

# Influence of ultraviolet-C irradiation treatment on quality and shelf life of mung bean sprouts

## during storage

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## Abstract

This research analyzed the impact of exposing mung bean sprouts to ultraviolet-C (UV-C) radiation for different periods (2, 5, and 10 min). Treated sprouts were preserved at 5°C and 85-95% relative humidity for 6 days. Irradiation for 10 min effectively reduced fresh weight loss, electrolyte leakage, and microbial count and maintained the firmness of sprouts. It also positively influenced the bioactive components, including antioxidants, total phenols, and protein, potentially providing health benefits to consumers. In addition, prolonged UV-C exposure for 10 min leads to oxidative stress, marked by a rise in malondialdehyde, proline, and hydrogen peroxide content. These compounds assist in stress reduction and preserve secondary metabolites. This research implies that postharvest 10 min UV-C irradiation offers a potential approach to uphold quality while maximizing the nutritional value of mung bean sprouts.

Keywords: bioactive components; enzymatic browning; mung bean sprouts; oxidative stress; UV-C radiation

# Highlights

- Use of UV-C irradiance is effective to preserve nutritional composition during storage.
- UV-C irradiation induces the production of bioactive compounds.
- UV-C irradiation assists in reducing microbial count.
- UV-C treatment improves the quality and shelf life of mung bean sprouts.

## **Practical Application**

Exposing mung bean sprouts to UV-C irradiation elevates the levels of bioactive compounds. UV-C equipment is now available for use in industrial applications. Sprouts can be maintained as fresh by using this technique in industries, and the concentration of health-promoting substances would rise or stay the same.

# Introduction

Diet has an immense role in maintaining good health and fighting against lifestyle diseases. Epidemiological studies indicate that a healthy diet prevents various lifestyle diseases. Plant-based diets are encouraged to reduce malnutrition and improve human health (Ezekekwu *et al.*, 2021). Mung bean (*Vigna radiata* L.) seeds are a protein-rich source of food. Besides protein and carbohydrates, seeds are rich in vitamins, phenolic acid, flavonoids, minerals, and dietary fibers. Mung beans have a substantial amount of lysine, a critical amino acid deficient in cereals.

However, due to the availability of antinutritional compounds, such as phytic acid, sprouting has proven to be an efficient strategy for boosting the nutritional value of legumes, including mung beans (Khattak et al., 2008). Sprouts are rich in organic acids, amines, phenols, sugars, sterols, and vital fatty acids (Geng et al., 2021). Mung bean sprouts, on the other hand, are highly perishable due to excess moisture content and strong metabolic activity. They decompose within a day under ambient conditions and in 5-6 days at lower temperatures. The most typical signs of degradation are dark pigmentation observed in the cotyledons and roots, and the emergence of rotting, sliminess, and musty odor. Preserving product quality and extending shelf life during storage is essential due to the quick decline in quality at relatively moderate temperatures.

Various post-harvest treatments, such as application of electrolyzed water (Rui *et al.*, 2011), plasma-activated water (Xiang *et al.*, 2019), and pulse electric treatment (Kramer *et al.*, 2015) were found to be effective against growth of microorganisms in mung bean sprouts. In addition, heat-shock treatment (Nishimura *et al.*, 2012) and exogenous adenosine triphosphate (ATP) (Chen *et al.*, 2018) were effective against the browning of mung bean sprouts. Recently, researchers observed that ethanol vapors, hot water dip, and ultraviolet (UV) irradiation have significantly increased antioxidant content and activity.

Post-harvest treatment by UV irradiation aims to enhance the quality and durability of produce (Adetuyi *et al.*, 2020). This treatment effectively improves antioxidant content and activates different defense-related compounds that induce resistance to pathogens (Kyere *et al.*, 2021). Since UV rays have superior germicidal activity, they prolong shelf life and minimize the microbial load. There has been scanty research on applying UV irradiations as postharvest treatments for mung bean sprouts. Ultraviolet-B (UV-B) irradiation helps to retain phenols, ascorbic acid (AA), and other bioactive compounds in sprouts (Gui *et al.*, 2018). However, current research shows that UV-B treatment has lower germicidal power. Furthermore, UV-B's influence on fresh produce's post-harvest quality may vary, and it is challenging to determine the appropriate dosage precisely. On the contrary, UV-C irradiance exhibits a consistent effect on the post-harvest quality of perishable products (Zhang *et al.*, 2021).

Literature search showed not a single detailed study regarding the effect of UV-C irradiation on mung bean sprouts at lower temperature storage. This research focuses on the influence of UV-C irradiation on the physical, biochemical quality, and microbial count of mung bean sprouts.

# **Materials and Methods**

## Preparation of sprout

Mung bean seeds were purchased from the Genetics and Plant Breeding Division, ICAR-IARI, New Delhi, India. Cleaned seeds were subjected to a 12-h soaking in water. After draining the remaining water, seeds were allowed to germinate in a sprout maker (Novelle Plast, New Delhi, India) for 24 h at 25°C.

#### UV-C irradiation treatments and storage condition

A closed chamber (60 cm [L] × 36 cm [W] × 40 cm [H]) was utilized for UV-C treatment. It was furnished with two germicidal tubes (UV 15 W; 28-mm diameter, 451.6mm length; Philips, Poland) that emitted radiation at a wavelength of 251.4 nm. For uniform distribution of light within the chamber, it was covered with black paper from outside and with aluminum foil inside. Before treatment, the chamber was operated for 15 min to stabilize the UV-C dosage. Approximately 100-g sprouts were positioned 15 cm from UV-C lights and exposed to 2-, 5-, and 10-min duration. Untreated sprouts represented the control group in a separate batch. After treatments, samples were placed into thermocol boxes (≈250-mL volume). Moistened filter papers were placed inside the boxes to maintain high humidity. All four lots were placed in cold storage (5°C, 85-95% relative humidity) for 6 days. Sampling for various parameters was done on 0, 2, 4, and 6-day of storage. Three sets of replicates (n = 3) for each treatment were chosen for quality assessment on each sampling day.

## Physiological and physical attributes

#### Physiological weight loss

Physiological weight loss (PWL) was assessed by precisely measuring the weight of samples in the beginning and at consistent intervals during storage. The outcomes were presented in percentage values reporting weight loss relative to the original fresh weight of sprouts.

#### Electrolyte leakage

Electrolyte leakage (EL) was assessed using the methodology outlined by He *et al.* (2007). Fifty individual sprouts were selected and immersed in double distilled water (250 mL) for 24 h at 150 rpm. The conductivity was analyzed using a conductivity meter, akin to the conductivity of viable seeds. After measurement, the same solution was kept at 100°C for 20 min. After 20 min of boiling, conductivity of the resulting solution was determined to be akin to the conductivity of non-viable seeds. The given formula was utilized to compute EL:

EL (%) = 
$$\frac{\text{Conductivity of live seeds}}{\text{Conductivity of dead seeds}} \times 100.$$

#### Firmness

Textural characteristics, specifically the firmness of sprouts, were evaluated by employing a texture analyzer (TA.XT Plus, Stable Micro Systems, England).

## Color

A colorimeter (Minolta Chroma Meter, CR-400, Tokyo, Japan) was used for color measurement of sprout in CIE LAB coordinates (L\*, a\*, and b\*). Hue angle (h\* = tan inverse [b\*/a\*]), color difference ( $\Delta E = [{\Delta L^*}^2 + {\Delta a^*}^2 + {\Delta b^*}^2]^{1/2}$ ), and chroma (C\* = [a\*<sup>2</sup> + b\*<sup>2</sup>]<sup>1/2</sup>) were computed. For each replication per treatment, a mean of 10 measurements was computed.

## Total soluble solids

Total soluble solids (TSS; <sup>°</sup>Brix) was assessed through a digital refractometer (Al-Dairi *et al.*, 2021). Sprouts were crushed and filtered using muslin cloth to obtain extracts. In our study, Atago-RX 7000i digital refractometer was used.

## Titratable acidity

Sprouts (5 g) were crushed in 20 mL distilled water. The macerate was then titrated against sodium hydroxide (0.1 N) after the addition of 2–3 drops of phenolphthalein indicator. Titratable acidity (TA) was computed with the given equation (Al-Dairi *et al.*, 2021):

$$TA = \frac{Titrate value (mL) \times 0.1 N NaOH \times 64.40}{Weight of sample (g) \times 1,000} \times 100,$$

## where:

64.40 = Equivalent weight of citric acid, 1,000 = Conversion of mg N/100 g to g N/100 g

#### Ascorbic acid

The method recommended by Cunniff (1995) in the *Official Methods of the AOAC International* was followed for analyzing ascorbic acid (AA). Sprouts (2 g) were homogenized with 3% metaphosphoric acid. After filtering, the extract (5 mL) was titrated against indophenol dye. The AA content in mg/100 g of sprouts was used to express the results.

## Total phenolic content

To analyze total phenolic content (TPC), 1g sprouts were crushed in 80% (v/v) methanol (10 mL) for extraction. After centrifugation, 100  $\mu$ L of supernatant was combined with 15% (v/v) sodium carbonate and 10% (v/v) FC reagent. The absorbance of the blank was measured at 765 nm and TPC concentration (mg of GAE/g) was computed using a gallic acid standard curve (Singleton and Rossi, 1965).

#### DPPH radical scavenging activity

Sprouts (500 mg) were macerated using 10 mL of methanol. After filtration, 3 mL 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was mixed with 0.1-mL extract (Shimada *et al.*, 1992). Absorbance was considered after 30 min of incubation at 517 nm, and the given equation was used to calculate antioxidant activity:

 $\begin{array}{l} \text{Antioxidant} \\ \text{activity (\%)} \end{array} = & \begin{array}{l} \begin{array}{l} \text{Absorbance of control} - \\ \hline \text{Absorbance of sample} \\ \hline \text{Absorbance of control} \end{array} \times 100. \end{array}$ 

#### Protein

Kjeldahl's method was followed to calculate protein content. A factor of 6.25 was applied to derive protein percentage after the nitrogen content was measured.

## Stress biomarkers test

#### Proline

Proline content was assessed following the procedure given by Bates *et al.* (1973). The process was initiated with the homogenization of 0.3 g sprouts in 6 mL of a 3% (v/v) sulfosalicylic acid solution. After centrifugation at 5,100×g for 5 min, 2 mL of this extract was mixed with 2 mL of ninhydrin and glacial acetic acid following incubation for 1 h at 95°C. Absorbance was considered after the addition of toluene. Proline content (µmol g<sup>-1</sup> FW) was determined with the help of L-proline.

## Malondialdehyde (MDA)

The process is initiated by homogenizing 2 g sprouts with 10 mL of trichloroacetic acid (TCA), followed by

centrifugation. A 1 mL extract and 2 mL of thiobarbituric acid were mixed in 20% TCA solution. After subjecting it to subsequent boiling and centrifugation, concentration ( $\mu$ M g<sup>-1</sup>) of supernatants was measured at 532 nm (Velikova *et al.*, 2000).

## Hydrogen peroxide $(H_2O_2)$

The 100 mg sprouts were extracted with 5 ml of 0.1% TCA solution. After centrifugation, 0.5 mL extract was blended to 1 M potassium iodide and 10 Mm phosphate buffer (pH 7). The reading was taken at 390 nm and the result was expressed as nmol/g FW (Velikova *et al.*, 2000).

## Enzyme activity

## Superoxide dismutase

Superoxide dismutase (SOD) enzyme activity was assessed following the method specified by Kono *et al.* (1978). A 1 g of sprouts were extracted in phosphate buffer (100 mM, pH 7) under cold conditions, followed by centrifugation at 10,000×g for 20 min. The crude extract (0.1 mL) was mixed to 0.6% (v/v) triton-X (0.1 mL), 96- $\mu$ M nitro blue tetrazolium, and sodium carbonate buffer solution (50 mM, pH 7). The reaction was initiated by adding 20 mM hydroxylamine hydrochloride (pH 6.0), and its absorbance was measured at 540 nm after 2 min of incubation. A unit of SOD activity was defined as the amount of enzyme required to inhibit chromogen production by 50%, and expressed as U kg<sup>-1</sup> of protein.

## Peroxidase

Peroxidase (POD) was determined with the help of Keesey's (1987) methodology. A 0.1 mL extract, 3 mL of pyrogallol, and phosphate buffer (0.1 M, pH 6.5) were mixed. The guaiacol dehydrogenation product (GDHP) was quantified at 436 nm. POD activity (Ukg<sup>-1</sup> of protein) measured the amount of enzyme needed to form 1.0- $\mu$ mol GDHP min<sup>-1</sup>.

## Polyphenol oxidase (PPO)

Hydroxymethyl aminomethane (THAM) hydrochloride, also known as Tris-HCl buffer (0.2 M, pH 7.5) was used to extract 2 g of sprouts. After centrifugation, the extract was combined with 4 mL of pyrocatechol and incubated for 30 min at 37°C. The reaction was stopped by adding 1 mL of 10% TCA, and the absorbance was measured at 430 nm. A 0.1 rise in optical density is equal to an enzyme unit (Kaul and Farooq, 1994).

## Microbial analysis

After dipping 10 g of sprouts in 100 mL of distilled water for 1 h, water samples were diluted serially. Aliquot of suitable dilution, 1 mL, was placed in triplicate in a sterilized Petri plate using a sterile pipette. Then, 10-15 mL of nutrient agar was added, stirred, and placed at room temperature for solidification. The inverted plates were incubated (37°C for 36 h) in a biochemical oxygen demand (BOD) incubator. Colony counting was performed on plates containing 30-300 colonies. The result was expressed as log cfu/g.

## Statistical analysis

All the measurements were performed in a completely randomized manner with three replications for each parameter, and the results were expressed as mean  $\pm$  standard error of mean (SEM). Using SPSS (IBM Corp., Armonk, New York, USA) Statistics (version 20), a two-way analysis of variance (ANOVA) with the Tukey test was employed to perform statistical analysis, and the necessary graphical representations were generated with MS Excel software.

# **Result and Discussion**

## Physiological and physical attributes

## Physiological weight loss

The data revealed an increase in PWL for all samples. The control sample exhibited higher (p < 0.05) PWL (17%) on the sixth day of storage. At the same time, no considerable difference was found among treated sprouts during storage (Figure 1A). The weight loss was 13%, 12%, and 11.8% in UV-C 10-, 5-, and 2-min-treated samples, respectively. Results indicated that UV-C irradiations reduced PWL during storage. Duarte-Sierra *et al.* (2019) observed similar results in UV-C-treated broccoli, which exhibited a lower weight loss, compared to untreated samples. According to Hosseini *et al.* (2019), UV-C irradiation effectively reduces microbial growth, minimizes tissue damage and weight loss, and preserves products' firmness.

## Electrolyte leakage

The UV-C-treated samples had relatively lower EL during storage, whereas a substantial (p < 0.05) rise was found in control samples. As represented in Figure 1B, maximum EL was observed in control (25.7%) treatment on the sixth day of storage. The EL was 11%, 16%, and 17% lower in UV-C 10-, 5-, and 2-min-treated samples, compared to control. The outcome supported a past research showing that UV-C treatment could lower the EL of oyster mushrooms (Wang *et al.*, 2017) and peaches (Zhou *et al.*, 2019). Civello *et al.* (2006) found that UV-C irradiance could promote polyamine accumulation and reduce lipid peroxidation in membrane, enhancing its stability. These findings showed that UV-C could delay the tissue EL of sprouts during storage.



Figure 1. Effect of UV-C treatments on (A) physiological weight loss, (B) electrolyte leakage and (C) firmness of stored mung bean sprouts. *Notes.* All the values are in triplicate (n = 3) and represented by mean  $\pm$  SE. Mean values with different letters are significantly different at  $\rho < 0.05$  using Tukey's test. Different small letters denote significant differences ( $\rho < 0.05$ ) among different treatments for the same day. Different capital letters denote significant difference ( $\rho < 0.05$ ) among different days for the same treatment. *Abbreviations.* UV: ultraviolet; n: number of replications; SE: standard error of mean.

#### Firmness

UV-C irradiance caused a considerable effect on firmness. The untreated sample softened more quickly than the irradiated sample. Firmness was found lower (p < 0.05) in control (7 N) and maximum in the UV-C 10 min-treated sample (18 N), followed by 5-min (15 N) and 2-min (13 N) treatments on the final day of storage (Figure 1C). A similar result was observed for strawberry (Li et al., 2019) and bitter gourd (Prajapati et al., 2021). Barka et al. (2000) and Stevens et al. (2004) reported that UV-C-irradiated fruit possessed a densely packed cell wall and middle lamella, compared to untreated fruits with a thinner cell wall detached from the plasma membrane. Furthermore, UV-C irradiation treatment inhibits the cell wall degrading enzymes, such as cellulase, and pectin methylesterase (Bu et al., 2013), leading to retention of higher firmness.

#### Color

Color is vital for quality assessment and consumer acceptance of any food product (Qiu *et al.*, 2021). Browning

led to a reduction in chroma and hue angle during storage. Chroma values of 2-min- (23.71) and 5-min-treated (24.44) sprouts were higher (p < 0.05) relative to 10-mintreated (22.94) and control (22.23) samples at the end of storage. Similarly, the hue angle of 5-min-treated (34.76) sample was significantly higher as compared to other treatments. As expected, the  $\Delta E$  value of UV-C 5-mintreated sprouts showed a considerably (p < 0.05) lower value (8.85) as compared to 2-min (9.97), 10-min (10.97), and control (11.46) samples on the sixth day of storage (Table 1).

The most likely explanation was that UV-C irradiation reduced oxidase activity and prevented the sprouts from browning. The outcome was consistent with an earlier research, suggesting that button mushroom browning might be decreased by UV-C treatment (Lu *et al.*, 2016). Here, sprouts having 10-min treatment showed early browning, compared to other treated sprouts. Prolonged UV-C exposure causes cell injury that may harm the product's visual quality. Meanwhile, mild UV-C

Parameters	Storage days	Treatments			
		Control	UV-C (2 min)	UV-C (5 min)	UV-C (10 min)
Chroma	2nd	25.53±0.66 <sup>C,a</sup>	25.30±1.35 <sup>A,B,a</sup>	26.16±0.02 <sup>C,a</sup>	26.21±0.12 <sup>B,C,a</sup>
	4th	23.57±1.214 <sup>A,B,b</sup>	25.12±0.61 <sup>A,B,a</sup>	25.32±1.02 <sup>B,a</sup>	24.12±1.37 <sup>B,a,b</sup>
	6th	22.23±0.33 <sup>A,c</sup>	23.71±0.88 <sup>A,a,b</sup>	24.44±0.46 <sup>A,a</sup>	22.94±0.39 <sup>A,c</sup>
Hue	2nd	40.11±0.81 <sup>C,a</sup>	41.12±0.87 <sup>B,C,a,b</sup>	42.36±0.52 <sup>C,b</sup>	40.98±1.21 <sup>C,a</sup>
	4th	35.84±1.11 <sup>B,a</sup>	39.12±0.61 <sup>B,c</sup>	39.67±0.43 <sup>B,C,c,d</sup>	37.65±0.90 <sup>B,a,b</sup>
	6th	31.65±0.51 <sup>A,a,b</sup>	33.54±0.92 <sup>A,b,c</sup>	34.76±0.71 <sup>A,c</sup>	32.12±1.04 <sup>A,b</sup>
ΔΕ	2nd	9.22±0.28 <sup>A,c</sup>	7.27±1.77 <sup>A,a,b</sup>	6.05±0.36 <sup>A,a</sup>	8.82±1.47 <sup>A,b</sup>
	4th	10.29±1.63 <sup>A,c</sup>	8.16±0.58 <sup>B,b</sup>	7.94±0.35 <sup>B,a</sup>	9.92±2.25 <sup>A,b</sup>
	6th	11.46±0.39 <sup>B,c,d</sup>	9.97±1.11 <sup>B,C,b</sup>	8.85±0.67 <sup>C,a</sup>	10.97±0.48 <sup>A,B,c</sup>

Table 1. Effect of UV-C treatment	on color changes in stored mung bean sprouts.
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All the values are in triplicate (n = 3) and represented by mean  $\pm$  SE. Mean values with different letters are significantly different at p < 0.05 using Tukey's test. Different small letters denote significant differences (p < 0.05) among different treatments for the same day. Different capital letters denote significant differences (p < 0.05) among different treatment.

UV: ultraviolet; ΔE: color difference; n: number of replications; SE: standard error of mean.

application is utilized to minimize browning reactions and ensure microbiological decontamination. This aligns with a previous research conducted by Wang *et al.* (2019), which showed that fresh-cut lotuses turned darker after being treated with intense UV-C irradiation.

#### Total Soluble Solids

The TSS level increased by 0.72 and 0.94 units (°Brix) in the 2- and 5-min-treated samples, respectively, on the second day, compared to the initial TSS value (9.77 °Brix). After that, a subsequent reduction was observed during the storage (Table 2). The initial rise in TSS level was attributed to stress-induced metabolic responses in sprouts. UV-C stress stimulates the synthesis of various compounds, including sugars and soluble solids, as a protective mechanism against stress (Idzwana *et al.*, 2020), leading to an initial rise in TSS content.

However, in the UV-C 10-min treatment, a continuous reduction in TSS content during storage had the lowest value (7.81 °Brix) at the end of storage. This was linked with prolonged UV-C treatment that caused increased oxidative damage, negatively impacted the pathways responsible for sugar accumulation, and resulted in a decline in TSS content (Pala *et al.*, 2013). Similarly, Stevens *et al.* (2004) suggested that TSS decreased due to conversion into energy under aerobic conditions. This was consistent with our study, where a reduction in TSS content was noted in all samples over the storage duration.

#### Titratable acidity

Titratable acidity decreased in all sprouts during storage. Treated sprouts displayed higher TA than untreated samples throughout storage (Table 2). TA declined and

able 2.	<ul> <li>Effect of UV-C treatments on total soluble solids (°Brix) a</li> </ul>	and titratable acidity (%) of stored mung bean sprouts.	

Parameter	Treatments	Storage days			
		0th	2nd	4th	6th
Total soluble solids (°Brix)	Control	9.97±0.73 <sup>C,a</sup>	9.58±0.07 <sup>C,a</sup>	8.97±0.33 <sup>AB,b</sup>	8.27±0.54 <sup>A,c</sup>
	UV-C (2 min) UV-C (5 min)	9.97±0.73 <sup>A,B,a</sup> 9.97±0.73 <sup>A,B,a</sup>	10.69±0.09 <sup>c,a</sup> 10.91±0.18 <sup>c,a</sup>	10.52±0.35 <sup>c,a</sup> 10.10±0.80 <sup>B,C,a</sup>	9.23±0.17 <sup>A,a</sup> 9.55±0.03 <sup>A,a</sup>
	UV-C (10 min)	9.97±0.73 <sup>C,a</sup>	9.36±0.17 <sup>C,a</sup>	8.66±0.11 <sup>BC,b</sup>	7.81±0.23 <sup>A,c</sup>
Titratable acidity (%)	Control	0.220±0.01 <sup>D,a</sup>	0.154±0.01 <sup>C,b</sup>	0.140±0.19 <sup>B,c</sup>	0.120±0.01 <sup>A,d</sup>
	UV-C (2 min)	0.220±0.01 <sup>D,a</sup>	0.171±0.07 <sup>C,a</sup>	$0.154 \pm 0.01^{B,a,b}$	0.128±0.01 <sup>A,c</sup>
	UV-C (5 min)	0.220±0.01 <sup>B,C,a</sup>	0.183±0.01 <sup>B,a</sup>	0.150±0.01 <sup>A,b</sup>	0.150±0.01 <sup>A,a</sup>
	UV-C (10 min)	0.220±0.01 <sup>D,a</sup>	0.210±0.00 <sup>B,C,a</sup>	$0.192 \pm 0.01^{B,a,b}$	$0.171 \pm 0.01^{A,a,b}$

All the values are in triplicate (n = 3) and represented by mean  $\pm$  SE. Mean values with different letters are significantly different at p < 0.05 using Tukey's test. Different small letters denote significant differences (p < 0.05) among different treatments for the same day. Different capital letters denote significant differences (p < 0.05) among different days for the same treatment. UV: ultraviolet; n: number of replications; SE: standard error of mean.

reached their lowest values on the sixth day of storage; 5-min (0.15%) and 10-min (0.17%) treatments had considerably (p < 0.05) higher values than control (0.12%) and 2-min (0.13%) treatment on the sixth day of storage. Higher TA was also observed in UV-C-irradiated peaches (Abdipour *et al.* 2019) and cherry tomatoes (Razali *et al.* 2021).

These variations in TA in UV-C-irradiated sprouts and other fruits suggest that UV-C treatment may regulate acid content during storage, thereby influencing the overall taste and quality. Reduced acidity of fruits and vegetables is linked with shorter shelf life.

#### Phytochemical responses

#### Ascorbic acid

The 10 min-treated sprouts showed higher (p < 0.05) AA content compared to the 2-min-, 5-min-treated, and control samples throughout the storage (Table 3). AA content in sprouts was found to be 58%, 33.6%, and 19% higher in UV-C 10-min, 5-min, and 2-min treatment, respectively, in comparison to control on the sixth day of storage.

The elevated levels of AA in sprouts exposed to UV-C irradiance could be due to advantageous defense

mechanisms, mainly suppression of ascorbate oxidase enzyme activity, which improves the capacity to withstand oxidative stress (Darré *et al.*, 2022). The results were similar to the previous studies on fresh-cut strawberries (Avalos-Llano *et al.*, 2020) and pineapple (Sari *et al.*, 2016), where UV-C irradiance helped to retain higher levels of AA.

#### Total phenolic content

TPC increases with storage time, reaching its maximum level on the sixth day of storage. The sample under 10-min treatment had higher (p < 0.05) phenolic content (30.18 mg GAE/g FW), compared to 5-min-treated (26.26 mg GAE/g FW) and 2-min-treated (24.49 mg GAE/g FW) samples and control (20.96 mg GAE/g FW) sample (Table 3). 10-min UV-C-irradiated sprouts had 43% more TPC than that in control.

A rise in TPC was noted when exposed to biotic and abiotic stressors (Matsuura *et al.*, 2017). Surjadinata *et al.* (2021) identified that injured carrot tissue exposed to UV-C irradiation generated reactive oxygen species (ROS) and jasmonic acid, signaling substances capable of activating phenylalanine ammonia-lyase (PAL) enzyme and raising phenolic levels. The UV-C irradiance's positive effect in increasing TPC was also reported in bitter gourd (Prajapati *et al.*, 2021) and grapes (Pinto *et al.*, 2022).

Table 3.	Effect of UV-C treatments on phytochemical attributes of stored mung bean sprouts.	
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Parameters	Treatments	Storage days (D)			
		Oth	2nd	4th	6th
Ascorbic acid (mg/100 g)	Control	37.97±0.25 <sup>D,a</sup>	29.45±3.40 <sup>C,a</sup>	21.52±1.96 <sup>B,a</sup>	11.41±1.53 <sup>A,a</sup>
	UV-C (2 min)	37.97±0.25 <sup>D,a</sup>	29.97±3.93 <sup>C,a</sup>	23.80±3.40 <sup>B,b</sup>	13.63±1.15 <sup>A,b</sup>
	UV-C (5 min)	37.97±0.25 <sup>D,a</sup>	31.73±5.19 <sup>C,b</sup>	22.67±3.93 <sup>B,b</sup>	15.25±1.34 <sup>A,b,c</sup>
	UV-C (10 min)	37.97±0.25 <sup>D,a</sup>	34.45±1.96 <sup>C,c</sup>	24.83±1.53 <sup>B,b,c</sup>	18.13±1.96 <sup>A,d</sup>
Total phenolic content	Control	10.4±1.35 <sup>A,b,c</sup>	13.48±1.03 <sup>A,B,a</sup>	17.28±0.32 <sup>C,a</sup>	20.96±1.38 <sup>C,D,a</sup>
(mg GAE/g FW)	UV-C (2 min)	10.4±1.35 <sup>A,b,c</sup>	14.05±1.13 <sup>B,a,b</sup>	19.08±0.51 <sup>C,b</sup>	24.49±0.85 <sup>D,b</sup>
	UV-C (5 min)	10.4±1.35 <sup>A,b,c</sup>	15.18±1.42 <sup>B,b</sup>	22.10±0.65 <sup>C,c</sup>	26.26±0.79 <sup>D,c</sup>
	UV-C (10 min)	10.4±1.35 <sup>A,b,c</sup>	15.58±1.03 <sup>B,b,c</sup>	25.8±0.32 <sup>C,c,d</sup>	30.18±1.13 <sup>D,d</sup>
DPPH inhibition (%)	Control	40.2±0.99 <sup>A,c</sup>	42.50±1.01 <sup>A,a</sup>	43.72±0.99 <sup>B,b,c</sup>	47.7±2.19 <sup>C,c,d</sup>
	UV-C (2 min)	40.2±0.99 <sup>A,c</sup>	47.11±0.99 <sup>A,B,b,c</sup>	50.01±3.45 <sup>B,d,e</sup>	62.6±5.54 <sup>C,f,g</sup>
	UV-C (5 min)	40.2±0.99 <sup>A,c</sup>	50.42±1.17 <sup>B,a,b</sup>	54.24±1.59 <sup>C,d,e</sup>	61.5±2.63 <sup>C,D,e,f</sup>
	UV-C (10 min)	40.2±0.99 <sup>A,c</sup>	55.73±2.64 <sup>A,B,d</sup>	57.53±2.63 <sup>B,f,g</sup>	66.1±2.96 <sup>C,g</sup>
Protein content (%)	Control	5.12±0.25 <sup>C,a</sup>	4.97±0.25 <sup>B,C,a</sup>	4.10±0.251 <sup>A,a</sup>	3.80±0.25 <sup>A,a</sup>
	UV-C (2 min)	5.12±0.25 <sup>D,a</sup>	5.12±0.25 <sup>C,b</sup>	4.66±0.51 <sup>B,b</sup>	3.97±0.44 <sup>A,b</sup>
	UV-C (5 min)	5.12±0.25 <sup>B,a</sup>	5.28±0.14 <sup>B,b,c</sup>	4.42±0.39 <sup>A,b</sup>	4.11±0.24 <sup>A,b</sup>
	UV-C (10 min)	5.12±0.25 <sup>B,a</sup>	5.43±0.44 <sup>B,c</sup>	4.83±0.25 <sup>A,B,b</sup>	4.53±0.25 <sup>A,c</sup>

All the values are in triplicate (n = 3) and represented by mean  $\pm$  SE. Mean values with different letters are significantly different at p < 0.05 using Tukey's test. Different small letters denote significant differences (p < 0.05) among different treatments for the same day. Different capital letters denote significant differences (p < 0.05) among different days for the same treatment. UV: ultraviolet; n: number of replications; SE: standard error of mean.

#### DPPH radical scavenging activity

Table 3 demonstrates dose-dependent variation in DPPH inhibition percentage of mung bean sprouts treated with UV-C irradiance. The 10-min-treated samples had significantly higher antioxidant activity (66.1%), followed by 5-min- (61.5%) and 2-min-treated (62.6%) samples, while the least antioxidant activity was recorded in control samples (47.7%) on the sixth day of storage.

Higher phenol and antioxidant activity may be attributed to an elevated secondary metabolite during storage. Earlier research done by Gogo *et al.* (2018) demonstrated a direct relationship between antioxidant activity and numerous secondary metabolites found in plants. Our study also proved that UV-C irradiation induced stress conditions that triggered a rise in secondary metabolites, such as phenols and antioxidants, depending on treatment dosage. This phenomenon was also observed in blueberries (González-Villagra *et al.*, 2020) and date fruits (Dassamiour *et al.*, 2022).

#### Protein content

UV-C irradiance displayed varied effects on the protein content of sprouts. Higher protein content was observed in treated sprouts, compared to control till the end of storage (Table 3). 10-min-treated samples had the highest protein content (4.53%). At the same time, the lowest content was found in untreated sample (3.80%) upon completion of storage duration. Comparable outcomes were observed in UV-C-treated cabbage (Liao *et al.*, 2016) and oyster mush-rooms (Wang *et al.*, 2017), which exhibited elevated protein content, correlating with an upsurge in protease activity.

#### Stress biomarkers

#### Proline

The primary source of proline in cells is exposure to biotic or abiotic stress. The proline content of sprouts increases with the exposure period to UV-C radiation (Table 4). UV-C 10-min treatment showed 84% higher proline content than control on the sixth day of storage.

Previous studies on bell peppers (Patel *et al.*, 2019) and bitter gourd (Prajapati *et al.*, 2021) showed that proline reduced cytosolic acidity related to different stress factors, including UV irradiation. It also reduced the concentration of H<sup>+</sup> ions generated from UV treatments, reducing the detrimental impact of UV-C and UV-B exposure.

#### Malondialdehyde

In this research, MDA content increased with increase in UV-C dose of sprouts. Peak MDA content (0.80 nmol/g FW) was found in 10-min UV-C-treated sample, followed by 5-min- (0.67 nmol/g FW), 2-min-treated (0.47 nmol/g FW) samples and control (0.31 nmol/g FW) on the sixth day of storage (Table 4).

Table 4.	Effect of UV-C treatments on	stress biomarkers and microbia	I count of stored mung bean sprouts.
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Parameters	Treatments	Storage days (D)			
		0th	2nd	4th	6th
Proline (µg/g)	Control	11.05±0.65 <sup>A,a</sup>	21.07±1.06 <sup>B,a</sup>	35.47±0.72 <sup>C,a</sup>	49.32±0.17 <sup>D,a</sup>
	UV-C (2 min)	11.05±0.65 <sup>A,a</sup>	29.66±0.37 <sup>B,a,b</sup>	35.52±0.23 <sup>B,C,a</sup>	51.55±0.87 <sup>D,a,b</sup>
	UV-C (5 min)	11.05±0.65 <sup>A,a</sup>	34.12±0.43 <sup>B,b,c</sup>	45.21±0.20 <sup>C,b,c</sup>	60.32±0.20 <sup>D,b</sup>
	UV-C (10 min)	11.05±0.65 <sup>A,a</sup>	39.32±0.14 <sup>B,c</sup>	51.82±0.20 <sup>C,c</sup>	67.50±44.4 <sup>D,c</sup>
MDA (nmol/g FW)	Control	0.17±0.04 <sup>A,a</sup>	0.19±0.06 <sup>A,a</sup>	0.24±0.04 <sup>B,a</sup>	0.32±0.17 <sup>C,a</sup>
	UV-C (2 min)	0.17±0.04 <sup>A,a</sup>	0.29±0.07 <sup>B,a</sup>	0.52±0.23 <sup>D,b</sup>	$0.47 \pm 0.07^{C,a,b}$
	UV-C (5 min)	0.17±0.04 <sup>A,a</sup>	$0.34 \pm 0.07^{B,a,b}$	0.60±0.20 <sup>C,b,c</sup>	0.67±0.20 <sup>C,b</sup>
	UV-C (10 min)	0.17±0.04 <sup>A,a</sup>	0.56±0.04 <sup>B,b</sup>	0.72±0.20 <sup>B,C,c</sup>	0.80±0.20 <sup>C,c</sup>
H <sub>2</sub> O <sub>2</sub> (nmol/g FW)	Control	0.19±0.02 <sup>A,a</sup>	0.27±0.02 <sup>B,a</sup>	0.32±0.06 <sup>C,a</sup>	0.34±0.03 <sup>C,b</sup>
	UV-C (2 min)	0.19±0.02 <sup>A,a</sup>	0.33±0.02 <sup>B,a</sup>	0.36±0.05 <sup>B,a</sup>	0.39±0.06 <sup>C,a</sup>
	UV-C (5 min)	0.19±0.02 <sup>A,a</sup>	0.40±0.05 <sup>B,a</sup>	0.41±0.05 <sup>B,a,b</sup>	0.42±0.06 <sup>B,a</sup>
	UV-C (10 min)	0.19±0.02 <sup>A,a</sup>	0.45±0.04 <sup>B,b</sup>	0.50±0.04 <sup>C,b</sup>	0.59±0.02 <sup>C,a</sup>
Microbial count (log cfu/mL)	Control	1.13±0.41 <sup>A,a</sup>	2.47±0.12 <sup>B,a</sup>	3.31±0.42 <sup>B,C,c</sup>	4.01±0.61 <sup>C,c</sup>
	UV-C (2 min)	1.13±0.41 <sup>A,a</sup>	2.13±0.38 <sup>B,a,b</sup>	2.62±0.14 <sup>B,b</sup>	3.15±0.09 <sup>C,b</sup>
	UV-C (5 min)	1.13±0.41 <sup>A,a</sup>	1.87±0.48 <sup>A,B,c</sup>	2.12±0.64 <sup>B,C,a,b</sup>	2.63±0.12 <sup>C,a,b</sup>
	UV-C (10 min)	1.13±0.41 <sup>A,a</sup>	1.61±0.26 <sup>B,d</sup>	1.98±0.16 <sup>C,a</sup>	2.15±0.43 <sup>D,a</sup>

All the values are in triplicate (n = 3) and represented by mean  $\pm$  SE. Mean values with different letters are significantly different at p < 0.05 using Tukey's test. Different small letters denote significant differences (p < 0.05) among different treatments for the same day. Different capital letters denote significant difference (p < 0.05) among different days for the same treatment.

UV: ultraviolet; MDA: malondialdehyde; H<sub>2</sub>O<sub>2</sub>: hydrogen peroxide; n: number of replications; SE: standard error of mean.

A rise in thiobarbituric acid reactive substances (TBARS) showed UV-induced damage from a compromised cell defense system. UV-C-treated capsicum (Mahdavian *et al.*, 2008) and bitter gourd (Prajapati *et al.*, 2021) showed changes in TBARS concentration, particularly in MDA. Plant membranes respond to oxidative stress by initiating the peroxidation of polyunsaturated fatty acids, ultimately generating MDA. Furthermore, it has been identified as a defense-related chemical because it has a favorable effect by triggering regulatory genes and providing cellular defense against oxidative stress (Morales and Munn'e-Bosch, 2019).

#### Hydrogen peroxide

UV-C treatment can trigger ROS production, including  $H_2O_2$  (Zhang *et al.*, 2021).  $H_2O_2$  serves various physiological roles, encompassing signaling and responding to stress conditions.

The highest levels of  $H_2O_2$  were observed on the sixth day in 10-min-irradiated sample (0.59 nmol/g FW),

followed by 5-min- (0.42 nmol/g FW) and 2-mintreated (0.39 nmol/g FW) samples, while the control (0.34 nmol/g FW) displayed the least  $H_2O_2$  content (Table 4). All treated sprouts had considerably higher  $H_2O_2$  values than untreated sprouts. Previous studies demonstrated increased  $H_2O_2$  levels following UV-C exposure in various fresh produce. For instance, UV-Ctreated *Clerodendrum volubile* (Adetuyi *et al.*, 2020) had increased  $H_2O_2$  content during the entire storage period, compared to the control. This indicates that UV-C irradiation causes oxidative stress and stimulates the generation of  $H_2O_3$ .

#### Antioxidant enzymes Superoxide dismutase

The most prevalent free radical in the body, superoxide, causes damage to cells. SOD is an enzyme that neutralizes and restores cells by eliminating the superoxide radical (Mustafi *et al.*, 2009). Although SOD was reduced in all treatments during storage, sprouts under UV-C 10-min and 5-min treatment had 1.75- and 1.6-fold higher SOD activity than control (Figure 2A).



Figure 2. Effect of UV-C treatments on antioxidant enzymes (A) superoxide dismutase, (B) peroxidase, and (C) polyphenol oxidase of stored mung bean sprouts. *Notes.* All the values are in triplicate (n = 3) and represented by mean  $\pm$  SE. Mean values with different letters are significantly different at p < 0.05 using Tukey's test. Different small letters denote significant differences (p < 0.05) among different treatments for the same day. Different capital letters denote significant difference (p < 0.05) among different days for the same treatment. *Abbreviations.* UV: ultraviolet; n: number of replications; SE: standard error of mean.

Our findings align with the research done by Jia *et al.* (2023) and Sun *et al.* (2022), where UV-C treatment increased SOD enzyme activity to withstand oxidative stress in jujube and pear fruits, respectively. This indicates that UV-C irradiation effectively enhances antioxidant defense mechanisms in fruits.

## Peroxidase

POD enzyme is essential for several biological processes, including oxidative stress defense. POD activities of sprouts showed variability among treatments as shown in Figure 2B. Sprouts exposed to UV-C irradiance showed 90% (10 min), 85% (5 min), and 50% (2 min) higher POD activity, relative to control on the final day of storage.

We observed a consistent trend with the research done by Ranjbaran *et al.* (2021), where UV-C application increased POD activity in grapes. POD enzyme is essential for several biological activities, particularly in plants, including oxidative stress defense.

## Polyphenol oxidase

Polyphenol oxidase significantly influences enzymatic browning as it facilitates the transformation of o-phenols to extremely unstable by-product o-quinones. According to Huang *et al.* (2017), fruits and vegetables lose shelf life because they react with other components to produce brown polymers.

On the final day of storage, 5-min irradiation treatment had significantly (p < 0.05) lowest PPO activity (80 U/min), followed by 10-min- (92 U/min) and 2-min treatment (110 U/min) and control (130 U/min) (Figure 2C). The results were consistent with the results in lily bulbs (Huang *et al.*, 2017) and apples (Yuan *et al.*, 2022), where UV-C application displayed reduction in PPO activity. Aligning to these observations, exposure to UV-C irradiance may modify a signaling pathway advantageous for sprouts because it involves the PPO enzyme's reduced activity and the maintenance of AA and phenolic component levels. These effects made it possible to maintain visual qualities for an extended duration.

## **Microbial count**

A steady increase in microbial count was observed during storage (Table 4). However, the total plate count exhibited significant (p < 0.05) higher values in untreated sprouts (4.01 log cfu/mL), followed by 2-min (3.15 log cfu/mL), 5-min (2.63 log cfu/mL), and 10-min-treatment (2.15 log cfu/mL) on the sixth day.

UV-C irradiation breaks down DNA and interferes microorganism's reproduction and metabolism ability, leading to cell death (Brem *et al.*, 2017). Additionally, UV-C encouraged the formation of antibacterial materials, such as phenols in pear fruits (Sun *et al.*, 2022). Studies suggested that UV-C irradiation reduces *Bacillus cereus* and *Escherichia coli* O157:H7 strain in watermelon beverages (Pendyala *et al.*, 2020).

# Conclusions

According to the findings of this research, mung bean sprouts exposed to UV-C irradiance for 10 min increased shelf life and preserved the quality. UV-C exposure increased nutraceutical substances, such as antioxidants and total phenols, except AA during storage. Additionally, it prevented weight loss, preserved firmness, and decreased the microbial count of sprouts stored for up to 6 days. Additionally, it was a safe approach to protect against diseases and disorders associated with storing fresh produce. These results could be applied to other Leguminosae products. However, more research is needed to determine the best dosages and duration of exposure for UV-C to fully realize its capabilities to extend a product's shelf life while preserving its bioactive compounds.

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# Data Availability

Access to the data that underpin the findings can be obtained by reaching out to the authors.

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