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# Optimizing the Hydrogen Peroxide Concentration During Soaking and the Germination Time: A Simple Strategy to Modify Phenolic Content and Enhance Antioxidant Capacity in Runner Bean Sprouts

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Runner bean (*Phaseolus coccineus* L.) is a crop of significant economic importance in Mexico, but its inclusion in diets remains limited to certain regions. The bioprocessing of legumes through germination expands their consumption possibilities. Applying oxidative stress during germination has been suggested to improve the functional qualities of legume seeds. In this study, response surface methodology (RSM) with a central composite rotatable design (13 treatments) was used to identify the optimal hydrogen peroxide soaking concentration ([$H_2O_2$]; 0–35 mM) and germination time (Gt; 0–96 h) to enhance germination percentage (GP), free phenolic content (FPC), free flavonoid content (FFC), and antioxidant capacity in black runner bean sprouts. Regression analysis generated predictive models for each response. Optimal conditions were identified as [$H_2O_2$] of 30 mM and Gt of 92 h, achieving a GP of 95.7%. Under these conditions, sprouts exhibited increases in FPC from 67.6 to 72.7 mg GAE/100 g dry weight (DW), FFC from 26.4 to 28.6 mg CE/100 g DW, $ABTS^{\bullet+}$ scavenging activity from 3,028 to 3,782 µmol TE/100 g DW, and oxygen radical absorbance capacity (ORAC) from 5,793 to 6,573 µmol TE/100 g DW compared to those germinated without $H_2O_2$ stress. Soaking with 30 mM $H_2O_2$ enhanced the content of ferulic and *p*-coumaric acids in free and bound phenolic fractions, whereas catechin and quercetin showed notable reductions in both fractions as a result of $H_2O_2$ treatment. These findings reveal that $H_2O_2$ treatment can modify the phenolic profile of runner bean sprouts, thereby boosting their nutraceutical value.

**Keywords:** ayocote bean, $H_2O_2$ soaking, *Phaseolus coccineus*, phenolic profile, sprouted beans

## ABBREVIATIONS

ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); ANOVA, analysis of variance; AOC, antioxidant capacity; C3GE, cyanidin 3-glucoside equivalents; CCRD, central composite rotatable design; CE, catechin equivalents; CV, coefficient of variation; DW, dry weight; FFC, free flavonoid content; FPC, free phenolic content; GAE, gallic acid equivalents; GP, germination percentage; Gt, germination time; [$H_2O_2$], hydrogen peroxide concentration; ORAC, oxygen radical absorbance capacity; $R^2$, coefficient of determination; ROS, reactive oxygen species; RSM, response

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surface methodology; TAC, total anthocyanin content; TE, Trolox equivalents.

## INTRODUCTION

Runner bean (*Phaseolus coccineus* L.), also known as ayocote bean, is indigenous to Mesoamerica and is adaptable to various climatic conditions, particularly in the tropical high-humidity zones of Mexico [Vargas Vázquez *et al.*, 2012]. Although it is a valuable nutritional resource, runner bean remains underutilized as a legume [Alvarado-López *et al.*, 2019]. Traditionally, beans are consumed after cooking [Osuna-Gallardo *et al.*, 2023]. Nevertheless, heightened awareness of health and sustainability issues has spurred interest in alternative methods of consuming legumes and in novel plant-based foods [Aloo *et al.*, 2021].

Sprout production constitutes an effective strategy for diversifying legume consumption [Wojdyło *et al.*, 2020]. Germination can increase the bioactive compound content, such as phenolic acids and flavonoids. These compounds are crucial in treating and preventing chronic degenerative diseases linked to oxidative stress, such as diabetes, hypertension, and cancers [Hernández-Miranda *et al.*, 2025]. Recent research suggests that applying stress during sprout production, particularly during pre-germination stages such as soaking, may enhance the levels of bioactive compounds in germinated legumes [León-López *et al.*, 2020; Yang *et al.*, 2019; Yu *et al.*, 2023]. Nevertheless, the extent of these effects depends on the specific germination conditions [da Silva *et al.*, 2021].

The response surface methodology (RSM) provides a powerful statistical framework for optimizing complex processes such as germination. Within RSM, the central composite rotatable design (CCRD) – which incorporates axial points at lower and upper extremes beyond the factorial range – facilitates accurate response prediction, nonlinear model development, curvature detection, and enhanced model robustness while optimizing multiple parameters with fewer experimental runs and efficient quadratic polynomial fitting [Andres *et al.*, 2020; Mahapatra *et al.*, 2025]. Consequently, this study aimed to evaluate the effects of 24-h soaking with hydrogen peroxide ($H_2O_2$) at different concentrations, as well as germination time, on the nutraceutical properties of black runner bean sprouts. The findings provide a basis for developing high-value ingredients for functional foods.

## MATERIALS AND METHODS

### Plant material

Black runner bean seeds (*P. coccineus*) used in this study were purchased at the local market in Tecpatán, Chiapas, Mexico, in 2019. The seeds were cleaned and stored in plastic bags at 4°C for subsequent germination assays and chemical determinations.

### Runner bean germination process

The germination protocol was adapted from León-López *et al.* [2020]. Black runner bean seeds were sanitized by immersion in a 0.5% sodium hypochlorite solution (1:5 *w/v*, ratio) for 10 min. The sanitizing solution was discarded and the seeds were then rinsed three times with purified water. Batches of 30 g of seeds were soaked for 24 h at room temperature with different $H_2O_2$ concentrations, as detailed in **Table 1**. After soaking, the seeds were rinsed with distilled water. The soaked seeds were sown uniformly on filter paper folds placed in plastic containers (20×30 cm). Germination was conducted in an incubator chamber (I-36VL Model, Percival Scientific Inc. Perry, IA, USA) at 25°C and 80–90% of relative humidity until germination time established in the experimental design was reached (**Table 1**). Germination percentage was calculated at the end of each incubation period. Each assay was performed in quadruplicate. Two replicates were used for physical characterization, while the remaining samples were dried and stored for subsequent chemical analysis.

**Table 1.** Central composite rotatable design (CCRD) consisting of 13 experiments produced by different combinations of two process variables and five levels: $X_1$ – soaking hydrogen peroxide concentration ($[H_2O_2]$) and $X_2$ – germination time (Gt).

| No.[1] | $[H_2O_2]$ (mM) ($X_1$) | Gt (h) ($X_2$) |
|---|---|---|
| 1 | 5 (–1) | 14 (–1) |
| 2 | 30 (+1) | 14 (–1) |
| 3 | 5 (–1) | 82 (+1) |
| 4 | 30 (+1) | 82 (+1) |
| 5 | 0 (–1.414) | 48 (0) |
| 6 | 35 (+1.414) | 48 (0) |
| 7 | 17.5 (0) | 0 (–1.414) |
| 8 | 17.5 (0) | 96 (+1.414) |
| 9 | 17.5 (0) | 48 (0) |
| 10 | 17.5 (0) | 48 (0) |
| 11 | 17.5 (0) | 48 (0) |
| 12 | 17.5 (0) | 48 (0) |
| 13 | 17.5 (0) | 48 (0) |

[1]Does not correspond to order of experiments. Values in parentheses correspond to coded levels.

### Experimental design

The independent variables were: $X_1$ – soaking hydrogen peroxide concentration ($[H_2O_2]$, 0–35 mM) and $X_2$ – germination time (Gt, 0–96 h). The experimental design employed a CCRD comprising 13 randomized experiments, as detailed in **Table 1**. These experiments were conducted in random order, and performed in triplicate.

### Evaluation of germination percentage

Germination percentage (GP) was evaluated in seeds treated with different $H_2O_2$ concentrations and germinated for different times. GP was calculated as the number of successfully germinated seeds, identified by the presence of visible radicles (>1 mm), divided by the total number of seeds sown *per* treatment, and then multiplied by 100 [León-López *et al.*, 2020].

### Estimation of sprout growth

After each germination period, the physical characteristics of the runner bean sprouts were recorded. Radicle length (mm) and radicle diameter (mm) were measured using a digital caliper (CALDI-6MP, Truper. S.A. de C.V., Mexico City, Mexico). The number of seeds that developed secondary roots was recorded to calculate the percentage of secondary root appearance.

### Flour obtaining process

The sprouted seeds obtained from each treatment were dried at 55°C for 24 h in a food dehydrator (Hamilton Beach, 32100a, 500 W, Glen Allen, VA, USA). After drying, the sprouts were ground in a coffee grinder (Hamilton Beach, 80350R, Glen Allen, VA, USA). The resulting flours were sifted through an 80-mesh sieve (sieve aperture: 0.180 mm).

### Preparation of flour extracts

For optimization purposes, a 0.5 g portion of each flour was extracted by orbital agitation in a horizontal rotary shaker (RKVSD, ART Inc., Laurel, MD, USA) (200 rpm, 25°C) with 4 mL of 80% (*v/v*) aqueous ethanol during 10 min [Osuna-Gallardo *et al.*, 2023]. The mixture was then centrifuged (Eppendorf 5810R, AG, Hamburg, Germany) at 4,000×*g* for 10 min, and the supernatant was collected and stored at −20°C for subsequent analysis.

### Determination of free phenolic content and free flavonoid content

The free phenolic content (FPC) was determined with the Folin-Ciocalteu colorimetric method of Singleton *et al.* [1999]. Twenty µL of appropriately diluted extracts obtained as described above were reacted by adding 180 µL of the Folin-Ciocalteu reagent. Following a 20-min incubation, the absorbance of the blue complex was measured at 750 nm using a microplate reader (Synergy HT Multi-Detection BioTek Instruments, Inc., Winooski, VT, USA). Results were calculated using a calibration curve of gallic acid (0–300 mg/L) and presented in mg of gallic acid equivalents (GAE) *per* 100 g of dry weight (DW) of sprouted seeds (mg GAE/100 g DW).

The free flavonoid content (FFC) was determined using the methodology proposed by Heimler *et al.* [2005]. In a 96-well plate, 20 µL of the extract were mixed with 100 µL of distilled water and 6 µL of 5% $NaNO_2$, rested for 5 min, then 12 µL of 10% $AlCl_3$ were added. After another 5 min, 40 µL of 1 M NaOH and 22 µL of water were incorporated, followed by a 30-min dark incubation at room temperature. Absorbance was read at 510 nm using a microplate reader (Synergy HT Multi-Detection BioTek Instruments, Inc., Winooski, VT, USA). Results were calculated using a catechin calibration curve (0–300 mg/L) and expressed in mg of catechin equivalents (CE) *per* 100 g of sprouted seed dry weight (mg CE/100 g DW).

### Determination of antioxidant capacity

The antioxidant capacity (AOC) of the sprouted seed flours was evaluated using the 2,2′-azinoazino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay [Re *et al.*, 1999]. Each extract (7.5 µL) was added to a 96-well plate and brought to a total volume of 300 µL *per* well with 292.5 µL of the $ABTS^{\bullet+}$ solution. The plate was incubated at room temperature for 10 min, followed by absorbance measurement at 735 nm (Synergy HT Multi-Detection BioTek Instruments, Inc., Winooski, VT, USA). Trolox served as the reference standard, with a calibration curve from 0 to 800 µg/mL. Results were expressed as µmol of Trolox equivalents (TE) *per* 100 g of seed flour on a dry weight basis (µmol TE/100 g DW).

Additionally, the AOC of unprocessed black runner beans and sprouted beans under optimized ($[H_2O_2]$ of 30 mM, Gt of 92 h) and control ($[H_2O_2]$ of 0 mM, Gt of 92 h) conditions was determined using the oxygen radical absorbance capacity (ORAC) assay [Ou *et al.*, 2001]. Peroxyl radicals were generated with 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), and free radical-induced fluorescence loss was measured in a microplate reader (Synergy HT Multi-Detection, BioTek Instruments, Inc., Winooski, VT, USA) at excitation/emission wavelengths of 485/538 nm. Results were expressed as µmol of Trolox equivalents (TE) *per* 100 g of seed flour on a dry weight basis (µmol TE/100 g DW).

### Regression analysis and optimization

The optimal soaking $H_2O_2$ concentration and Gt to maximize GP, FPC, FFC, and AOC in the ABTS assay of sprouted black runner beans were established using the response surface methodology (RSM). The experimental design (CCRD) comprised 13 randomized runs (**Table 1**), varying $[H_2O_2]$ (0–35 mM) and Gt (0–96 h). PG, FPC, FFC, and AOC were modelled as quadratic responses according to Equation (1):

$$Y = \beta_0 + \sum_{i=1}^{2}\beta_i X_i + \sum_{i=2}^{2}\beta_{ii} X_i^2 + \sum_{i=1}^{2}\sum_{i=j+1}^{2}\beta_{ij} X_i X_j + \varepsilon \quad (1)$$

where: Y is the predicted response variable ($Y_1$ = PG, $Y_2$ = FPC, $Y_3$ = FFC, $Y_4$ = AOC); $\beta_0$, $\beta_i$, $\beta_{ii}$, and $\beta_{ij}$ are the regression coefficients for the intercept, linear, quadratic, and interaction terms, respectively; $X_i$ and $X_j$ represent the independent variables ($X_1 = [H_2O_2]$, $X_2$ = Gt); and ε denotes the experimental error.

### Sprouts characterization

#### Proximate chemical composition

The moisture (method 925.09), protein (method 920.87), ash (method 923.03), and lipid (method 922.06) contents of black

runner beans and sprouted beans under optimized and control conditions were determined according to the official AOAC International methods [AOAC, 2005]. The carbohydrate content was calculated by subtracting the sum of moisture, protein, fat, and ash from 100 g of sample. All results were expressed in g/100 g DW.

### Total anthocyanin content

The total anthocyanin content (TAC) was determined after extraction of a weighed 0.1 g portion of flours of unsprouted, control sprouted, and optimal sprouted beans into a 2 mL vial with 1 mL of acidified methanol (95% methanol and 1 M HCl, 85:15, *v/v*). The samples were vortexed for 10 min (Genie 2 mixer, model G560, Scientific Industries, Inc., Bohemia, NY, USA) and then centrifuged (Eppendorf 5810R, AG, Hamburg, Germany) at 3,000×*g* for 10 min, and the supernatants were collected. Sample absorbance was immediately recorded at 535 nm ($A_{535}$) and 700 nm ($A_{700}$) using a microplate reader (Synergy HT Multi-Detection BioTek Instruments, Inc., Winooski, VT, USA). TAC was quantified using the following Equation (2) [Abdel-Aal & Huel, 1999]:

$$TAC = \frac{A_{535} - A_{700}}{\varepsilon} \times V_{extract} \times MW \times \frac{1}{w_{sample}} \quad (2)$$

where: ε is cyanidin 3-glucoside molar absorption coefficient (23,900 L/(cm×mol), $V_{extract}$ is total extract volume (L), MW is cyanidin 3-glucoside molecular weight (449.2 g/mol), and $w_{sample}$ is weight of the sample (g).

Results were expressed as mg of cyanidin 3-glucoside equivalents (C3GE) *per* 100 g of sprouted bean dry weight (C3GE/100 g DW).

### Composition of free and bound phenolic fractions

The free phenolic fraction was obtained as described in "Preparation of flour extracts" section. The bound phenolics were extracted from the precipitate remaining after free phenolic extraction [Mora-Rochín *et al.*, 2010]. The precipitate was treated with 10 mL of 2 M NaOH, heated at 95°C for 30 min, and stirred for 1 h at room temperature. The mixture was neutralized with 2 mL concentrated HCl, vortexed for 2 min, and defatted by adding 5 mL of hexane. The defatted fraction was then extracted four times with 5 mL of ethyl acetate each time (vortexed for 10 min and centrifuged at 3,000×*g* for 10 min *per* extraction). The combined ethyl acetate fractions were evaporated to dryness under reduced pressure in a Speed Vac Concentrator SC 250 EXP (Thermo Scientific Inc., Sunnyvale, CA, USA), and reconstituted in 1 mL of 50% methanol.

The phenolic compound profile of the free and bound phenolic fractions was determined using a Dionex UltiMate 3000 high-performance liquid chromatography (HPLC) system with a photodiode array detector (DAD3000) (Thermo Fisher Scientific, New York, NY, USA) according to the procedure previously used by Valdez-Morales *et al.* [2014]. The injection volume was 10 µL. Separation was performed on a C18 Acclaim 120 Å analytical column (C18, 5 µm, 120 Å, 4.6×250 mm) from Dionex (Thermo Fisher Scientific, New York, NY, USA), at room temperature using a gradient elution of acetic acid–acidified water (pH 2.8) (A) and acetonitrile (B). The gradient program varied the proportion of solvents over 45 min as follows: 95% A (0–8 min); 6–12% B (8–14 min); 12–20% B (14–18 min); 20–35% B (18–24 min); 35–95% B (24–27 min); 95–60% B (27–31 min); 60–40% B (31–34 min); 40–20% B (34–38 min); and 20–5% B (38–45 min). The flow rate was 0.5 mL/min. Detection was set at wavelengths of 280, 320, and 360 nm. Chromatographic peaks were identified by comparing their retention times and UV-Vis spectra with those of authentic standards. Samples were injected in triplicate, and data were analyzed using Chromeleon 7.0.200 software (Thermo Fisher Scientific, Sunnyvale, CA, USA). Results were expressed as µg/g seed flour DW.

### Statistical analysis

The effect of $H_2O_2$ treatment on seedling growth, chemical composition, AOC, free phenolic content, free flavonoid content, and phenolic profile was evaluated using a one-way analysis of variance (ANOVA). Mean comparisons were performed with Tukey's test at the 95% confidence level ($p<0.05$).

## RESULTS AND DISCUSSION

### Effect of the soaking with $H_2O_2$ and germination time on the growth performance of runner bean sprouts

Radicle length, diameter, and the number of secondary roots were measured in sprouts as outlined in the experimental design (**Table 1**). Elevated $H_2O_2$ concentrations consistently promoted radicle elongation at both 48 h and 82 h of germination (**Table 2**, **Figure 1**). At 48 h, radicle length increased from 17.3 mm (0 mM $H_2O_2$) to 23.9 mm (35 mM $H_2O_2$). At 82 h, values ranged from 41.0 mm (5 mM $H_2O_2$) to 46.3 mm (30 mM $H_2O_2$). The effect was more pronounced at 48 h, highlighting that $H_2O_2$ substantially enhances early root development in runner bean sprouts. No root growth was detected with germination times shorter than 14 h, regardless of $H_2O_2$ treatment. Secondary roots were absent at short Gt. At 48 h, secondary root formation was not induced irrespective of the $H_2O_2$ concentration applied, and at 82 h, 30 mM $H_2O_2$ increased secondary roots by 36 percentage points compared to 5 mM. The presence of secondary roots was primarily determined by Gt and further influenced by $H_2O_2$ treatment at high concentrations. These results emphasize the importance of optimizing both Gt and $H_2O_2$ concentration to maximize root development.

Previous studies have shown that exogenous $H_2O_2$, applied at optimal concentrations, promotes adventitious root formation and elongation across various plant species [Li & Jia, 2013; Roussos, 2023]. In accordance with these findings, Barba-Espin *et al.* [2010] reported that pea seeds imbibed with $H_2O_2$ at concentrations of 0, 5, 10, and 20 mM exhibited a clear stimulation of seedling length, with the highest concentration showing the most pronounced effect. Exogenous $H_2O_2$ at suitable doses acts as a priming agent, boosting seedling vigor while limiting oxidative damage [Wojtyla *et al.*, 2016].

**Table 2.** Length and diameter of radicles, and percentage of seeds that developed secondary roots after soaking runner bean in an $H_2O_2$ solution in different concentrations ([$H_2O_2$]) and germinating for different times (Gt).

| No.[1] | [$H_2O_2$] (mM) | Gt (h) | Length (mm) | Diameter (mm) | Secondary roots (%) |
|---|---|---|---|---|---|
| 1 | 17.5 | 0 | 0.0 | 0.0 | NP |
| 2 | 5 | 14 | 0.0 | 0.0 | NP |
| 3 | 30 | 14 | 0.0 | 0.0 | NP |
| 4 | 0 | 48 | 17.3 | 2.1 | NP |
| 5* | 17.5 | 48 | 18.9 | 2.3 | NP |
| 6 | 35 | 48 | 23.9 | 2.5 | NP |
| 7 | 5 | 82 | 41.0 | 3.0 | 14.5 |
| 8 | 30 | 82 | 46.3 | 3.4 | 50.5 |
| 9 | 17.5 | 96 | 45.8 | 3.1 | 56.9 |

[1]Does not correspond to order of the experiments. *Central point repeated by 5. NP, secondary roots were not present.

Reactive oxygen species (ROS), particularly $H_2O_2$, are key signaling molecules in seed physiology, shifting the view from harmful by-products of aerobic respiration to regulators of developmental transitions. During imbibition, $H_2O_2$ accumulates through mitochondrial, peroxisomal, and nicotinamide adenine dinucleotide phosphate oxidase activity, facilitating endosperm weakening and hormonal modulation by enhancing gibberellin biosynthesis while reducing abscisic acid and ethylene levels [Černý *et al.*, 2018]. In cereals, $H_2O_2$ also promotes α-amylase activation and programmed cell death in the aleurone layer *via* DELLA protein interactions, processes essential for nutrient mobilization [Nazir *et al.*, 2020]. In turn, antioxidant systems, both enzymatic and non-enzymatic, balance oxidative stress while permitting $H_2O_2$ to act as a signaling molecule [Chu *et al.*, 2022]. Moreover, nuclear accumulation of $H_2O_2$ at radicle protrusion may regulate redox-sensitive transcription factors, leading to gene expression changes linked to phytohormone signaling [Wang *et al.*, 2025]. Collectively, these findings highlight the multifaceted role of $H_2O_2$ in coordinating germination success and phytochemical outcomes.

## Mathematical models of response variables

As shown in **Table 3**, the process variables, *i.e.*, [$H_2O_2$] and Gt, significantly influenced the response variables, including GP, FPC, FFC, and AOC. Using multiple regression analysis, quadratic polynomial equations for each response variable were accurately fitted to the experimental data of germination conditions. The response surfaces and the obtained contour plots were then analyzed to visualize these effects (**Figure 2**). The validity and adequacy of the predictive models were confirmed by considering the statistical parameters, including a high coefficient of determination ($R^2$) and adjusted $R^2$ (>0.80 for both), a very low *p*-value (<0.05), a coefficient of variation (CV) below 10%, and a non-significant lack-of-fit test ($p$>0.05) (**Table 4**).

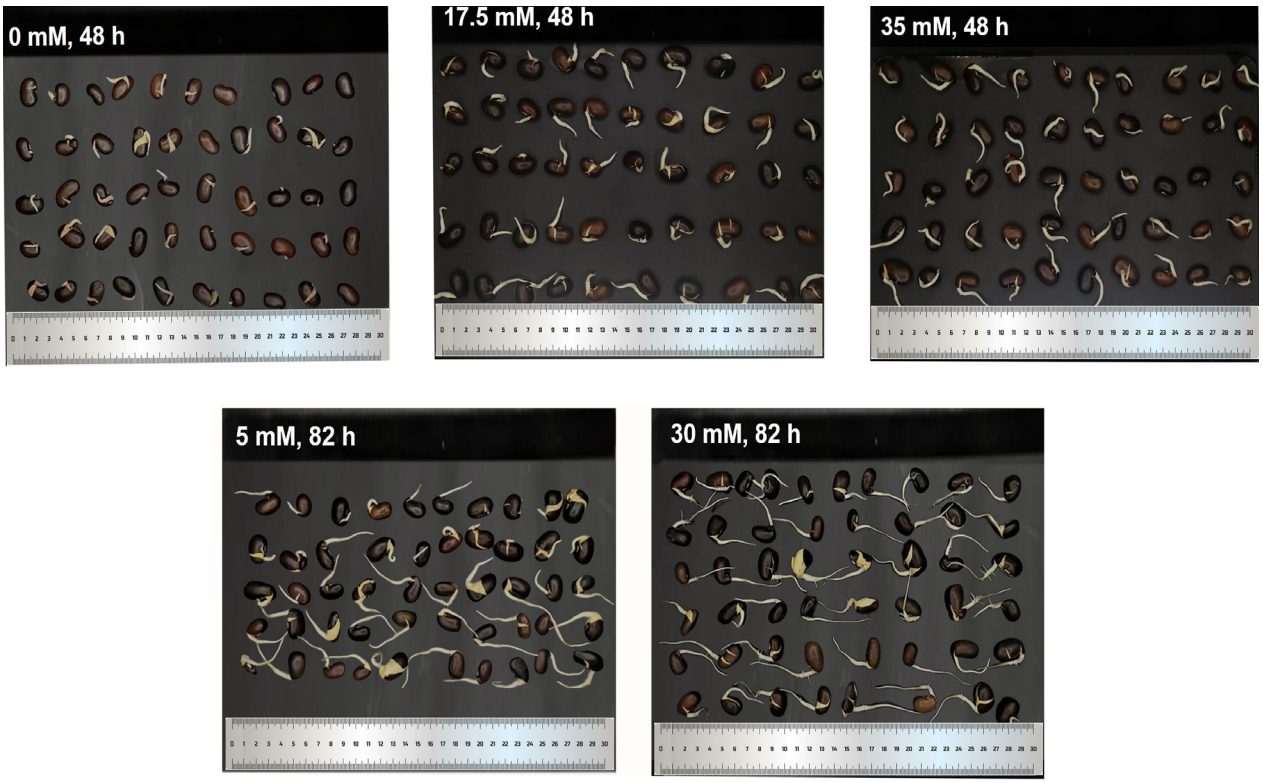


**Figure 1.** Appearance of runner bean sprouts at different germination times (48–82 h) after soaking in $H_2O_2$ solutions of varying concentrations (0–35 mM).

### Germination percentage

The process variables, [$H_2O_2$] and Gt, affected the germination performance in black runner bean sprouts, with GP ranging from 14.0 to 98.0%, depending on the experimental conditions (**Table 3**). The analysis of variance yielded a significant quadratic model for GP ($p$<0.0001), which includes the linear terms of [$H_2O_2$] and Gt, the quadratic term Gt ($Gt^2$), and the interaction between both variables ([$H_2O_2$]×Gt) (**Table 4**).

The contour plot (**Figure 2A**) shows a clear GP increase with longer Gt. The treatment with 35 mM $H_2O_2$ for 48 h resulted in GP values similar to those achieved after more than 82 h at lower $H_2O_2$ concentrations, suggesting that oxidative elicitation expedited germination. The highest GP was observed at $H_2O_2$ concentrations above 30 mM combined with Gt exceeding 48 h. Comparable responses have been documented in other legumes treated with $H_2O_2$ during imbibition [Barba-Espín *et al.*, 2012; León-López *et al.*, 2020; Santhy *et al.*, 2014]. This behavior is explained by the inductive effect of $H_2O_2$ in breaking seed dormancy and increasing germination. The oxidative stress caused by the natural accumulation of $H_2O_2$ enhances the production of ROS that diffuse from the seed surface to its interior and, by interacting with other molecules, inhibit the action of abscisic acid cytokinins, and indole-3-acetic acid, while simultaneously increasing the biosynthesis and inhibiting the catabolism of gibberellic acid, thereby promoting the germination process [Delis-Hechavarría *et al.*, 2021; Wojtyla *et al.*, 2016].

### Free phenolic content

The FPC in black runner bean sprouts was affected by the process variables, ([$H_2O_2$] and Gt), showing values ranging from 46.9 to 71.8 mg GAE/100 g DW across the 13 treatments (**Table 3**). The analysis of variance yielded a significant quadratic model for FPC ($p$<0.0001), which includes the linear and quadratic terms of [$H_2O_2$] and Gt, and the interaction between both variables ([$H_2O_2$]×Gt) (**Table 4**).

The contour plot (**Figure 2B**) shows the interaction of the process variables [$H_2O_2$] and Gt with FPC in runner bean sprouts, where increasing [$H_2O_2$] resulted in the highest FPC. A similar trend was observed with increasing Gt; however, the stimuli caused by the chemical stress induced by different $H_2O_2$ concentrations (0, 17.5, and 35 mM) at the same Gt (48 h) showed significant increases in FPC. The highest FPC (71.8 mg GAE/100 g DW) was obtained at Gt of 96 h and [$H_2O_2$] of 17.5 mM (**Table 3**), demonstrating that the synergy between high peroxide concentrations and prolonged germination times maximized FPC in black runner bean sprouts. Previous studies have reported similar positive effects of germination time and $H_2O_2$ stress on total phenolic content in wheat sprouts, *Ficus deltoidea* Jack plant,

**Table 3.** Experimental rotatable central composite design used to obtain treatment combinations of different concentrations of $H_2O_2$ ([$H_2O_2$]) and germination time (Gt) for producing runner bean sprouts, along with the experimental values obtained for the selected response variables.

| No.[1] | Process variables | | Response variables | | | |
|---|---|---|---|---|---|---|
| | [$H_2O_2$] (mM) | Gt (h) | GP (%) | FPC (mg GAE/100 g DW) | FFC (mg CE/100 g DW) | AOC (μmol TE/100 g DW) |
| 1 | 5 | 14 | 14.0 | 51.0 | 16.3 | 2,117 |
| 2 | 30 | 14 | 45.3 | 55.7 | 19.7 | 2,106 |
| 3 | 5 | 82 | 94.3 | 69.0 | 23.5 | 2,889 |
| 4 | 30 | 82 | **98.0** | 70.0 | **28.3** | **3,526** |
| 5 | 0 | 48 | 57.6 | 59.9 | 19.7 | 2,416 |
| 6 | 35 | 48 | 90.2 | 65.9 | 25.9 | 2,948 |
| 7 | 17.5 | 0 | 0.0 | 46.9 | 15.7 | 1,980 |
| 8 | 17.5 | 96 | 95.9 | **71.8** | 26.6 | 3,426 |
| 9 | 17.5 | 48 | 81.7 | 60.9 | 21.0 | 2,579 |
| 10 | 17.5 | 48 | 75.3 | 61.3 | 21.2 | 2,670 |
| 11 | 17.5 | 48 | 74.3 | 61.7 | 21.1 | 2,713 |
| 12 | 17.5 | 48 | 80.8 | 61.5 | 21.2 | 2,652 |
| 13 | 17.5 | 48 | 79.6 | 60.6 | 21.2 | 2,661 |

[1]Does not correspond to order of the experiments. GP, germination percentage; FPC, free phenolic content; FFC, free flavonoid content; AOC, antioxidant capacity in ABTS assay; GAE, gallic acid equivalent; CE, catechin equivalent; TE, Trolox equivalent, DW, dry weight. Values in bold mean maximum values.

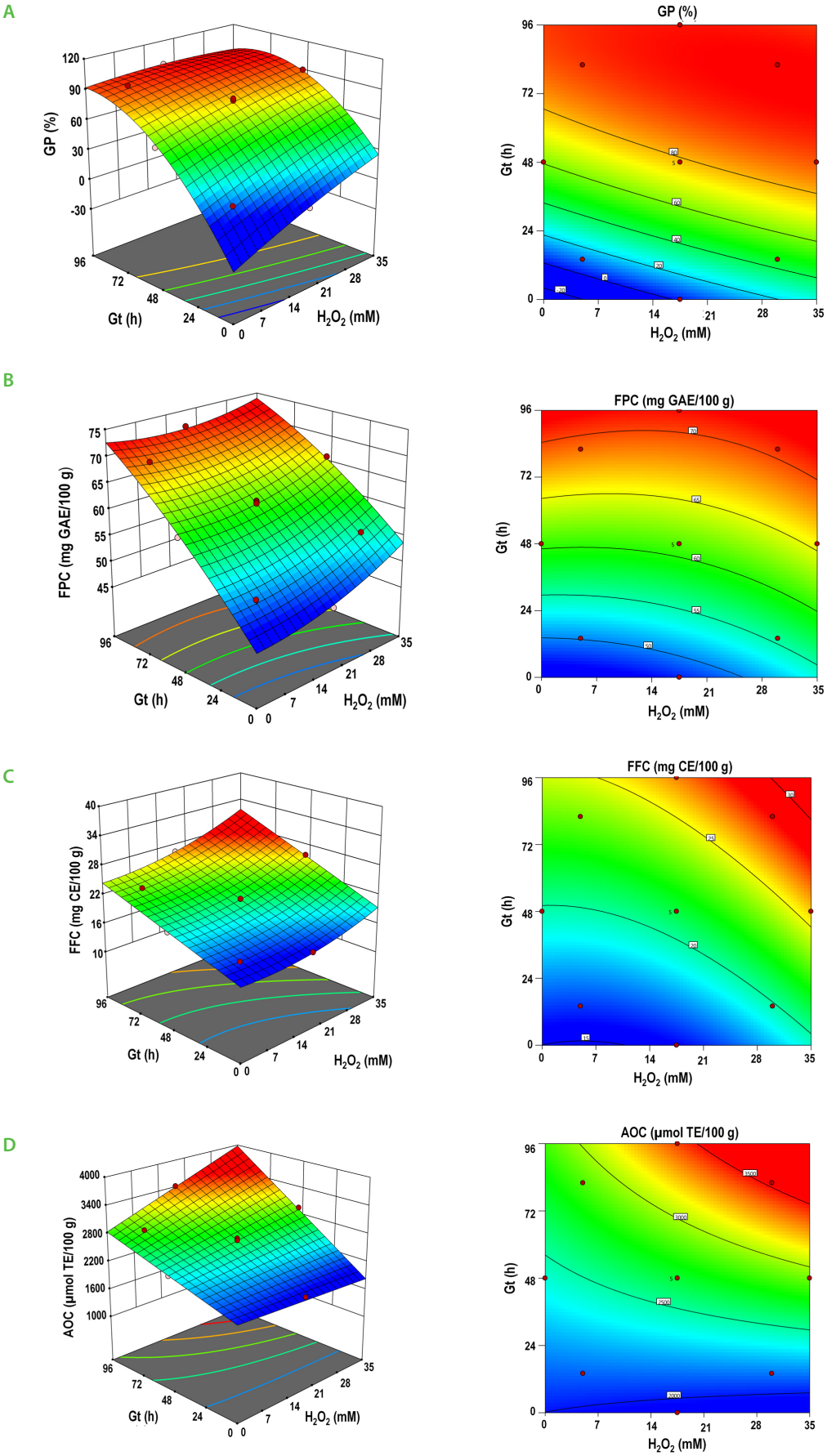


**Figure 2.** Response surface and contour plots for the effect of the soaking hydrogen peroxide concentration ($H_2O_2$), and germination time (Gt) on the response variables: (**A**) germination percentage (GP), (**B**) free phenolic content (FPC), (**C**) free flavonoid content (FFC), and (**D**) antioxidant capacity (AOC) of black runner bean sprouts.

**Table 4.** Parameters of predicted quadratic polynomial model of regression analysis for the response variables including germination percentage (GP), free phenolic content (FPC), free flavonoid content (FFP), and antioxidant capacity (AOC) after adjusting the experimental data.

| Parameter | GP | FPC | FFC | AOC |
|---|---|---|---|---|
| $\beta_0$ | 77.15 | 61.20 | 21.14 | 2,667.97 |
| $\beta_1$, $[H_2O_2]$ ($X_1$) | 10.08*** | 1.78*** | 2.10*** | 171.15*** |
| $\beta_2$, Gt ($X_2$) | 33.54*** | 8.43*** | 3.89*** | 529.30*** |
| $\beta_{11}$, $[H_2O_2]$ ($X_{12}$) | NS | 0.91*** | 0.80*** | NS |
| $\beta_{22}$, Gt ($X_{22}$) | 14.46*** | –0.84** | NS | NS |
| $\beta_{12}$, $[H_2O_2]$×Gt ($X_1X_2$) | –6.84*** | –0.92** | 0.37*** | 160.14*** |
| P-value for model | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| $R^2$ | 0.992 | 0.995 | 0.999 | 0.993 |
| $R^2_{ajusted}$ | 0.988 | 0.992 | 0.999 | 0.991 |
| P-value for lack of fit | $0.476^{NS}$ | $0.483^{NS}$ | $0.331^{NS}$ | $0.628^{NS}$ |
| CV (%) | 4.93 | 1.08 | 0.55 | 1.69 |

**Significant at $p<0.01$; ***significant at $p<0.001$; NS, not significant at $p\geq0.05$; $[H_2O_2]$, $H_2O_2$ concentration; Gt, germination time; R, coefficient of determination; CV, coefficient of variation.

and chia sprouts, supporting the present findings [Dziki *et al.* 2015; Nurnaeimah *et al.*, 2020; Gómez-Velázquez *et al.*, 2021]. Dziki *et al.* [2015] evaluated the influence of germination time (2, 4, 6, and 8 days) on the total phenolic compound content in wheat sprouts, finding that the highest values were obtained at 8 days of germination. On the other hand, several authors observed the same positive effect when stressing seeds or plants with $H_2O_2$, finding that concentrations of 16 and 30 mM improved the total phenolic content in ethanolic extracts of *F. deltoidea* [Nurnaeimah *et al.*, 2020], and concentrations of 10 and 20 mM significantly increased phenolic content in chia sprouts (*Salvia hispanica* L.) [Gómez-Velázquez *et al.*, 2021].

The observed increase in FPC in $H_2O_2$-treated sprouts may result from *de novo* synthesis and metabolic transformation. Enzymes such as L-tyrosine ammonialyase and L-phenylalanine ammonialyase are highly responsive to both germination duration and $H_2O_2$ elicitation [Świeca, 2016]. Additionally, seeds can upregulate defense enzymes like peroxidase and polyphenol oxidase to manage the rapid increase in ROS under stress conditions [Nurnaeimah *et al.*, 2020].

### Free flavonoid content

The free flavonoid content in black runner bean sprouts was affected by the $H_2O_2$ soaking concentration and Gt showing values ranging from 15.7 to 28.3 mg CE/100 g DW (**Table 3**). The analysis of variance yielded a significant quadratic model for FFC, which includes the linear terms of $[H_2O_2]$ and Gt, the quadratic terms of $[H_2O_2]$, and the interaction term of both variables ($[H_2O_2]$×Gt) (**Table 4**).

The response surface (**Figure 2C**) shows a progressive increase in FFC with increasing Gt, reaching a maximum at the longest durations tested. $H_2O_2$ concentration exerted a secondary but meaningful effect, enhancing flavonoid accumulation at intermediate doses, particularly in combination with extended Gt. These findings indicate that flavonoid biosynthesis is primarily regulated by developmental processes associated with germination and is further amplified by oxidative signals. $H_2O_2$ functions as a signaling molecule that modulates the expression of genes involved in flavonoid biosynthetic pathways, while prolonged germination supports sustained metabolic activity and substrate availability. Comparable responses have been reported in Dalia bean [Mendoza-Sánchez *et al.*, 2016], quinoa [Świeca, 2016], *F. deltoidea* [Nurnaeimah *et al.*, 2020], and chia seeds [Gómez-Velázquez *et al.*, 2021], demonstrating the robustness of $H_2O_2$ elicitation across various plants.

### Antioxidant capacity

The AOC in black runner bean sprouts ranged from 1,980 to 3,526 µmol TE/100 g DW and was affected by both process variables (**Table 3**). The analysis of variance yielded a significant two-factor interaction model for AOC, which includes the linear terms of $[H_2O_2]$ and Gt, as well as the interaction of both variables ($[H_2O_2]$×Gt) (**Table 4**).

The contour plot (**Figure 2D**) shows that AOC increased as both $H_2O_2$ concentration and Gt rose, mirroring the trends observed for FPC and FFC. Notably, the highest AOC value (3,526 µmol TE/100 g DW) was achieved by soaking seeds in 30 mM $H_2O_2$ and germinating for 82 h, indicating that high

peroxide concentrations can reduce the time required to reach near-maximum AOC, whereas at shorter germination times (*e.g.*, 14 h) peroxide concentration had little effect on AOC. The observed increase in AOC during germination is primarily attributed to elevated levels of phenolic compounds, as the FPC and FFC significantly correlated with AOC.

In line with previous studies on amaranth, lentil, Dalia bean, and chia sprouts [Gómez-Velázquez *et al.*, 2021; Mendoza-Sánchez *et al.*, 2016; Perales-Sánchez *et al.*, 2014; Świeca, 2015], these findings suggest that flavonoids and other phenolics enhanced by $H_2O_2$ treatment are key contributors to the improved antioxidant potential of black runner bean sprouts.

## Optimization of hydrogen peroxide soaking concentration and germination time

For optimizing process conditions, the RSM was used through the graphical method to determine the optimal combination of the [$H_2O_2$] and Gt that maximize the values of the GP, FPC, FFC, and AOC in the sprouts. The overlaid contour plot (**Figure 3**) was used to determine the best combinations of the process variables. The dark green area shows the region with the best combination of process variables to achieve the highest levels of the four response variables, from which the optimal conditions of [$H_2O_2$] (30 mM) and Gt (92 h) were selected.

The predicted values at the optimal point under the aforementioned conditions were: GP=96.7%, FPC=72.3 mg GAE/100 g DW, FFC=29.6 mg CE/100 g DW, and AOC=3,737 µmol TE/100 g DW. To validate the accuracy of the prediction model, runner bean seeds were germinated under the optimal conditions, and the response variables GP, FPC, FFC, and AOC were determined. Results are shown in **Table 5**. Growth performance and proximate chemical composition of sprouts obtained under optimal conditions were also analyzed.

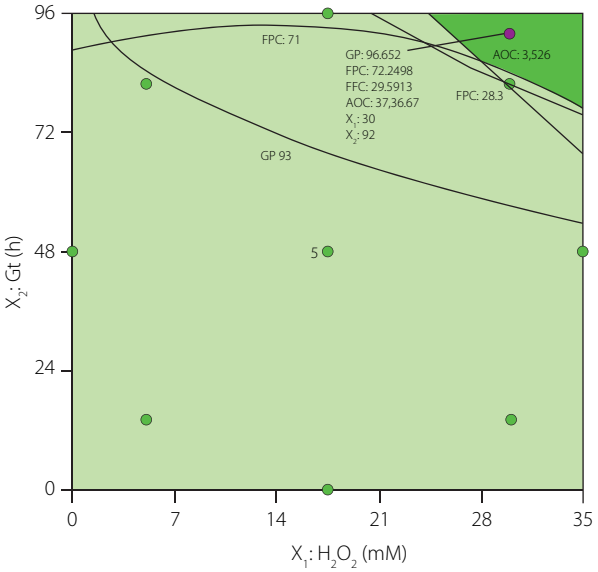


**Figure 3.** Optimization graph for the response variables: germination percentage (GP), free phenolic content (FPC), free flavonoid content (FFC), and antioxidant capacity (AOC) of black runner bean sprouts. $X_1$, soaking hydrogen peroxide concentration ($H_2O_2$); $X_2$, Germination time (Gt).

## Effect of the optimal treatment on growth performance and proximate chemical composition of runner bean sprouts

To evaluate the effect of soaking treatment with $H_2O_2$ on the growth of runner bean sprouts, radicle length and diameter, as well as the percentage of secondary roots, were analyzed. Sprouts obtained under optimal conditions were compared with those obtained by soaking without $H_2O_2$ for 92 h (control sprouts). The radicle length in the optimal and control treatments differed significantly ($p<0.05$), with the former having a 32.0% higher value (**Table 5**). This indicates a positive trend in the development of structures in runner bean sprouts due to the inductive effect of the $H_2O_2$ treatment. However, the radicle's diameter did not show significant differences ($p\geq0.05$) between the two treatments. Regarding the percentage of seeds with secondary roots, the optimal treatment registered a higher percentage compared to the treatment without the stressor, with 76.7% and 43.0%, respectively, showing a difference, which suggests that $H_2O_2$ accelerates the emergence of lateral structures in the seed's root system. The root is a fundamental organ whose main function is to absorb water and minerals; therefore, a greater length implies a higher probability of success for the establishment, development, and survival of the seedling. Interestingly, Saleh *et al.* [2019] found a correlation between the increase in their length and the rise in antioxidant capacity as well as the content of phenolic compounds and flavonoids in different legumes. Other authors have observed the same inductive trend of $H_2O_2$ on root growth in various seeds [Barba-Espín *et al.*, 2012; Chaplygina *et al.*, 2020]. These results could be explained by the interaction between the redox state and plant hormones orchestrated by $H_2O_2$ in the induction of proteins related to signaling and plant development during the early growth of seedlings [Barba-Espin *et al.*, 2010]. In addition, the natural accumulation of $H_2O_2$ promotes the energy metabolism required for the growth of the radicle and plumule rather than promoting water absorption in the early stage of germination through an increase in osmotic regulators; it mobilizes sugar reserves derived from stored starch, inhibits catabolism, and promotes the biosynthesis of gibberellic acid, a growth and development regulatory substance in seedlings [Delis-Hechavarría *et al.*, 2021; Song *et al.*, 2023].

The unsprouted runner black beans contained 18.96 g of protein, 2.25 g of lipids, 5.50 g of ash, and 73.29 g of total carbohydrates in 100 g DW (**Table 5**). This proximate composition largely coincides with those reported by various authors for different bean varieties [Alvarado-López *et al.*, 2019; Corzo-Ríos *et al.*, 2020; Osuna-Gallardo *et al.*, 2023], with minimal variations that could be explained by environmental conditions during cultivation and harvest, the grain variety, and the methodology used. The protein and lipid content in the sprouts obtained under optimal conditions and control sprouts showed significant increases ($p<0.05$) compared to the unsprouted seeds (**Table 5**). This increase can be attributed to the loss of dry weight due to the oxidation of carbohydrates during seed respiration and the activation of certain enzymes during

**Table 5.** Proximate composition, phenolic content, and antioxidant capacity of unsprouted runner bean and sprouted under control and optimal conditions, as well as growth performance of sprouts.

| Parameter | Unsprouted bean | Sprouted bean | |
|---|---|---|---|
| | | Control conditions (0 mM $H_2O_2$, 92 h) | Optimal conditions (30 mM $H_2O_2$, 92 h) |
| Germination percentage (%) | – | 82.5±5.8$^b$ | 95.7±1.2$^a$ |
| Radicle length (mm) | – | 53.5±9.0$^b$ | 70.7±11.0$^a$ |
| Radicle diameter (mm) | – | 3.2±0.5$^a$ | 3.3±0.5$^a$ |
| Secondary roots (%) | – | 43.0±1.1$^b$ | 76.7±6.6$^a$ |
| Protein (g/100 g DW) | 18.96±0.26$^b$ | 21.73±0.56$^a$ | 22.76±0.50$^a$ |
| Lipids (g/100 g DW) | 2.25±0.09$^b$ | 2.75±0.08$^a$ | 2.76±0.03$^a$ |
| Ash (g/100 g DW) | 5.50±0.21$^a$ | 5.76±0.17$^a$ | 5.91±0.08$^a$ |
| Total carbohydrates (g/100 g DW) | 73.29±0.56$^a$ | 69.60± 0.81$^b$ | 68.72±0.61$^b$ |
| Free phenolic content (mg GAE/100 g DW) | 58.8±1.6$^c$ | 67.6±0.9$^b$ | 72.7±1.8$^a$ |
| Free flavonoid content (mg CE/100 g DW) | 22.3±1.5$^c$ | 26.4±1.4$^b$ | 28.6±1.5$^a$ |
| Total anthocyanin content (mg C3GE/100 g DW) | 9.5±0.2$^a$ | 3.8±0.2$^b$ | 3.0±0.3$^c$ |
| ABTS assay (µmol TE/100 g DW) | 2,472±56$^c$ | 3,028±38$^b$ | 3,782±58$^a$ |
| ORAC (µmol TE /100 g DW) | 4,982±303$^c$ | 5,793±280$^b$ | 6,573±283$^a$ |

Different lowercase letter superscripts in the same row show significant difference ($p$<0.05). ABTS assay, assay with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); ORAC, oxygen radical absorbance capacity; GAE, gallic acid equivalent; CE, catechin equivalent; TE, C3GE, cyanidin 3-glucoside equivalent; Trolox equivalent, DW, dry weight.

the germination process [Nazih *et al.*, 2025]. No significant difference ($p$≥0.05) was found between the proximate chemical composition of the control sprouted seeds and the seeds sprouted under optimized conditions, contrary to what was reported by León-López *et al.* [2020], who reported a significant increase in protein content in germinated chickpea grains under chemical stress with $H_2O_2$.

### Effect of the optimal treatment on phytochemicals and antioxidant properties of runner bean sprouts

The free phenolic content measured in unsprouted runner bean (58.8 mg GAE/100 g DW) was consistent with values reported by Osuna-Gallardo *et al.* [2023], but lower than those observed in other *P. coccineus* varieties [Alvarado-López *et al.*, 2019]. Germination resulted in significant ($p$<0.05) increases in FPC for both the optimal (to 123.7%) and control (to 115.0%) treatments compared to the unsprouted seeds (**Table 5**). Additionally, the optimal $H_2O_2$ treatment yielded significantly ($p$<0.05) higher FPC than the control, supporting findings in other seeds or grains, including chickpea, chia, and barley, where $H_2O_2$ elicitation promoted phenolic accumulation [Delis-Hechavarría *et al.*, 2021; Gómez-Velázquez *et al.*, 2021; León-López *et al.*, 2020]. A higher phenolic content is noteworthy, as these compounds contribute substantially to the antioxidant activity of sprouts.

The free flavonoid content also increased significantly ($p$<0.05) in both optimal (to 128.1%) and control (to 118.1%) treatments relative to the unsprouted seeds (**Table 5**). The increase in FFC under the optimal $H_2O_2$ treatment is consistent with previous studies in other species exposed to hydroxyl peroxide elicitation [Gómez-Velázquez *et al.*, 2021; Uchegbu & Amulu, 2015], highlighting the role of $H_2O_2$ as an effective stressor to enhance flavonoid biosynthesis.

For total anthocyanin content, the value obtained in this study (9.5 mg C3GE/100 g DW) was lower than those previously reported for bean of some *P. coccineus* varieties [Alvarado-López *et al.*, 2019], but higher than those observed by Osuna-Gallardo *et al.* [2023], who reported values close to 4 mg C3GE/100 g for unprocessed runner bean. In our study, germination led to a significant ($p$<0.05) reduction in TAC in both optimal and control treatments compared to the unsprouted seeds (**Table 5**). James *et al.* [2020] reported a decrease in anthocyanin content as germination time increased in different legumes, probably because various enzyme systems are mobilized and activated during germination, leading to anthocyanin loss through oxidation and leaching. Meanwhile, Dueñas *et al.* [2006] evaluated the effect of germination and elicitation on the phenolic profile of bean seeds (*Phaseolus vulgaris* L.) germinated for 8 days and observed a reduction in total and some specific anthocyanins, reporting

that germination in the presence of inducers or stressors caused a more extensive decrease in anthocyanin content even below the quantification limit. The behavior recorded in the present study for the anthocyanin content in black runner bean soaking under the induction of chemical stress with $H_2O_2$ is in agreement with these findings, since a greater reduction was observed in the optimal germination treatment than in the control one (**Table 5**).

The antioxidant capacity (AOC) of unsprouted seeds and sprouts germinated under both optimal and control conditions was evaluated as $ABTS^{\bullet+}$ scavenging activity and ORAC. According to Munteanu & Apetrei [2021], the ABTS assay operates *via* a mixed mechanism (involving both electron and hydrogen atom transfer), whereas the ORAC assay relies primarily on hydrogen-atom-transfer mechanisms. The use of both methods provided a more comprehensive assessment of antioxidant properties. The value obtained by the ABTS assay for the unsprouted seed (2,472 µmol TE/100 g DW) was consistent with that reported by Osuna-Gallardo *et al*. [2023], who reported 2,657.94 µmol TE/100 g of unprocessed runner bean. However, it was higher than the values reported by Orak *et al*. [2016] for ten white bean (*P. vulgaris*) varieties (350–517 µmol TE/100 g), and also higher than those reported by Weidner *et al*. [2018] for four *P. vulgaris* varieties (421–640 µmol TE/100 g DW). Optimal germination treatment and the control germination treatment showed significant increases ($p<0.05$) of 53.0% and 22.5%, respectively, compared with the unsprouted bean (**Table 5**). Likewise, a significant increase ($p<0.05$) of 24.9% was observed as a result of the soaking treatment with $H_2O_2$ when comparing the optimal sprouts to the controls. This increase in the AOC in the sample germinated under optimal conditions could be associated with the stimulation of the synthesis of compounds with high antioxidant activity, such as phenolic compounds, due to the chemical stress produced by the application of $H_2O_2$ during germination [León-López *et al*., 2020].

Several authors have emphasized the increase in AOC during the germination process using $H_2O_2$ as an inducing stress agent. For instance, Gómez-Velázquez *et al*. [2021] recorded an increase in the AOC of chia seeds germinated with 10, 20, and 30 mM $H_2O_2$, obtaining increases in the range of 29–37% compared to seeds germinated without the stressor. Similarly, León-López *et al*. [2020] reported that in white chickpea germination, chemical $H_2O_2$ elicitation produced an increase of 14.8% when comparing their optimal treatment ([$H_2O_2$] of 30 mM, Gt of 72 h) with control seeds without elicitor ([$H_2O_2$] of 0 mM, Gt of 72 h). Conclusions from these studies agree with the present findings regarding the increase in AOC measured by the ABTS assay in runner bean sprouts treated with $H_2O_2$.

The ORAC showed the same trend as that determined by ABTS assay (**Table 5**). Optimal and control germination treatments showed significant increases ($p< 0.05$) of 31.9% and 16.3%, respectively, compared with ungerminated runner beans, moreover, a significant increase ($p<0.05$) of 13.5% was observed between runner beans germinated under optimal conditions and those germinated under control conditions. The ORAC of the ungerminated seed (4,982 µmol TE/100 g DW) was close to that reported by Alvarado-López *et al.* [2019], who reported values of 5,162; 3,694; 2,557; and 2,031 µmol TE/100 g, for four varieties of *P. coccineus* bean: purple, black, brown, and white, respectively. However, Osuna-Gallardo *et al.* [2023] recorded an ORAC value of 3,866 µmol TE/100 g of runner bean flour, which was lower than those reported in this study. These variations may be associated with differences in the phenolic profiles among bean varieties, as well as with the extraction and quantification techniques used.

In summary, these results demonstrate that $H_2O_2$ treatment during soaking significantly enhanced the antioxidant capacity of black runner bean sprouts, supporting their potential use as nutraceutical ingredients with improved health-promoting properties.

A variety of phenolic acids, flavonoids, and anthocyanins have been reported in runner bean varieties [Baeza-Jiménez & López-Martínez, 2024; López-Martínez, 2020]. Particularly, gallic acid, sinapic acid, ferulic acid, chlorogenic acid, *p*-coumaric acid, protocatechuic acid, and 4-hydroxybenzoic acid have been identified in unprocessed black runner beans [Baeza-Jiménez & López-Martínez, 2024]. Flavonoids, such as kaempferol 3-glucoside, catechin, and epicatechin, were also reported in unprocessed black runner beans. In this study, gallic, syringic, ferulic, chlorogenic, and *p*-coumaric acids were identified in unsprouted runner beans (**Table 6**). Regarding flavonoids, consistent with Baeza-Jiménez & López-Martínez [2024], catechin and quercetin were identified; in addition, rutin was detected in the free phenolic fraction of the unsprouted seeds. Germination processes can modify the phenolic profile [Domínguez-Arispuro *et al.,* 2018; Yu *et al.,* 2023], and even soaking stress can elicit an increase in some phenolic contents [León-López *et al*., 2020].

Among the main findings, the significant ($p<0.05$) increase in the content of gallic acid and syringic acid, and the presence of caffeic acid, stand out as a result of germination in the free phenolic fraction (**Table 6**). The content of catechin and quercetin also significantly increased ($p<0.05$) in the free phenolic fraction as a result of the control germination treatment. The stress treatment with $H_2O_2$ during soaking induced specific changes in the free phenolic fraction compared to the unprocessed seed and the control sprouts; notably, a significant increase in ferulic acid and *p*-coumaric acid was observed. In contrast, the content of catechin and quercetin decreased significantly ($p<0.05$) compared to the control sprouts.

Although the content of bound phenolic compounds was not used as a response variable for the RSM optimization, nor were their antioxidant properties individually measured, it was considered important to analyze the phenolic profile of this fraction to fully discuss the metabolic changes that occurred. Ferulic and *p*-coumaric acids, which increased in both free and bound phenolic fractions in runner bean sprouts obtained under optimal conditions, serve as potent antioxidants with applications across food, nutraceuticals, and pharmaceuticals. Ferulic acid supports metabolic health by enhancing glucose and lipid metabolism and mitigating oxidative stress, while

**Table 6.** Phenolic compound profile of unsprouted runner bean and sprouted under control and optimal conditions (μg/g dry weight).

| Phenolic compound | Unsprouted bean | | Sprouted bean | | | |
|---|---|---|---|---|---|---|
| | | | Control conditions (0 mM $H_2O_2$, 92 h) | | Optimal conditions (30 mM $H_2O_2$, 92 h) | |
| | Free | Bound | Free | Bound | Free | Bound |
| Gallic acid | 158.5±3.6$^{c}$ | 72.7±1.3$^{f}$ | 218.0±6.8$^{a}$ | 120.6±2.3$^{d}$ | 197.2±3.3$^{b}$ | 97.3±2.4$^{e}$ |
| Syringic acid | 7.8±0.5$^{d}$ | 10.4±0.7$^{d}$ | 52.9±2.4$^{c}$ | 137.4±3.7$^{a}$ | 57.0±4.0$^{c}$ | 76.3±1.8$^{b}$ |
| Ferulic acid | 89.2±1.7$^{c}$ | 184.2±4.8$^{b}$ | 43.9±2.5$^{d}$ | 86.8±2.7$^{c}$ | 190.1±3.9$^{b}$ | 356.2±7.2$^{a}$ |
| Chlorogenic acid | 26.7±0.1$^{b}$ | ND | 25.5±0.7$^{b}$ | 15.3±1.5$^{c}$ | 27.3±2.1$^{b}$ | 42.8±0.7$^{a}$ |
| Caffeic acid | ND | ND | 14.0±0.4$^{a}$ | 14.2±0.3$^{a}$ | 14.04±0.1$^{a}$ | 7.1±0.0$^{b}$ |
| *p*-Coumaric acid | 25.4±0.2$^{e}$ | 81.7±2.4$^{b}$ | 24.2±2.7$^{e}$ | 39.1±0.14$^{d}$ | 67.6±2.7$^{c}$ | 205.9±4.1$^{a}$ |
| Catechin | 53.3±3.6$^{d}$ | 72.5±1.9$^{c}$ | 85.7±3.7$^{b}$ | 178.6±4.7$^{a}$ | 76.8±1.4$^{bc}$ | 82.4±4.6$^{b}$ |
| Quercetin | 14.8±0.9$^{d}$ | 41.4±1.5$^{b}$ | 38.2±2.4$^{b}$ | 103.4±4.1$^{a}$ | 20.1±0.9$^{cd}$ | 25.3±2.3$^{c}$ |
| Rutin | 128.4±3.1$^{b}$ | ND | 125.1±4.7$^{b}$ | 189.8±5.9$^{a}$ | 120.0±6.3$^{b}$ | 13.3±0.8$^{c}$ |
| Total phenolics | 504.1±13.0$^{d}$ | 462.9±12.6$^{d}$ | 613.4±26.1$^{c}$ | 871.0±26.2$^{a}$ | 756.1±24.8$^{b}$ | 899.4±24.0$^{a}$ |

Different lowercase letter superscripts show significant differences across rows ($p$<0.05). ND, not detected.

also exerting anti-inflammatory, antimicrobial, neuroprotective, and cardiovascular effects [Jacobo-Velázquez, 2025; Kumar *et al.*, 2025]. Meanwhile, *p*-coumaric acid provides anti-inflammatory, antidiabetic, anticancer, cardioprotective, and hepatoprotective benefits, positioning both phenolic acids as promising candidates for drug formulations and chronic disease prevention [Kaur & Kaur, 2022; Kumar *et al.*, 2025].

## CONCLUSIONS

The present study demonstrates that $H_2O_2$ treatment before black runner bean seed germination is an effective strategy to enhance sprout quality. $H_2O_2$ elicitation improved germination performance, stimulated radicle growth, increased the accumulation of total phenolic compounds, and enhanced the antioxidant capacity of sprouts. Optimal conditions, 30 mM $H_2O_2$ combined with prolonged germination (around 92 h), resulted in higher levels of certain free and bound phenolic acids and flavonoids, as well as improved $ABTS^{\bullet+}$ scavenging activity and ORAC of the sprouts. The use of response surface methodology (RSM) was critical for identifying these optimal elicitation conditions, offering a robust statistical framework that facilitates reproducibility and potential scale-up. These findings highlight the promise of $H_2O_2$-assisted sprouting for the development of functional flours with enhanced nutritional and potential health benefits.

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## CONFLICT OF INTERESTS

Authors declare no conflicts of interest.

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