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Study on the Effect of Low-Dose Irradiation on Vitamin C Content in Choy Sum (*Brassica integrifolia* (West.) O.E. Schulz.)

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Abstract: Postharvest preservation of fresh leafy vegetables using ionizing radiation offers effective phytosanitary control without thermal damage. In this study, the retention of L-ascorbic acid (vitamin C) in fresh choy sum (*Brassica integrifolia* (West.) O.E. Schulz.) subjected to low-dose X-ray irradiation (0, 0.25, 0.50, 0.75, and 1.00 kGy) was investigated. A reversed-phase high-performance liquid chromatography (RP-HPLC) method was optimized and validated on an ODS C18 column under an isocratic mobile phase of 0.05 M KH_2PO_4 (pH 2.8) and methanol (90:10, v/v) with spectrophotometric detection at 245 nm, demonstrating high instrumental precision (RSD 0.07% for retention time and 0.76% for peak area at 1.0 mg/L, n = 5) and satisfactory spike recovery in the choy sum matrix (97.7 ± 0.8%). HPLC quantitation indicated that L-ascorbic acid decreased progressively with increasing absorbed dose ($p < 0.05$); however, irradiation at 0.25 kGy preserved 96.6% of the initial vitamin C (33.65 ± 0.58 vs. 34.82 ± 0.65 mg/100 g FW in the non-irradiated control). At 1.00 kGy, retention remained at 80.3% (27.95 ± 0.46 mg/100 g FW), with dose-dependent degradation adequately described by an apparent first-order model ($k = 0.221$ kGy^{-1}, $R^2 = 0.985$). Comparative iodometric back-titration overestimated the baseline content (38.45 ± 0.72 mg/100 g FW) and showed an apparent retention of only 49.2% at 1.00 kGy, reflecting interference from co-extracted non-specific endogenous reductants and optical masking of the starch–triiodide endpoint. These findings demonstrate that low-dose irradiation up to 0.25 kGy preserves more than 96% of endogenous L-ascorbic acid in fresh *Brassica integrifolia*, while doses up to 1.00 kGy preserve approximately 80%, and establish validated RP-HPLC as a more reliable technique than redox titrimetry for nutritional retention studies.

Keywords: *Brassica integrifolia*; choy sum; low-dose irradiation; L-ascorbic acid; RP-HPLC; iodometric titration; degradation kinetics.

1. Introduction

Postharvest deterioration of fresh leafy greens represents a substantial economic challenge in horticultural supply chains due to elevated baseline respiration, rapid moisture transpiration, and pathogenic microbial decay. Ionizing radiation is widely recognized as an advanced non-thermal phytosanitary treatment capable of arresting parasitic proliferation and extending the postharvest commercial window without causing structural thermal denaturation [1]. When administered at sub-kilogray doses (≤ 1.0 kGy), low-dose irradiation maintains physical cellular turgidity and curtails macromolecular degradation compared with traditional chemical disinfection or thermal pasteurization [1, 2]. However, the radiolytic dissociation of cellular water molecules induces reactive oxygen species (ROS), including hydroxyl radicals (•OH), hydrogen peroxide (H_2O_2), and superoxide anion radicals ($O_2^{\bullet -}$), which can precipitate secondary oxidative cascades targeted at redox-sensitive endogenous micronutrients [2].

Choy sum (*Brassica integrifolia* (West.) O.E. Schulz.) is an economically prominent leafy vegetable widely cultivated and consumed across tropical and subtropical Asia. The commodity is agronomically appreciated for its rapid cultivation turnover and favorable nutritional profile, delivering essential dietary minerals, soluble dietary fiber, and health-promoting secondary metabolites such as polyphenols, carotenoids, and glucosinolates [3, 4]. A critical determinant of postharvest quality in *Brassica* vegetables is the retention of L-ascorbic acid (vitamin C). Ascorbic acid is an essential water-soluble antioxidant indispensable for human cellular collagen biosynthesis, enzymatic cofactor functions, and immune modulation [5, 6]. Because primates and humans lack functional L-gulonolactone oxidase to biosynthesize ascorbic acid *de novo*, human nutritional adequacy depends entirely on the intake of fresh dietary produce [5].

Nevertheless, the enediol structure of the furanone ring renders L-ascorbic acid highly vulnerable to oxidation, converting it into dehydroascorbic acid and irreversibly degrading it into 2,3-diketogulonic acid upon exposure to radiolytically generated free radicals, oxygen, and metabolic oxidases [2, 7]. While numerous studies have documented general nutritional stability in irradiated horticultural crops, empirical degradation

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kinetics and processing thresholds specifically in *Brassica integrifolia* subjected to varying sub-kilogray irradiation regimens remain inadequately defined. Furthermore, classical titrimetric analytical procedures frequently encounter severe matrix interferences in pigmented leafy tissues, highlighting the necessity of high-performance liquid chromatography (HPLC) for unambiguous quantification [7, 8]. Therefore, this study systematically evaluates the dose-dependent degradation kinetics of L-ascorbic acid in *Brassica integrifolia* under low-dose X-ray irradiation (0.00–1.00 kGy) using validated RP-HPLC and benchmarks the chromatographic findings against conventional iodometric back-titration.

2. Materials and Methods

2.1 Plant Material

Fresh choy sum (*Brassica integrifolia* (West.) O.E. Schulz.) specimens were purchased at commercial maturity from the Go Supermarket distribution facility in Da Nang, Vietnam. Vegetable stems and foliage were systematically inspected and graded. Only sound specimens exhibiting vibrant green coloration, crisp petiole turgor, uniform stem caliber (1.2–1.5 cm diameter), and an absence of mechanical bruising, chlorosis, or pest infestation were retained. Selected specimens were secured in ventilated polyolefin containers at 4 ± 1 °C and conveyed to the analytical laboratory within 90 min of procurement to establish baseline chemical parameters.

2.2 Analytical Methods

2.2.1 Standard Solutions and Mobile Phase Preparation

An L-ascorbic acid stock standard solution (500 mg/L) was prepared by dissolving 50.0 mg of analytical-grade L-ascorbic acid standard (≥ 99.0% purity; Sigma-Aldrich) in HPLC-grade double-distilled water acidified to pH 2.8 with concentrated orthophosphoric acid (H_3PO_4). The primary stock solution was stored in amber volumetric flasks at 4 °C in the dark and freshly prepared prior to each analytical sequence to preclude photo- and oxidative degradation [7].

The chromatographic mobile phase comprised an aqueous potassium dihydrogen phosphate buffer and methanol mixture (90:10, v/v). Specifically, 5.85 g of analytical-grade KH_2PO_4 was dissolved in 900 mL of double-distilled water, titrated to pH 2.8 with H_3PO_4, and blended with 100 mL of HPLC-grade methanol (10%, v/v) [8]. The mobile phase was vacuum filtered through a 0.45 μm cellulose nitrate membrane filter and degassed ultrasonically for 10 min prior to chromatographic elution.

2.2.2 Sample Extraction of Choy Sum

Fresh choy sum (*Brassica integrifolia*) tissue (10.0 g) was accurately weighed, comminuted, and homogenized using a chilled porcelain mortar and pestle in the presence of an extraction medium acidified with H_3PO_4 (pH 2.8) to stabilize endogenous L-ascorbic acid against autoxidation. To eliminate interfering endogenous reducing agents, pigments, and soluble macromolecular proteins, 5.0 mL of 5% (w/v) lead acetate ($Pb(CH_3COO)_2$) solution was introduced prior to adjusting the final volume to 100 mL with double-distilled water [7, 8]. The suspension was filtered through Whatman No. 42 filter paper to remove particulate debris, diluted eightfold (f = 8) with acidified extraction medium (pH 2.8), passed through a 0.22 μm PTFE syringe filter, and sonicated for 5 min to eliminate microbubbles. All pre-treatment and extraction protocols were conducted under subdued ambient lighting at low temperature (< 4 °C).

2.2.3 Quantitative Determination by RP-HPLC

Quantitative chromatography was conducted on an HPLC system equipped with an isocratic delivery pump, an autosampler, a temperature-controlled column compartment, and a UV-Vis photodiode array detector. Chromatographic separation was achieved on an ODS C18 reversed-phase column (250 mm × 4.6 mm, 5 μm particle size). The isocratic mobile phase (0.05 M KH_2PO_4 buffer, pH 2.8 : methanol = 90:10, v/v) was delivered at a flow rate of 1.0 mL/min with an injection volume of 20 μL. Detection was performed at the absorption maximum (λmax = 245 nm). Linear calibration profiles ($R^2 > 0.99$) were constructed using standard ascorbic acid concentrations spanning 0.1 to 10.0 mg/L [8].

The vitamin C content of the vegetable specimens (mg/100 g FW) was calculated according to Equation (1):

$$C = (C_x \times V \times f \times 100) / m \qquad (1)$$

where C_x is the L-ascorbic acid concentration in the injected aliquot derived from the linear calibration equation (mg/L), V is the primary extract volume (0.100 L), f is the pre-injection dilution factor (f = 8), and m is the fresh sample mass (m = 10.0 g).

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2.2.4 Low-Dose X-Ray Irradiation Treatment

Choy sum samples were partitioned into five treatment lots: a non-irradiated control (0.00 kGy) and four irradiated lots administered target absorbed doses of 0.25, 0.50, 0.75, and 1.00 kGy. Irradiation was conducted at the Son Son Irradiation Plant (Son Son Corporation, Ho Chi Minh City, Vietnam) using an industrial X-ray irradiation system driven by an electron-beam accelerator at ambient temperature. Absorbed doses were delivered and controlled according to the routine dosimetry procedure of the irradiation facility [15, 16]. Non-irradiated control samples were transported, handled, and held under identical environmental conditions without beam exposure. Each treatment lot was analyzed in triplicate (n = 3) by RP-HPLC and iodometric back-titration on the day of treatment.

2.2.5 Comparative Determination by Iodometric Back-Titration

For comparative validation, vitamin C content was determined by iodometric back-titration with standard sodium thiosulfate. Because aqueous triiodide (I_3^-) solutions are unstable, the active oxidant was generated in situ by reaction between KIO_3 and excess KI in an acidic medium (Eq. 2). The generated I_3^- quantitatively oxidizes L-ascorbic acid ($C_6H_8O_6$) to dehydroascorbic acid ($C_6H_6O_6$) (Eq. 3), and the unconsumed excess of I_3^- is back-titrated with standard $Na_2S_2O_3$ using starch as the indicator (Eq. 4):

$$IO_3^- + 8I^- + 6H^+ \rightarrow 3I_3^- + 3H_2O \quad (2)$$

$$C_6H_8O_6 + I_3^- \rightarrow C_6H_6O_6 + 3I^- + 2H^+ \quad (3)$$

$$I_3^- + 2S_2O_3^{2-} \rightarrow 3I^- + S_4O_6^{2-} \quad (4)$$

The L-ascorbic acid content of the aliquot was quantified from the stoichiometric difference between the total in situ generated I_3^- and the back-titrated excess I_3^-, and expressed as mg per 100 g fresh weight (FW) [7, 14].

2.3 Statistical Analysis

All experimental treatments and chemical assays were conducted in three independent replicates (n = 3). Experimental data are expressed as mean ± standard deviation (SD). Differences among treatment groups were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at $p < 0.05$ using Minitab 19 statistical software.

3. Results and Discussion

3.1 RP-HPLC Method Optimization and Instrumental Performance

Chromatographic operating parameters were optimized using L-ascorbic acid standard solutions. The UV absorption spectrum of ascorbic acid in acidic aqueous medium (pH 2.8, H_3PO_4) exhibited an absorption maximum at λmax = 245 nm that showed no bathochromic or hypsochromic shift across 10–30 mg/L (molar absorptivity ≈ 1.0–1.3 × 10^4 $L \cdot mol^{-1} \cdot cm^{-1}$); this wavelength was selected for quantitative monitoring [7, 8]. Mobile phase pH and organic modifier proportion were evaluated with a 0.5 mg/L standard (n = 3). Raising the aqueous phase pH to 4.2 and 5.2 shortened the retention time by 8.1% and 15.3% and reduced the peak area by 53.8% and 66.4%, respectively, attributable to solute deprotonation above pKa1 ≈ 4.17 and accelerated enediol autoxidation [9, 10]. At pH 2.8, increasing the methanol fraction from 10% to 20% and 30% (v/v) decreased analyte retention and reduced the peak area by 40.3% and 71.7% [9, 11]. Consequently, an isocratic mobile phase of 0.05 M KH_2PO_4 (pH 2.8) and methanol (90:10, v/v) was selected, yielding adequate retention (tR ≈ 2.37 min) and baseline resolution from the unretained solvent front. The analytical performance characteristics under these conditions are given in Table 1.

Table 1: Analytical performance characteristics of the optimized RP-HPLC method for L-ascorbic acid determination

Analytical parameter	Result
Detection wavelength	λmax = 245 nm (stable across 10–30 mg/L)
Mobile phase (isocratic)	0.05 M KH_2PO_4 (pH 2.8) : methanol = 90:10 (v/v); 1.0 mL/min
Retention time (tR)	≈ 2.37 min
Instrumental precision (1.0 mg/L, n = 5)	RSD 0.07% (tR); RSD 0.76% (peak area)
Linear range and equation	0.1–10.0 mg/L; $Y = 2.73 \times 10^4 X + 1.25 \times 10^3$ ($R^2 > 0.99$)
Practical limit of quantification (LOQ)	0.10 mg/L
On-column recovery (5.0 mg/L, n = 5)	90.4 ± 3.9%

Y: chromatographic peak area (mAU·s); X: L-ascorbic acid concentration (mg/L). On-column recovery = (peak area with analytical column / peak area in flow-injection mode without column) × 100.

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Both precision indices complied with commonly applied acceptance criteria (RSD ≤ 1.0% for retention time and ≤ 5.0% for peak area) [12, 13]. At standard concentrations above 10.0 mg/L, the detector response deviated from linearity; vegetable extracts were therefore diluted eightfold to fall within the validated linear range. The on-column recovery of 90.4 ± 3.9% confirms that L-ascorbic acid underwent neither irreversible stationary-phase adsorption nor on-column degradation during chromatographic elution [12, 13].

3.2 Method Validation in Choy Sum Matrix

To establish analytical trueness and assess whether co-extracted endogenous constituents of choy sum (*Brassica integrifolia*) cause signal suppression or enhancement, a post-extraction spike recovery test was conducted. Diluted extracts were fortified with standard L-ascorbic acid at 2.0 mg/L and analyzed in triplicate (n = 3) alongside unfortified aliquots under identical chromatographic conditions. Recovery was computed as $R\ (\%) = [(S_{fortified} - S_{unspiked}) / (b \times C_{added})] \times 100$, where b is the calibration slope (2.73×10^4 mAU·s·L·mg^{-1}) and C_{added} = 2.0 mg/L. The results are presented in Table 2.

Table 2: Analytical recovery and matrix evaluation of L-ascorbic acid in choy sum (*Brassica integrifolia*) extracts via standard addition (2.0 mg/L fortification, n = 3)

Run	Unspiked extract tR (min)	Unspiked extract S (mAU· s)	Fortified extract (+2.0 mg/L) tR (min)	Fortified extract (+2.0 mg/L) S (mAU· s)	Recovery R (%)
1	2.370	119,850	2.371	173,084	97.5
2	2.368	118,620	2.369	172,456	98.6
3	2.372	120,410	2.373	173,371	97.0
Mean	2.370	119,627	2.371	172,970	97.7
± SD	0.002	916	0.002	468	0.8

The mean spike recovery of 97.7 ± 0.8% demonstrates that co-extracted matrix components (such as soluble polysaccharides, trace pigments, and non-target organic acids) produced negligible matrix effects on chromatographic quantitation, confirming the analytical trueness and repeatability of the validated RP-HPLC procedure.

3.3 Effect of Irradiation Dose on Vitamin C Retention Determined by HPLC

The residual L-ascorbic acid content and corresponding retention in control and irradiated choy sum determined by RP-HPLC are summarized in Table 3. Absorbed doses not exceeding 1.00 kGy are the regulatory ceilings applied to fresh produce for sprouting inhibition, ripening delay, and insect disinfestation [15, 16]. The retention time of L-ascorbic acid remained stable across all dose levels (2.367–2.371 min), indicating no co-eluting interference arising from irradiation.

Table 3: L-ascorbic acid content and retention in control and irradiated choy sum (*Brassica integrifolia*) determined by RP-HPLC

Absorbed dose (kGy)	Retention time tR (min)	L-ascorbic acid (mg/100 g FW)	Retention (% of control)	Loss (%)
0.00 (control)	2.371 ± 0.010	34.82 ± 0.65^{a}	100.00	0.00
0.25	2.368 ± 0.012	33.65 ± 0.58^{b}	96.64	3.36
0.50	2.370 ± 0.009	31.14 ± 0.42^{c}	89.43	10.57
0.75	2.367 ± 0.015	29.28 ± 0.51^{d}	84.09	15.91
1.00	2.369 ± 0.011	27.95 ± 0.46^{e}	80.27	19.73

Values represent mean ± standard deviation (n = 3). Different superscript letters (a–e) indicate significant differences among doses at $p < 0.05$ (one-way ANOVA followed by Duncan's multiple range test). Retention (%) = [C(irradiated) / C(control)] × 100.

As detailed in Table 3, L-ascorbic acid decreased progressively with increasing absorbed dose, with every dose increment producing a statistically significant reduction ($p < 0.05$). Nevertheless, degradation was minimal at 0.25 kGy (loss of 3.36%, corresponding to 96.64% retention) and increased moderately to 10.57%, 15.91%, and 19.73% at 0.50, 0.75, and 1.00 kGy, respectively. Regression of $\ln(C/C_0)$ against absorbed dose showed that the data were well described by an apparent first-order model ($k = 0.221$ kGy^{-1}, $R^2 = 0.985$). Over this narrow sub-kilogray range, a zero-order linear model gave a comparable fit ($R^2 = 0.987$); thus, the rate constant k serves mainly as a descriptor of tissue radiosensitivity. Retention of more than 80% of the initial vitamin C at 1.00 kGy indicates that the cellular architecture and co-occurring endogenous antioxidants of

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Brassica integrifolia effectively buffer the cascade of radiolytically generated ROS [1, 2]. Compared with the substantial losses typically reported for conventionally canned or frozen vegetables [17], low-dose irradiation largely preserves the micronutrient quality of the produce.

3.4 Comparative Evaluation Against Iodometric Back-Titration

To benchmark analytical specificity, the same samples were analyzed concurrently by classical iodometric back-titration. A direct comparison of the chromatographic and titrimetric results is given in Table 4.

Table 4: Comparison of L-ascorbic acid content and retention in choy sum (*Brassica integrifolia*) determined by iodometric back-titration and RP-HPLC

Absorbed dose (kGy)	Titration (mg/100 g FW)	Retention, titration (%)	HPLC (mg/100 g FW)	Retention, HPLC (%)	Difference Δ (%)
0.00	38.45 ± 0.72^{a}	100.00	34.82 ± 0.65^{a}	100.00	0.00
0.25	32.18 ± 0.60^{b}	83.69	33.65 ± 0.58^{b}	96.64	−12.95
0.50	22.65 ± 0.48^{c}	58.91	31.14 ± 0.42^{c}	89.43	−30.52
0.75	19.52 ± 0.40^{d}	50.77	29.28 ± 0.51^{d}	84.09	−33.32
1.00	18.92 ± 0.35^{d}	49.21	27.95 ± 0.46^{e}	80.27	−31.06

Values represent mean ± standard deviation (n = 3). Different superscript letters within a column indicate significant differences at $p < 0.05$ (one-way ANOVA followed by Duncan's multiple range test). Δ = retention (titration) − retention (RP-HPLC), in percentage points.

Although iodometric titration captured the overall negative relationship between irradiation dose and ascorbic acid content, it revealed major quantitative discrepancies. In the non-irradiated control, titration overestimated the ascorbic acid content by 10.4% (38.45 vs. 34.82 mg/100 g FW by HPLC), arising from non-specific oxidation of co-extracted cellular reductants (e.g., glutathione, cysteine-containing peptides, and low-molecular-weight phenolics) by triiodide [7, 14]. Furthermore, titration indicated apparent losses of up to 50.79% at 1.00 kGy, a discrepancy of more than 33 percentage points compared with HPLC. This divergence occurs because non-specific endogenous antioxidants are concurrently depleted by radiolysis, producing an exaggerated apparent loss in titrimetric monitoring. In addition, co-extracted chlorophyll pigments caused optical interference that masked the starch–triiodide endpoint. In contrast, RP-HPLC resolved L-ascorbic acid from interfering pigments and polar organic acids, demonstrating that chromatographic separation is necessary for accurate postharvest nutritional evaluation.

4. Conclusion

This study established and validated a sensitive and robust reversed-phase HPLC method on an ODS C18 column with UV-Vis detection at 245 nm for monitoring L-ascorbic acid retention in fresh choy sum (*Brassica integrifolia*). The isocratic mobile phase (0.05 M KH_2PO_4, pH 2.8 : methanol = 90:10, v/v) suppressed ionization of L-ascorbic acid, providing good chromatographic resolution ($t_R \approx 2.37$ min) and high instrumental precision (RSD 0.07% for retention time and 0.76% for peak area). The method showed good linearity over 0.1–10.0 mg/L ($R^2 > 0.99$), a practical LOQ of 0.10 mg/L, an on-column recovery of 90.4 ± 3.9%, and a matrix spike recovery of 97.7 ± 0.8%.

Low-dose X-ray irradiation (0.25–1.00 kGy) caused dose-dependent but modest reductions in L-ascorbic acid. Chromatographic monitoring showed retention of 96.64%, 89.43%, 84.09%, and 80.27% at 0.25, 0.50, 0.75, and 1.00 kGy, respectively (apparent first-order rate constant $k = 0.221$ kGy^{-1}). In contrast, iodometric back-titration overestimated the baseline content and magnified the apparent radiation losses because of interference from non-specific endogenous reductants and chlorophyll masking. These results indicate that irradiation at doses ≤ 0.25 kGy preserves more than 96% of vitamin C in fresh *Brassica integrifolia*, while doses up to 1.00 kGy preserve approximately 80%. Future investigations should evaluate the corresponding microbiological efficacy and shelf-life behavior under commercial refrigerated distribution.

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