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Research article

Changes in bioactive compounds, antioxidant, and enzymatic activities in immature *Cucurbita ficifolia* fruit during postharvest storage

Amanda Mora-Cuevas[1], Elia Nora Aquino-Bolaños[1], Liliana Lara-Capistran[2], José Luis Chávez-Servia[3], Eloy Rodríguez-deLeón[4], Manuel Alberto Santos-Basurto[5] and Jimena Esther Alba-Jiménez[6,*]

[1] Centro de Investigación y Desarrollo en Alimentos (CIDEA), Universidad Veracruzana, Xalapa, Veracruz, México
[2] Facultad de Ciencias Agrícolas, Universidad Veracruzana, Xalapa, Veracruz, México
[3] CIIDIR-Oaxaca, Instituto Politécnico Nacional, Oaxaca, México
[4] Facultad de Química, Universidad Autónoma de Querétaro (UAQ), Querétaro, Mexico
[5] Science Department, Arkansas State University, Campus Querétaro, Querétaro, México
[6] SECIHTI-Centro de Investigación y Desarrollo en Alimentos (CIDEA), Universidad Veracruzana, Xalapa, Veracruz, México

*** Correspondence:** Email: jimalba@uv.mx; Tel: +522283045426.

Abstract: Chilacayote (*Cucurbita ficifolia* Bouché) is a fruit rich in bioactive compounds; however, in its immature state, it is highly perishable. This limited shelf life requires the application of postharvest technologies, such as refrigeration. The objective of this study was to evaluate the changes in antioxidant content and enzymatic activity in immature *C. ficifolia* fruit during storage. Immature fruits were stored for 15 days at 5 and 10 °C [95% relative humidity (RH)] and room temperature (22 °C, 83% RH). Weight loss, total polyphenols and flavonoids, ascorbic acid, and antioxidant activity were assessed every five days; phenolic compounds were identified by HPLC, and enzymatic activity was also monitored. The results indicate that the greatest weight loss occurred at 22 °C (9.2%). Concentration of total polyphenols and flavonoids was higher at 10 and 22 °C (0.24–0.27 mg EAG g^{-1} FW and 0.061–0.076 mg ECAT g^{-1} FW, respectively). A decrease in ascorbic acid content was observed across all temperatures (averaging 17.1 mg EAA 100 g^{-1} FW). Higher antioxidant activity was recorded at 5 and 10 °C for FRAP (1.03 and 0.93 µmol Etrolox g^{-1} FW, respectively) and at 10 and 22 °C for DPPH (1.5 and 1.3 µmol ET g^{-1} FW, respectively). Four phenolic compounds were identified: p-hydroxybenzoic acid, p-coumaric acid, ferulic acid, and caffeic

acid. p-Hydroxybenzoic acid (noted anti-inflammatory, antioxidant, antidiabetic, and antimicrobial properties) was the most abundant at 10 and 22 °C. Furthermore, polyphenol oxidase (PPO) and peroxidase (POD) enzymatic activities were lower at 10 and 22 °C (0.047–0.060 U min^{-1} g^{-1} FW and 1.01–0.89 U min^{-1} g^{-1} FW, respectively). Consequently, a storage temperature of 10 °C is proposed to preserve the bioactive compounds of immature chilacayote for up to 15 days.

Keywords: *Cucurbita ficifolia*; postharvest; bioactive compounds; antioxidant activity; polyphenoloxidase; peroxidase

1. Introduction

The *Cucurbita* genus is a cornerstone of the Cucurbitacea family, comprising the diverse group of plants known as squashes. *Cucurbita ficifolia*, commonly known as fig-leaf gourd or *chilacayote*, is a species well-adapted to temperate regions at altitudes of 1000–3000 meters above sea level (masl), though it remains sensitive to frost [1]. Its edible components include leaves, young stems, seeds, and fruit at various stages of maturity. While immature fruits are staples in numerous traditional dishes, mature fruits are typically reserved for confectionery and desserts. Morphologically, the immature fruit is characterized by a globular shape, a green-to-mottled coloration, and a soft pericarp [2]. Based on its respiratory patterns, *C. ficifolia* is classified as a non-climacteric fruit [3]. Nutritionally, it is a significant source of carbohydrates, vitamins, and minerals (notably iron), as well as bioactive compounds such as phenolic acids, flavonoids, and vitamin C. These phytochemicals are associated with functional properties beneficial in preventing or treating chronic cardiovascular diseases due to their antioxidant, anti-inflammatory, and hypoglycemic activities [4,5].

During the postharvest phase, fruits undergo critical physiological and metabolic shifts—primarily respiration—that determine quality during storage [6]. Immature fig-leaf gourds exhibit a high respiration rate [6], which, coupled with a thin epicarp that increases susceptibility to mechanical damage, results in high perishability and a limited shelf life. Despite its potential, *C. ficifolia* holds secondary economic status compared to species like *Cucurbita pepo*, leaving its optimal storage parameters largely under-researched. Although refrigeration is the most accessible technology to delay senescence and extend shelf life, certain fruits are prone to chilling injury, leading to quality degradation. Therefore, this study aimed to evaluate postharvest fluctuations in bioactive compound content and enzymatic activity under different storage temperatures to establish alternatives for extending shelf life, promoting consumption, and enhancing commercial production.

2. Materials and methods

2.1. Biological material and sample preparation

Immature *C. ficifolia* fruits were harvested approximately 15 days after anthesis at Rancho el Atorón, Coatepec, Veracruz (19°29'32.9"N 96°56'39.0"W). Selection criteria included color, weight between 600 and 700 g, and the absence of mechanical damage. Fruits were subsequently stored unpackaged for 15 days under three temperature conditions: 5 and 10 °C with 95% relative humidity (RH), and at room temperature (22 ± 2 °C) with 83% RH. At each sampling point (0, 5, 10, and 15 days),

two rot-free fruits per temperature treatment were selected, and their pulp was analyzed for antioxidant compounds and enzymatic activity.

2.1.1. Extract preparation

Three types of extracts were prepared from finely chopped fresh pulp: methanolic extract [5 g pulp of fresh weight (FW) in 10 mL of acidified methanol to 1% HCl], for the determination of total polyphenols and flavonoids and activity antioxidant; meta-phosphoric extract (5 g pulp of FW in 50 mL of extracting solution [7]), for ascorbic acid analysis; and enzymatic extract [10 g of FW and 6 mg of PVPP in 10 mL of phosphate buffer (0.2 M, pH 6.5)], for polyphenol oxidase (PPO) and peroxidase (POD) activity assays. The methanolic and meta-phosphoric extracts were homogenized (WiseTis, HG-15A) at 10,300 rpm for 1 min, and then centrifuged (Hettich zentrifuge, Universal 32R, Tuttlingen, Germany) at 4,000 rpm for 20 min at 4 °C. The enzyme extract was homogenized (WiseTis, HG-15A) at 10,300 rpm for 2 min, incubated for 1 h at 4 °C, and centrifuged (Hettich zentrifuge, Universal 32R, Tuttlingen, Germany) at 4000 rpm for 30 min at 4 °C. All extracts were filtered through a double layer of fine mesh, and the resulting supernatant was used for the subsequent analyses.

2.2. Measured parameters

2.2.1. Weight loss

Weight loss was determined using four immature fruits per treatment, weighed individually at the beginning of the experiment and every 5 days during storage using a digital scale (Queen Sense, LCD3-10). Cumulative weight loss was calculated as the difference between the initial and final weight, expressed as a percentage of the initial weight [8].

2.2.2. Analysis of compounds with antioxidant activity

Total polyphenols: Total polyphenol content was determined according to the method described by Moreno-Quiroga et al. [9]. Briefly, a 0.4 mL aliquot of a methanolic extract sample was mixed with 1 mL of distilled water and 200 mL of Folin–Ciocalteu reagent; the mixture was then left to stand for 5–8 min. Subsequently, 2 mL of 7% (w/v) Na_2CO_3 and 1.4 mL of distilled water were added, and the mixture was vortexed and incubated for 1 h at room temperature in the dark. Finally, absorbance was measured at 750 nm using a spectrophotometer (Jenway 6305, Stratfordshire, United Kingdom). Quantification was performed using a gallic acid (GA) standard calibration curve (concentration of 0.02–0.5 mg mL^{-1}), and results were expressed as milligrams of gallic acid equivalents per gram of fresh weight (mg GAE g^{-1} FW).

Total flavonoids: Total flavonoid content was analyzed following the methodology of Moreno-Quiroga et al. [9]. A 0.25 mL sample of methanolic extract was mixed with 0.075 mL of $NaNO_2$, incubated for 5 min, and stirred. Subsequently, 0.15 mL of $AlCl_3$ was added and allowed to stand for 1 min, followed by the addition of 0.5 mL of NaOH and 2.025 mL of distilled water. After stirring, the absorbance was read at 510 nm in a spectrophotometer (Jenway 6305, Staffordshire, United Kingdom). For quantification, a catechin (CAT) standard calibration curve was used (concentration of 0.02–0.5

mg mL^{-1}), and results were expressed as milligrams of catechin equivalents per gram of fresh weight (mg CATE g^{-1}).

Ascorbic acid: Ascorbic acid content was determined following the AOAC [7] method. A 10 mL meta-phosphoric extract was titrated with the 2,6-dichloroindophenol salt until a persistent faint pink color was observed. The volume of titrant consumed was recorded. Quantification was performed using an ascorbic acid (AA) calibration curve (concentration of 0.006–0.05 mg mL^{-1}). Results were expressed as milligrams of ascorbic acid equivalent per 100 grams of fresh weight (mg AAE 100 g^{-1} FW).

2.2.3. *In vitro* antioxidant activity

Ferric reducing antioxidant power (FRAP) method: Antioxidant activity was evaluated using the FRAP method, as described by Moreno-Quiroga et al. [9]. Briefly, 0.1 mL of the methanolic extract was mixed with 3 mL of FRAP reagent (consisting of sodium acetate-acetic acid buffer at pH 3.6, 10 mM TPTZ, and 20 mM $FeCl_3$ $6H_2O$, in a 10:1:1 ratio). The mixture was incubated for 30 min in a water bath at 37 °C. Absorbance was measured at 593 nm using a UV-VIS spectrophotometer (model YR01849). Quantification was based on a Trolox standard curve (concentration of 0.09–0.99 μmol mL^{-1}). Results were reported as micromoles of Trolox equivalent per gram of fresh weight (μmol TE g^{-1} FW).

DPPH method: The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined according to Moreno-Quiroga et al. [9]. A 100 μL aliquot of methanolic extract was added to 2.9 mL of a DPPH solution (6 mg of DPPH diluted to 100 mL with 80% v/v methanol) in the absence of light. The mixture was incubated at room temperature for 30 min, and the absorbance was measured at 517 nm using a spectrophotometer (Shimadzu UV1800, Kyoto, Japan). For quantification, a Trolox standard curve (0.13–0.79 μmol Trolox mL^{-1}) was used. Results were expressed as micromoles of Trolox equivalent per 100 grams of fresh weight (μmol TE 100 g^{-1} FW).

2.2.4. Identification of phenolic compounds by HPLC-DAD

Phenolic compounds were identified using high-performance liquid chromatography (HPLC) following the method described by Zeb [10], with minor modifications. The analysis was performed on a Waters Alliance system (Milford, MA, USA) equipped with a quaternary pump (model e2695) and a diode array detector (DAD) (model 2998). Samples from days 0 and 15, stored at the three specified temperatures, were analyzed. For extract preparation, 50 mg of lyophilized immature *C. ficifolia* fruit tissue was weighed and mixed with 5 mL of methanol. The sample was placed in a sonicator for 30 minutes, which was repeated three times.

The resulting extract was adjusted to a final volume of 25 mL with methanol and filtered through 0.45 μm nylon acrodiscs into HPLC vials. Separation was achieved using a C18 column (5 μm, 150 × 4.5 mm) as the stationary phase. The mobile phase consisted of (A) 12.5 mM aqueous acetic acid and (B) acetonitrile (CH_3CN). The gradient elution was programmed as follows: 0–2 min, 5% B; 5 min, 15% B; 20 min, 50% B; and 25 min, 5% B. The flow rate was maintained at 1.0 mL min^{-1} with a detection wavelength of 280 nm and an injection volume of 20 μL. The column temperature was kept constant at 35 °C to ensure the reproducibility of the chromatographic profiles. Individual phenolic compounds were quantified using standard calibration curves, and results were expressed in μg g^{-1} dry weight (DW).

2.2.5. Enzyme activity

Polyphenoloxidase enzyme (PPO): Polyphenoloxidase enzymatic activity was determined according to the method described by Zhou et al. [11]. Briefly, 1.5 mL of phosphate buffer (0.2 M, pH 6.5) was placed in test tubes and pre-incubated in a water bath at 50 °C. The reaction mixture was prepared by adding 0.75 mL of catechol (0.73 M) as a substrate and 0.75 mL of enzyme extract, with thorough stirring after each addition. Absorbance was monitored at 420 nm for 3 min using a spectrophotometer (Shimadzu UV1800, Kyoto, Japan). PPO activity was expressed as absorbance units per minute per gram of sample (U PPO min^{-1} g^{-1} FW).

Peroxidase enzyme (POD): Peroxidase (POD) activity was measured following the procedure described by Zhou et al. [11]. A 2.25 mL aliquot of phosphate buffer (0.2 M, pH 5) was added to test tubes and maintained in a water bath at 20 °C. The mixture was prepared by sequentially adding 0.2 mL of 0.25% hydrogen peroxide, 0.75 mL of 0.01% guaiacol, and 0.1 mL of enzyme extract, ensuring homogenization after each step. Absorbance was recorded at 470 nm for 3 min using a spectrophotometer (Shimadzu UV1800, Kyoto, Japan). POD activity was expressed as absorbance units per minute per gram (U POD min^{-1} g^{-1} FW).

2.2.6. Statistical analysis

The study followed a completely randomized factorial design with two factors: storage temperature at three levels (5, 10, and 22 °C) and storage period at four levels (0, 5, 10, and 15 days). Response variables were measured on independent experimental units over time, using four replicates for each temperature across all four time levels (n = 48). Analysis of variance (ANOVA) was performed using a general linear model for statistical assessment; mean comparisons between groups were conducted using Tukey's test ($p \leq 0.05$). Results are expressed as mean ± standard deviation, and analyses were performed using SAS statistical software (SAS, 2006).

3. Results and discussion

3.1. Weight loss

Weight loss results are illustrated in Figure 1. The highest loss was observed at room temperature (9.2%), whereas storage at 5 and 10 °C resulted in losses of 4.2% and 3.2%, respectively, both remaining below the 5% threshold. In this regard, Urías et al. [12] reported losses of 16% and 12% in *C. pepo* fruits stored at 10 and 20 °C for 12 days, respectively. These values are notably higher than the ones recorded in the present study.

The observed weight loss could be mainly due to fruit transpiration, a physiological process reported to account for over 97% of total weight loss in fresh produce [13]. Since a loss exceeding 6% typically results in diminished commercial quality and the onset of wilting [14], the weight loss recorded at room temperature suggests that, under these conditions, the fruit may no longer be marketable.

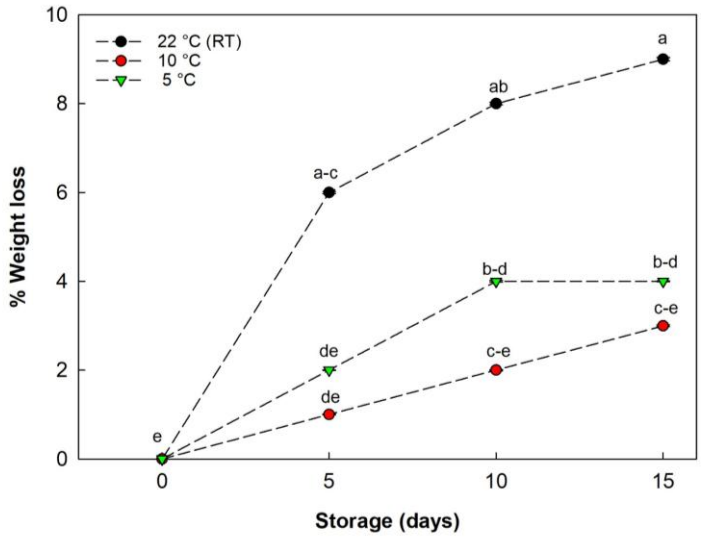

Figure 1. Weight loss (%) of immature *Cucurbita ficifolia* Bouché fruit stored at different temperatures. Different letters indicate statistically significant differences ($p \leq 0.05$).

3.2. Total polyphenols and flavonoids

Total polyphenol content increased across all three temperatures after 5 days of storage (Figure 2A). Values at 10 °C and at room temperature remained stable until day 15 (0.24–0.27 mg GAE g^{-1} FW). In contrast, at 5 °C, content increased until day 10 before declining by day 15 (0.23 mg GAE g^{-1} FW), showing no significant differences compared to the other storage temperatures. These concentrations are lower than those reported by Sharma and Rao [15] and Mokhtar et al. [16] for immature *Cucurbita maxima* and *Cucurbita moshata* Duchesne fruits, which yielded values of 0.56 ± 0.00 mg GAE g^{-1} FW and 0.78 ± 0.01 mg GAE g^{-1} FW, respectively.

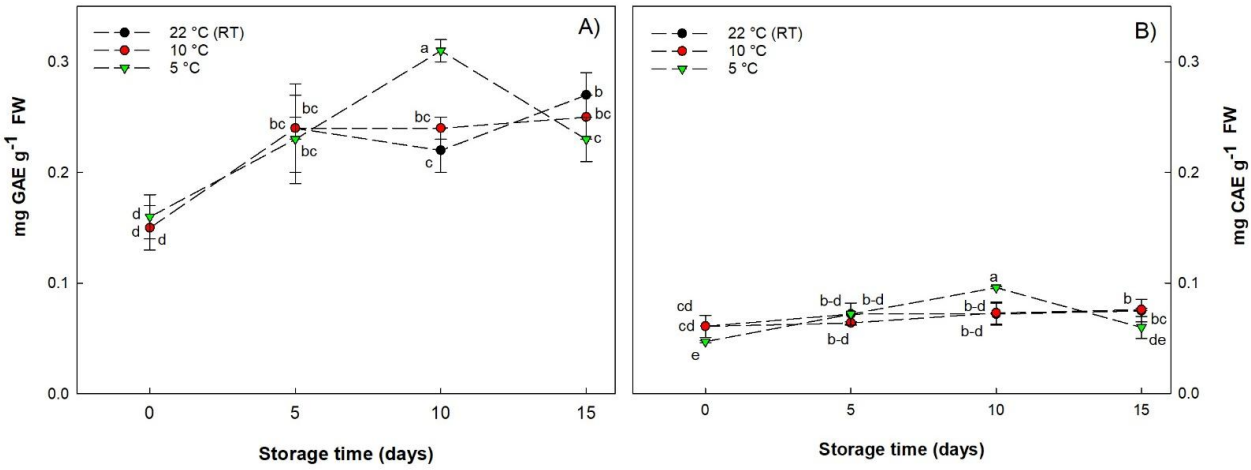

Figure 2. Total phenolic (A) and flavonoid (B) content in immature *Cucurbita ficifolia* Bouché fruit stored at different temperatures. Different letters indicate statistically significant differences ($p \leq 0.05$).

Previously, it was reported [8] that phenolic compounds in immature *Cucurbita moshata* fruit remained stable for 35 days when stored at 24.73 °C and 36.83% RH, which contrasts with the findings of the present study. Conversely, in melon fruits (*Cucumis melo* L.) stored at 4 °C and 80%–90% RH for 60 days, phenolic content increased during the first 30 days before subsequently declining [17]. This follows a trend similar to that observed in this study in *Cucurbita ficifolia* fruits stored at 5 °C.

Total phenolic content increased during the first five days of storage across all evaluated temperatures, suggesting that immature *Cucurbita ficifolia* fruits remained metabolically active postharvest and adjusted their secondary metabolism in response to storage conditions [18]. However, the most pronounced increase occurred at 5 °C, peaking on day 10. This indicates that low-temperature storage elicited a more intense transient response than at 10 °C or ambient temperature. Such behavior may be associated with cold-induced oxidative stress, as low temperatures disrupt cellular homeostasis, promote the accumulation of reactive oxygen species (ROS), and activate antioxidant defense mechanisms, including the synthesis of phenolic compounds [19]. Nevertheless, the decrease observed by day 15 suggests that this accumulation was transient, likely resulting from the oxidation, degradation, or consumption of these compounds during ROS detoxification [20].

Total flavonoid content remained stable throughout the storage period at both 10 °C and room temperature (0.061–0.076 mg CATE g^{-1} FW). In contrast, at 5 °C, values increased until day 10 (0.047–0.096 mg CATE g^{-1} FW), before decreasing by day 15 (0.060 mg CATE g^{-1} FW) (Figure 2B). De Lira et al. [8] reported, in immature fruit of *Cucurbita moshata*, a range of 0.009–0.021 mg CATE g^{-1} FW, which is lower than the concentrations observed in this study.

The trend observed at 5 °C aligns with findings previously reported [17] in melon (*Cucumis melo* L.) fruits stored at 4 °C and 80%–90% HR for 60 days, where an initial increase occurred during the first days of storage followed by a decrease after day 30. Regarding room temperature, de Lira et al. [8] reported that in immature *Cucurbita moshata* fruits stored at 24.73 °C and 36.83% HR, total flavonoid content increased slightly up to 35 days. This contrasts with the present study, in which the flavonoid levels remained unchanged. Generally, the increase in these secondary metabolites may be attributed to low-temperature stress, which stimulates phenylpropanoid metabolism [21]. Various biotic and abiotic factors induce stress in plant products, which respond through the catabolism and synthesis of secondary metabolites to adapt to the new environmental conditions [22].

3.3. Ascorbic acid

Figure 3 illustrates the ascorbic acid content in immature chilacayote throughout the storage period. The most significant reduction occurred during the first 5 days, with concentrations dropping by 16% and 40%; this decline was more pronounced in samples stored at room temperature. Following day 5, ascorbic acid levels remained statistically stable in fruits kept at room temperature. In contrast, those stored at 5 and 10 °C exhibited a slight further decrease between days 10 and 15, presenting final values of 19, 14.5, and 17.8 mg AAE 100 g^{-1} FW for fruits stored at 5 and 10 °C and room temperature, respectively. These values are consistent with the 9.73 mg AAE 100 g^{-1} FW reported by Stryjecka et al. [23] for *C. ficifolia* pulp and align with values reported for *C. maxima* (14 mg AAE 100 g^{-1} FW).

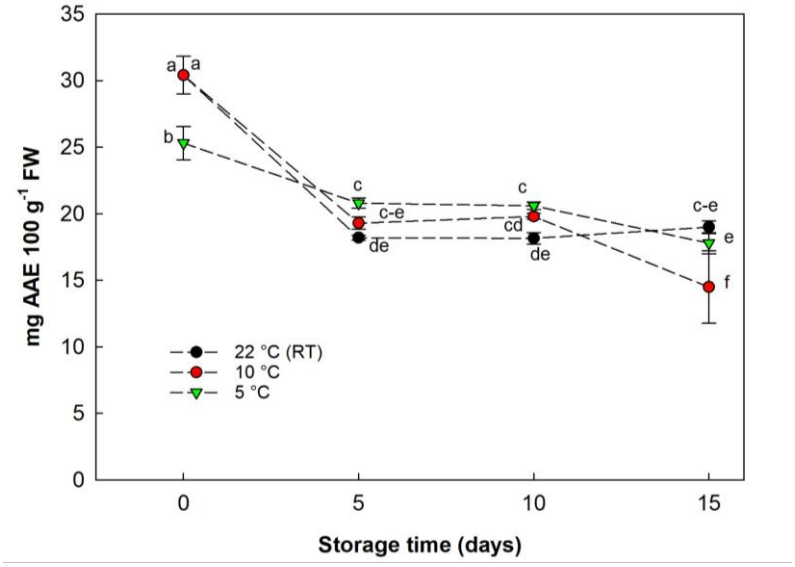

Figure 3. Ascorbic acid content in immature *Cucurbita ficifolia* Bouché fruit stored at different temperatures. Different letters indicate statistically significant differences ($p \leq 0.05$).

Andrejiová et al. [24] evaluated changes in ascorbic acid across five varieties of *C. moschata* stored at 15 °C for 52 days. They reported initial concentrations ranging from 13.9 to 18.7 mg AAE 100 g^{-1} FW, followed by a decrease of over 50% in all varieties (resulting in values between 6 and 9 mg 100 g^{-1} FW). These findings are consistent with the values observed in the present study under refrigeration temperatures. Similarly, Kopta et al. [25] observed a comparable trend in *C. maxima* var. Duch fruits stored at 20 °C and 65% RH for four months; they reported that ascorbic acid content declined from 12.4–15 mg AAE 100 g^{-1} FW in freshly harvested fruit to 5.3–5.6 mg 100 g^{-1} FW after storage.

The observed reduction in ascorbic acid content may be linked to the ripening process itself, occurring independently of temperature or optimal storage conditions. Reactive oxygen species (ROS), particularly hydrogen peroxide, increase during ripening. Ascorbate acts as an antioxidant by scavenging ROS and maintaining cellular redox balance across various compartments, including chloroplasts, peroxisomes, and mitochondria [26]. Furthermore, it serves as a substrate for ascorbate oxidase. This enzyme, which remains active throughout ripening and plays a vital role in fruit development, is notably abundant in members of the Cucurbitaceae family [27].

3.4. Antioxidant activity

Antioxidant activity, as measured by the FRAP method, increased across all three temperatures from day 0 to day 15. The highest values were recorded at 5 °C (1.03 µmol TE g^{-1}) and 10 °C (0.93 µmol TE g^{-1}) (Figure 4). These results are comparable to those reported by Kostecka-Gugala et al. [28], who found an antioxidant activity of 0.714 µmol TE g^{-1} for *C. ficifolia*, and values ranging from 0.2 to 1.4 µmol TE g^{-1} for *C. maxima*, *C. pepo*, and *C. moschata* cultivars.

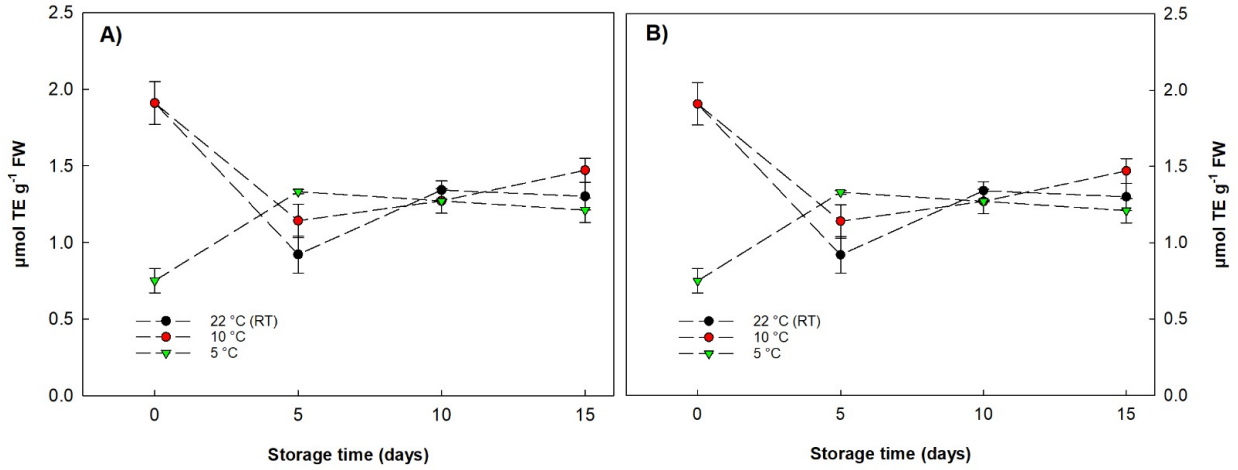

Figure 4. Antioxidant activity of immature *Cucurbita ficifolia* Bouché fruit stored at different temperatures: (A) ferric reducing antioxidant power (FRAP) and (B) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Different letters indicate statistically significant differences ($p \leq 0.05$).

The observed increase in antioxidant activity (FRAP) likely stems from temperature-driven effects on immature *C. ficifolia fruit*. At room temperature, this trend may be linked to postharvest metabolic shifts; as the tissue remains physiologically active after harvest, it undergoes metabolic reconfiguration during storage [18]. Conversely, at 5 and 10 °C, the increase appears related to cold-acclimation-induced stress, which triggers secondary metabolite synthesis as a defensive response [29]. A similar phenomenon has been reported in zucchini, where cold exposure stimulates reactive oxygen species production and activates non-enzymatic antioxidant pathways [30]. Moreover, this increase aligned with elevated total polyphenol levels suggests these compounds contribute significantly to the fruit's reducing capacity—a finding consistent with reports associating phenolic content with FRAP-measured antioxidant activity [31].

Regarding the antioxidant activity measured by the DPPH method, all treatments initially exhibited 1.8 µmol TE g^{-1} FW. After five days of storage, a significant decline was observed across all temperatures, with values reaching 1.4, 1.15, and 0.9 µmol TE g^{-1} FW for 5, 10, and 22 °C, respectively. Subsequently, fruits stored at 5 °C showed no significant fluctuations until the end of the experiment. In contrast, those stored at 10 °C and room temperature (22 °C) exhibited significant increases, reaching final values of 1.5 µmol TE g^{-1} FW and 1.3 µmol TE g^{-1} FW, respectively. These results are higher than those reported for various watermelon cultivars stored at 5 ± 0.5 °C for 15 days, which ranged from 1.4 to 0.4 µmol TE g^{-1} FW. Furthermore, a similar decreasing trend during storage has been previously documented [32].

The observed reduction in DPPH radical scavenging activity may be linked to the degradation of other antioxidant constituents, such as ascorbic acid, which followed a similar downward trend during storage. Kostecka-Gugala et al. [28] reported that *Cucurbita* fruit extracts with higher polyphenol concentrations do not always exhibit the highest *in vitro* antioxidant activity; rather, this capacity appears to depend on the methodology employed and the specific phenolic profile. Additionally, the contribution of other compounds, such as ascorbic acid, specific carbohydrates, and pigments that may exert antioxidant effects, should be considered [33].

3.5. Identification of phenolic compounds

Five phenolic compounds were identified in immature chilacayote using the HPLC-DAD at the onset and conclusion of storage across at all three temperatures. Hydroxybenzoic acid was the predominant compound (975.7–1743.5 μg g^{-1} DW), followed by p-coumaric acid (91.9–168.1 μg g^{-1} DW), ferulic acid (20.3–55.1 μg g^{-1} DW), and, to a lesser extent, caffeic acid (2.3–11.3 μg g^{-1} DW) (Table 1). While vanillic acid was also detected, it occurred in trace amounts across all samples and was therefore not quantified.

Table 1. Phenolic compound concentrations found by HPLC-DAD in the immature fruits of *C. ficifolia* Bouché.

Retention time (min)	Phenolic compound	Day 0	Day 15		
			5 °C	10 °C	22 °C
		Concentration (μg g^{-1} DW)			
9.7	Hydroxybenzoic acid	975.7 ± 35.1	394.4 ± 15.5	1140.3 ± 2.3	1743.5 ± 7.9
10.7	Caffeic acid	2.8 ± 0.6	11.3 ± 2.0	2.3 ± 0.6	6.0 ± 0.6
12.7	P-coumaric acid	93.7 ± 3.8	168.1 ± 13.1	91.9 ± 1.9	112.4 ± 1.5
13.4	Ferulic acid	55.1 ± 5.3	20.3 ± 2.8	26.9 ± 9.1	38.9 ± 9.1

C. ficifolia Bouché fruits at various ripening stages (from 5 to 55 days post-anthesis) have been previously characterized [3]. That study reported that the highest phenolic compound concentrations and hypoglycemic effects occurred in 15- and 25-day-old fruits. Identified compounds included gallic, chlorogenic, and syringic acids, in addition to catechins.

Similarly, Moreno-Quiroga et al. [9] identified the primary phenolic compounds in the pulp of *C. ficifolia* Bouché fruits from different populations in Oaxaca, Mexico. Their findings confirmed the presence of 4-hydroxybenzoic, 4-hydroxyphenylacetic, coumaric, ferulic, and vanillic acids, along with vanillin. These phenolic compounds, in addition to chlorogenic acid, were also reported in two *C. pepo* cultivars (Nimba and Astra Polka) [34]. Furthermore, Mokhart et al. [16] identified syringic, cinnamic, protocatechuic acids, caffeic, ferulic, and coumaric acids, as well as vanillin, in *C. moschata* fruits at different ripeness stages; notably, chlorogenic acid was not detected in that species.

Regarding concentration shifts during storage, *C. ficifolia* fruits stored at room temperature showed increases in hydroxybenzoic, caffeic, and p-coumaric acids, while ferulic acid concentrations decreased. A similar trend was observed for fruits stored at 10 °C, characterized by an increase in hydroxybenzoic acid and a decrease in caffeic and ferulic acids, whereas p-coumaric acid levels remained stable. Finally, in fruits stored at 5 °C, caffeic and p-coumaric acids concentrations increased, while the remaining compounds declined (Table 1).

The observed changes may be attributed to fruit development during storage [16]. In *C. moschata*, ferulic acid concentration typically decreases with ripening, while caffeic acid increases during development, and coumaric acid rises only during the early stages. These patterns align with the findings of the present study, with the exception of the caffeic acid decrease observed in fruits stored at 10 °C. Furthermore, these variations may be linked to cold stress, which enhances phenylalanine ammonia lyase (PAL) activity [21], leading to an immediate increase in cinnamic, p-coumaric, and

caffeic acid concentrations. This mechanism would account for the elevated levels of these acids at the lowest temperature, indicating higher physiological stress compared to other storage conditions.

Hydroxybenzoic acid was the primary phenolic compound identified in immature *C. ficifolia* fruit at both 10 and 22 °C. This is significant because hydroxybenzoic acid derivatives are widely associated with antioxidant, anti-inflammatory, antidiabetic, and antimicrobial properties [35]. Consequently, its high abundance may contribute, at least partially, to the fruit's functional value.

3.6. Enzyme activity

PPO activity at 5 °C reached the highest recorded values (0.095–0.092 U min^{-1} g^{-1} FW) and remained constant throughout the storage period (Figure 5). In contrast, PPO activity decreased in fruits stored at 10 °C (0.085–0.047 U min^{-1} g^{-1} FW) and at room temperature (0.085–0.060 U min^{-1} g^{-1} FW) until day 15. These values are lower than those reported by [17] for *Cucumis melo* fruits stored at 6 ± 1 °C (80%–90% RH) for 60 days, where PPO activity was 0.8 U min^{-1} g^{-1} FW and POD activity ranged from 2.6 to 1.5 U min^{-1} g^{-1} FW. However, the present results are consistent with those reported by Hu et al. [36] for mature *C. moschata* Duch fruits stored for 6 days at 4 °C, which showed average PPO activity values between 0.05 and 0.06 U min^{-1} g^{-1} FW.

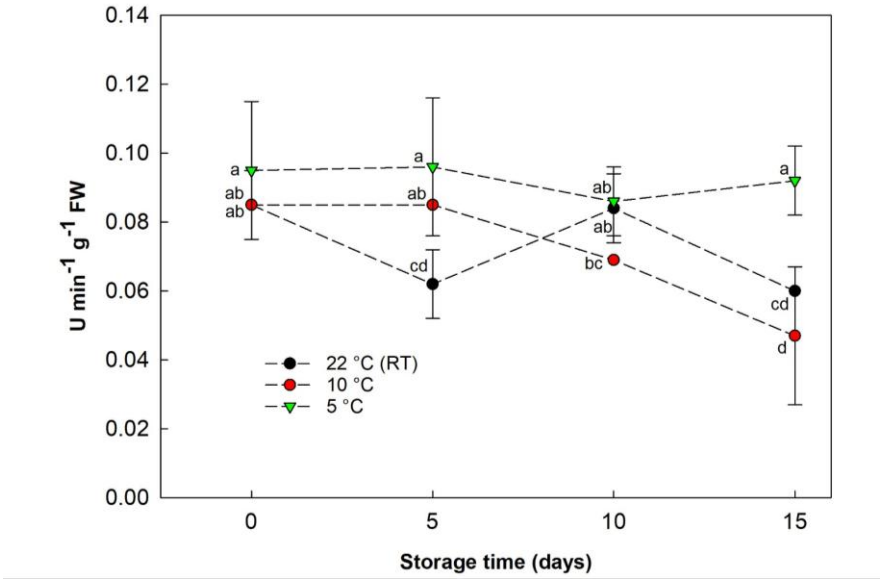

Figure 5. Polyphenol oxidase (PPO) activity in immature *Cucurbita ficifolia* Bouché fruit stored at different temperatures. Different letters indicate statistically significant differences ($p \leq 0.05$).

PPO activity in immature *Cucurbita ficifolia* fruit remained low across all evaluated conditions, suggesting low susceptibility to enzymatic browning during storage. This may be attributed to the maintenance of tissue cellular integrity, which limits the interaction between the enzyme, phenolic compounds, and oxygen [37]. Nevertheless, the higher PPO activity recorded at 5 °C could be linked to low temperatures stimulating enzymatic activity as a response to chilling stress [38]. A similar

behavior has been reported in *C. pepo*, where PPO activation was observed around day 5 of cold storage as part of an early response to chilling injury [30].

POD activity was highest at 5 °C (0.35–2.09 U min^{-1} g^{-1} FW), compared to 10 °C (0.33–1.01 U min^{-1} g^{-1} FW) and room temperature (0.33–0.89 U min^{-1} g^{-1} FW) throughout the storage period (Figure 6). Hu et al. [36] reported average POD activity values between 1.1 and 1.2 U g^{-1} FW for ripe *C. moschata* Duch fruits stored for 6 days at 4 °C, which is consistent with the findings of the present study. The trend observed at 5 °C aligns with previous reports [39], where a significant increase in peroxidase activity was observed in *C. pepo* fruits stored at 4 °C for 12 days.

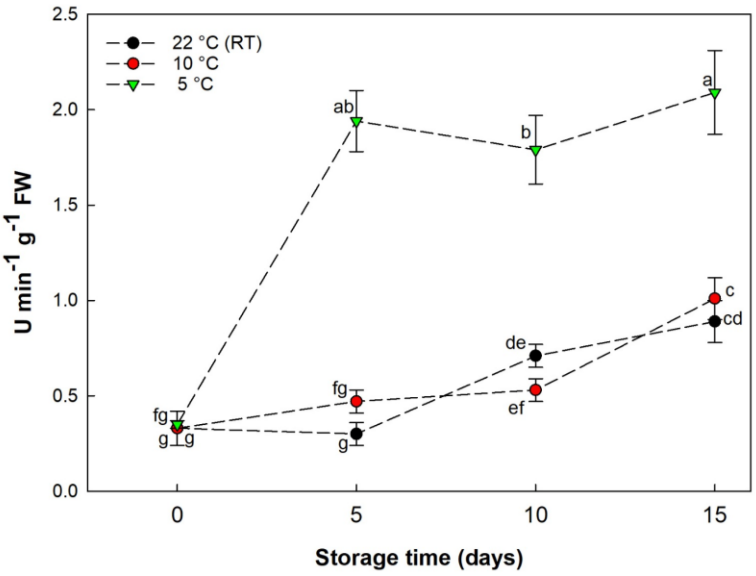

Figure 6. Peroxidase (POD) activity in immature *Cucurbita ficifolia* Bouché fruit stored at different temperatures. Different letters indicate statistically significant differences ($p \leq 0.05$).

The elevated POD activity may be attributed to the accumulation of peroxides in plant cells when fruits are exposed to low temperatures [40]. This triggers an increase in peroxidase activity, which acts as an antioxidant defense mechanism by detoxifying hydrogen peroxide [41]. Zhou et al. [42] noted that this behavior represents an early response in cold-sensitive tissues. Similarly, previous studies [39–41] found that *C. pepo* is susceptible to chilling injury at temperatures below 5 °C, which induces an increase in POD activity. A similar trend was observed in immature *C. ficifolia* (chilacayote), suggesting that comparable physiological damage may be occurring; however, further studies are required to confirm this hypothesis.

4. Conclusions

The cold storage of chilacayote immature fruits prevented greater weight loss as compared to those fruits stored at room temperature, which is relevant since mass loss is a major quality indicator involved in consumer acceptability. When comparing both cold storage temperatures (5 and 10 °C), the fruits showed a similar *in vitro* antioxidant activity, though fruits stored at 5 °C showed a clear increase in the activity of the peroxidase and polyphenol oxidase enzymes, assumed to be correlated

to cold stress and chilling injury, which could contribute to a rapid deterioration of the fruits and directly impact their quality. The results then suggest that a temperature of 10 °C would be optimal for storage; it is low enough to prevent a significant weight loss throughout the storage time and maintain high antioxidant activity, while promoting a high concentration of p-hydroxybenzoic acid associated with enhanced metabolic function, but not low enough to induce significant cold damage, being able to extend the shelf-life of the fruits for at least 15 days, while maintaining the expected quality.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare that there is no interest conflict.

Author contributions

J.E.A.J & E.N.A.B (Conceptualization, Methodology, Investigation, Review and Editing), M.A.S.B (Writing-original draft preparation, Review and Editing), L.L.C & J.L.C.S (Review, Data curation and Formal analysis), E.R.d (Review, Methodology and Formal analysis). All authors have read and agreed to the published version of the manuscript.

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