively stable chroma values. However, these subtle shifts in color intensity were not visually perceptible and did not affect the commercial appeal of the grapes [45]. After 4 weeks, the hue angle of isochoric impregnated grapes remained higher than that of refrigerated grapes stored with solution, indicating better preservation of greenish hues. The color changes observed during senescence are primarily attributed to anthocyanin degradation and other pigment breakdown processes, alongside structural disruptions that allow enzymatic oxidation and browning [46, 47].

Figure 3 provides a visual comparison of fresh, refrigerated, and isochoric cold-stored grapes. No obvious differences in appearance were detected among fresh and stored grapes after 1 week (Figure 3A). However, when cross-sectioned, isochoric-treated grapes (ICS and ICS + s) appeared more translucent than refrigerated ones (RF and RF + s) at the same time point (Figure 3B). Both RF and ICS grapes without solution exhibited visible shriveling after 4 weeks of storage, which is consistent with the higher weight loss reported in Figure 2. In contrast, grapes stored with the impregnation solution (RF + s and ICS + s) showed a similar overall appearance, with only one out of six grapes exhibiting slight browning after 4 weeks. This observation is supported by the relatively stable L^* , a^* , and b^* values and lower ΔE values for RF + s compared with ICS and ICS + s. Although ICS and ICS + s showed higher ΔE values than RF treatments, these changes mainly reflect reduced lightness and chroma rather than pronounced browning, as indicated by the hue angle values remaining within the green–yellow range.

3.5 | Textural Properties

Figure 4 presents the results of TPA, illustrating changes in grape hardness, gumminess, springiness, and cohesiveness over storage. The postharvest treatment and the presence or absence of a solution significantly influenced the hardness of grapes during storage. The most pronounced softening occurred in refrigerated (RF) and isochoric cold-stored (ICS) grapes that were not stored in a solution, with hardness reaching its lowest point by the fourth week. This rapid decline in firmness is likely linked to the enzymatic degradation of pectic substances within the cell wall, leading to the breakdown of structural pectin backbones [48]. A gradual decline in hardness was observed in both refrigerated (RF and RF + s) and isochoric-stored grapes without a solution (ICS), with moisture loss being a key factor contributing to softening [49]. However, grapes stored in a Ca^{2+} -enriched solution exhibited a slower decline in hardness, as calcium facilitated the formation of structural cross-links between uronic acid carboxyl groups, reinforcing the fruit's texture [49]. Similar calcium-induced firmness retention has been reported for zucchini [50] and apricots [51]. Isochoric impregnated grapes stored with solution (ICS + s) maintained the highest hardness. These results suggest that ICS in a Ca^{2+} -enriched solution may facilitate calcium penetration into fruit tissues, which might have contributed to improved texture retention [49]. Since texture degradation is a major factor affecting fruit quality, ICS appears to mitigate this deterioration, as demonstrated in prior research on sweet cherries [16], grape tomatoes [13], and blueberries [20].

Springiness (Figure 4B) refers to the fruit's ability to recover its shape after being compressed, an indicator of its elastic properties. Cohesiveness (Figure 4C) is measured as the ratio of the work done during the second compression to that of the first, indicating the structural integrity of the sample. Gumminess (Figure 4D), a combined measure of hardness and cohesiveness, describes how the fruit resists deformation under applied pressure [52]. During storage, both springiness and gumminess decreased in grapes stored without solution. In contrast, grapes stored with solution (RF + s and ICS + s) maintained consistent springiness and gumminess throughout the storage period. Meanwhile, cohesiveness remained relatively stable across all samples. These findings emphasize the critical role of storage solution in preserving grape texture and delaying structural degradation over time.

3.6 | TSP Content

Figure 5 illustrates the TSP content in grapes following postharvest treatments and throughout the storage period. After 1 week, no significant differences in TSP levels were observed between refrigerated grapes stored with or without a solution (RF and RF + s). However, isochoric impregnated grapes, regardless of storage conditions (ICS and ICS + s), exhibited higher TSP content compared to their refrigerated counterparts. By the 4th week, TSP levels declined across all treatments. Despite this reduction, grapes stored in a solution (RF + s and ICS + s) retained significantly higher TSP levels than those stored without a solution (RF and ICS).

Previous research has documented a gradual decline in TSP content during cold storage of grapes [37, 53]. This reduction might be attributed to PPO activity, which facilitates the oxidation of

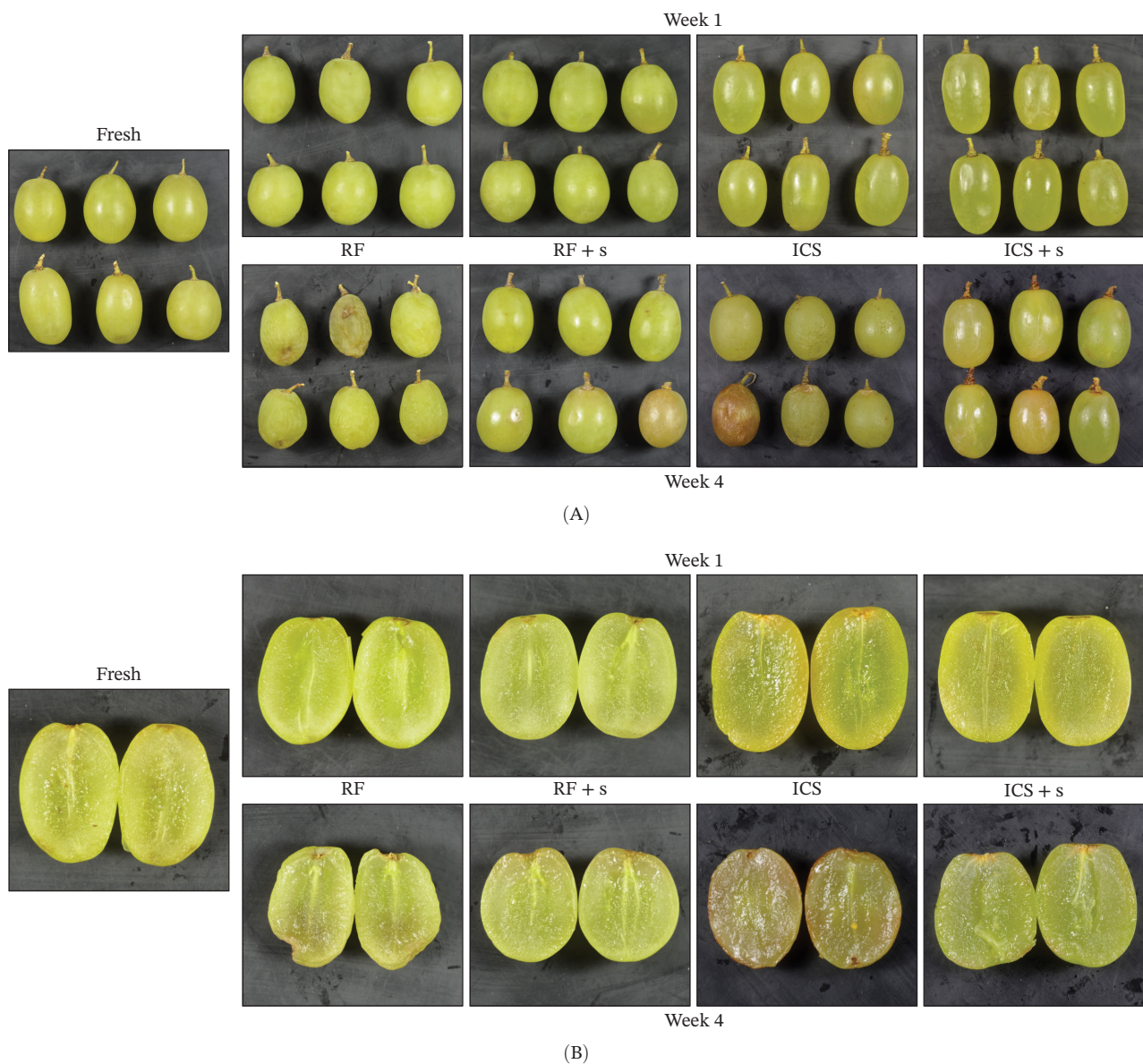


FIGURE 3 | The appearance of fresh and postharvest treated green grapes. ICS, isochoric cold storage with the solution (Week 1) then refrigerated without the solution (Week 2–4); ICS + s, isochoric cold storage with the solution (Week 1) then refrigerated with the solution (Week 2–4); RF, refrigerated with solution (Week 1) then refrigerated without the solution (Week 2–4); RF + s, refrigerated with solution. (A) whole grapes and (B) halved grapes.

phenolic compounds to quinones in the presence of oxygen [44]. Another possible factor contributing to TSP degradation might be the decline in phenylalanine ammonia-lyase (PAL) activity during senescence [54]. Although PPO and PAL activities were not directly measured in this study, the observed decline in TSP is consistent with well-established postharvest biochemical trends reported for grapes during cold storage. Environmental factors, including exposure to oxygen and light, also significantly influence the stability of phenolic antioxidants in fruits during postharvest storage [55, 56].

4 | Conclusion

This study demonstrates that ICS is an effective technology for extending the shelf-life of grapes while preserving their quality.

Compared with conventional refrigeration, ICS not only slows microbial growth, minimizes weight loss, and maintains total phenolic content, but it also offers potential energy savings due to reduced cooling requirements and prevention of ice crystal formation [12, 57, 58]. The use of impregnation solutions containing sucrose and CaCl_2 further enhances these benefits, highlighting ICS's potential for broader commercial applications. Grapes stored with ICS can be marketed either with the impregnation solution or after its removal, providing flexibility in postharvest handling. Overall, ICS presents a sustainable and innovative alternative for food producers seeking efficient preservation strategies with extended shelf-life and improved product quality. Future work should evaluate the sensory attributes and consumer perception of grapes stored under ICS to support commercial application. Additionally, techno-economic and scalability evaluations are recommended to determine the feasibility and

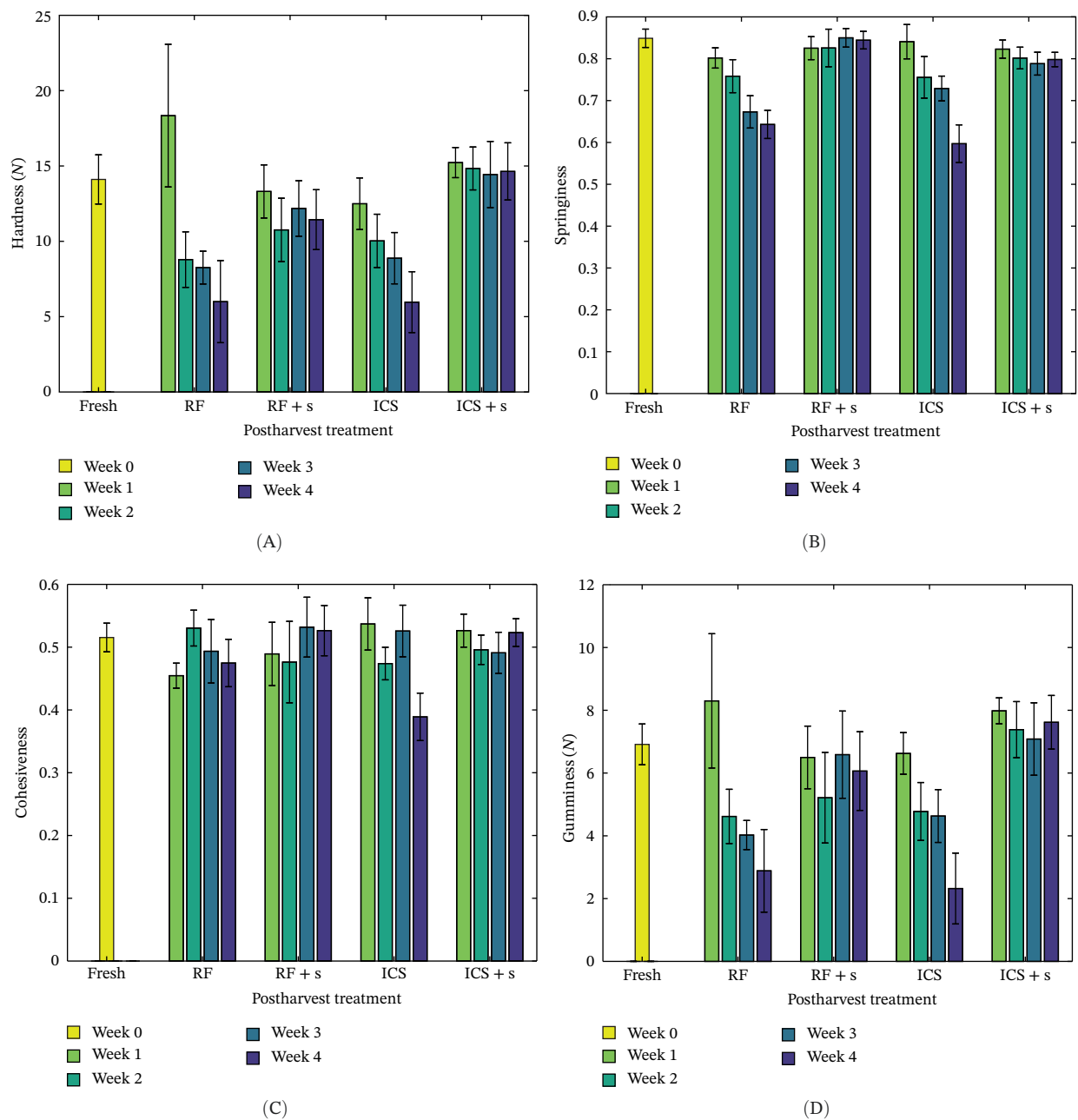


FIGURE 4 | (A) Hardness (N), (B) springiness, (C) cohesiveness, (D) gumminess (N) of fresh and postharvest treated green grapes. ICS, isochoric cold storage with the solution (Week 1) then refrigerated without the solution (Week 2–4); ICS + s, isochoric cold storage with the solution (Week 1) then refrigerated with the solution (Week 2–4); RF, refrigerated with solution (Week 1) then refrigerated without the solution (Week 2–4); RF + s, refrigerated with solution. Data represents means and interval plots at 95% confidence interval.

industrial implementation potential of ICS. In addition to economic benefits of lower energy use, previous analyses suggest that ICS could be implemented large-scale using cost-effective

and commercially available equipment [59], although additional techno-economic and scalability evaluations are recommended to determine the potential for industrial applications of ICS.

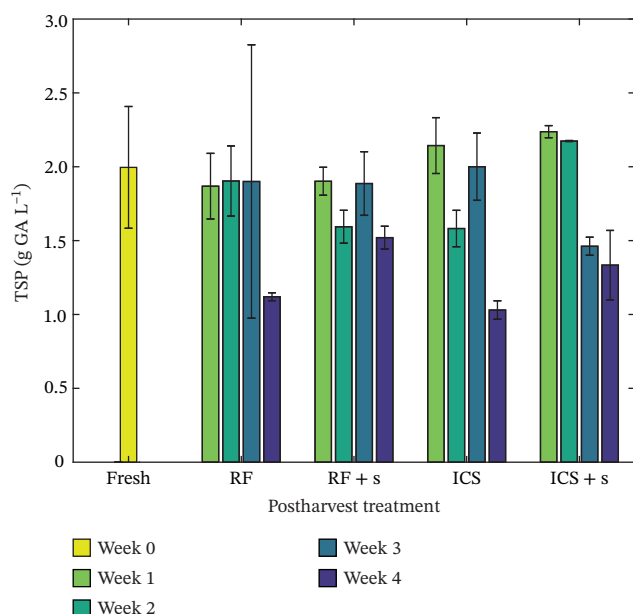


FIGURE 5 | Total soluble phenolic (TSP, g gallic acid L⁻¹, dry basis) content of fresh and postharvest treated green grapes. ICS, isochoric cold storage with the solution (Week 1) then refrigerated without the solution (Week 2–4); ICS + s, isochoric cold storage with the solution (Week 1) then refrigerated with the solution (Week 2–4); RF, refrigerated with solution (Week 1) then refrigerated without the solution (Week 2–4); RF + s, refrigerated with solution. Data represents means and interval plots at 95% confidence interval.

Author Contributions

Sumeyye Atci and Valerie S. McGraw: conceptualization, methodology, validation, formal analysis, investigation, data curation, visualization, writing – original draft preparation, writing – reviewing and editing. **Gabriel Pinto Bermeo:** investigation, formal analysis, data curation. **Tara McHugh and Matthew J. Powell-Palm:** funding acquisition. **Boris Rubinsky:** funding acquisition, conceptualization. **Cristina Bilbao-Sainz:** conceptualization, writing – reviewing and editing, supervision, project administration, funding acquisition.

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Conflicts of Interest

Boris Rubinsky and Matthew J. Powell-Palm have a financial stake in BioChoric Inc., a private entity working on the commercialization of isochoric technologies. Valerie S. McGraw is also affiliated with BioChoric Inc. The other authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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