Research Article **Open Access**

Laser-primed wheat as a source of phenolic bioactive compounds with antioxidant and cytoprotective potential

[1,2]Tamar V. Sanikidze*, [3]Andre D.L. Batako, [4]Baoxiu Qi, [3]Juan Ahuir Torres, [1,2]Irakli Chkhikvishvili, [5]Nana Bakradze, [1]Eka Shekiladze, [5]Alexander Sharashenidze, [2]Maia Enukidze, [2]Marine Machavariani, [2]Nunu Gogia, [1]Marina Tsimakuridze, [6]Lela Chkhitauri, [5]Teimuraz Dumbadze, [1]Sophio Kalmakhelidze

[1]Department of Physics, Biophysics, Biomechanics and Informative Technologies, Tbilisi State Medical University, Tbilisi, Georgia; [2]V. Bakhutashvili Institute of Medicol Biotechnologies, Tbilisi State Medical University, Tbilisi, Georgia; [3a]General Engineering Research Institute, Faculty of Health, Innovation, Technology & Science, Liverpool John Moores University; [4]School of Pharmacy and Biomolecular Science. Faculty of Health, Innovation, Technology & Science, Liverpool John Moores University; [5]Georgian Technical University, Tbilisi, Georgia; [6]European University, Tbilisi, Georgia.

***Corresponding author:** Tamar Sanikidze, PhD, Professor, TbilisiState Medical University, 33 Vaja Pshavela av, Tbilisi - 380060, Georgia

Submission Date: December 31st, 2025, **Acceptance Date:** February 16th, 2026; **Publication Date**: February 18th, 2026

Please Cite this Article as: Sanikidze T.V., Batako A.D.L., Qi B., Ahuir Torres J., Chkhikvishvili I., Bakradze N., Shekiladze E., Sharashenidze A., Enukidze M., Machavariani M., Gogia N., Tsimakuridze M., Chkhitauri L., Dumbadze T., Kalmakhelidze S. Laser-primed wheat as a source of phenolic bioactive compounds with antioxidant and cytoprotective potential. *Bioactive Compounds in Health and Disease*. 2026; 9(2): 73–88. DOI: https://doi.org/10.31989/bchd.v9i2.1896

ABSTRACT

Background: Wheat is a major dietary staple and an important source of phenolic bioactive compounds with antioxidant and cytoprotective properties. However, environmental stress and conventional farming methods can limit the accumulation of these beneficial compounds and affect their functional quality. A promising strategy to enhance the natural synthesis of bioactive compounds is still under investigation. The functional relevance of laser-induced phenolic enrichment in human cell systems remains largely unexplored.

Objective: To determine the effect of CO_2 laser seed priming on wheat phenolic content and functional (antioxidant and cytoprotective) activity.

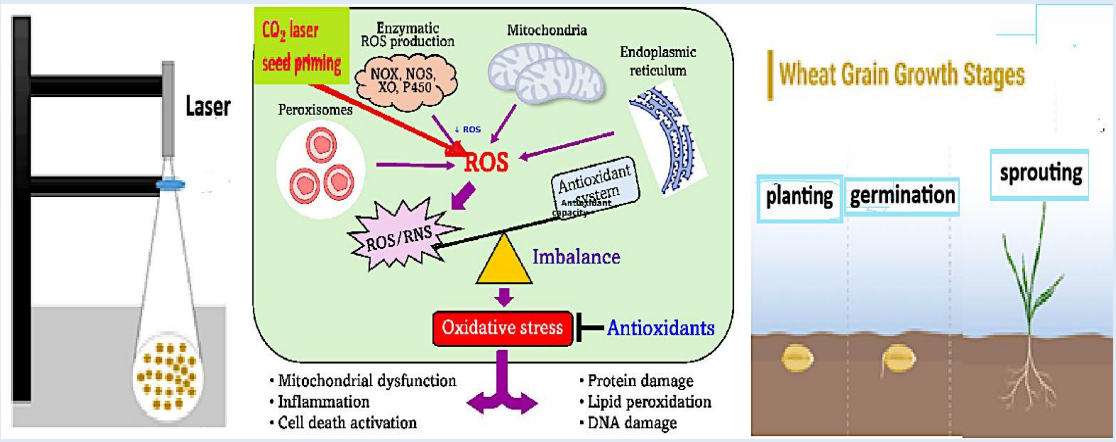
Methods: This study assessed the effects of continuous-wave CO_2 laser seed priming in the wheat cultivar “Red Doly” at three developmental stages: grain, germination, and green sprouts. Total free and bound phenolics, as well as phenolic profiles, were determined using High-Performance Liquid Chromatography (HPLC). Antioxidant capacity was evaluated through radical-scavenging assays. Cytoprotective activity was examined in Jurkat T lymphocytes under oxidative stress induced by hydrogen peroxide.

Results: Laser priming enhanced germination and early growth (5–25% increase vs. control). Total free phenolic content increased significantly, particularly in green sprouts ($p < 0.01$). The antioxidant capacity increased at all developmental stages and correlated strongly with phenolic content ($r = 0.81$, $p = 0.0004$). Caffeic acid was most abundant in the free fractions, while ferulic acid dominated in the bound fractions. Phenolic extracts from laser-treated sprouts significantly improoved Jurkat cell viability and reduced oxidative cytotoxicity ($p < 0.001$), with more pronounced effects observed for free-phenolic–enriched fractions.

Conclusions: This study establishes a functional connection between laser-induced metabolic activation and the protection of human cells by integrating compound identification, biomarker validation, and preclinical cellular efficacy testing (Steps 1, 2, 5, 6, and 8 of the Functional Food Development Model). CO_2 laser priming offers a scalable, chemical-free approach to producing phenolic-enriched functional wheat ingredients. Notably, this research is one of the first to demonstrate that phenolic enrichment induced by CO_2 laser priming results in measurable cytoprotective effects in a human cell-based model.

Novelty: This study is one of the first to establish a connection between CO_2 laser priming-induced phenolic enrichment in wheat and a functional biological outcome. It demonstrates significant cytoprotection of human Jurkat T lymphocytes under oxidative stress. The research also includes detailed phenolic profiling using HPLC and validation of antioxidant biomarkers.

Keywords: CO_2 laser seed priming; Wheat phenolics; Antioxidant capacity; Cytoprotective activity; Functional foods; Wheat sprouts; Oxidative stress



Graphical Abstract: Effect of laser priming on quantity and quality of phenolic compounds of wheat.

INTRODUCTION

Cereals are crucial commodities in global agriculture, with wheat (Triticum spp.) being one of the most important crops. Wheat is a rich source of complex carbohydrates, dietary fiber, and plant-based protein. It also contains essential micronutrients, including

magnesium, zinc, selenium, and B-group vitamins. The health benefits of wheat are largely attributed to bioactive compounds, including phenolic acids, flavonoids, tocopherols, carotenoids, and bioactive peptides. These compounds exhibit antioxidant, anti-inflammatory, and antimicrobial properties [1-6], which can help reduce risks of cardiovascular disease and type 2 diabetes, colorectal cancer and diverticular disease, as well as improve gut microbiota composition [7-8]. Additionally, phenolic compounds and other phytochemicals found in cereal products act as functional bioactive components with antioxidant properties that may positively influence health outcomes [9].

Wheat is highly susceptible to environmental stresses, which can be categorized as abiotic (such as drought, heat, salinity, nutrient deficiency, and waterlogging) and biotic (including fungal diseases, bacterial infections, and insect pests). These stresses can disrupt membrane integrity and photosynthetic efficiency, impair root development, reduce grain filling, and decrease grain weight. Additionally, they can alter ionic balance, enzyme activity, and germination capacity, severely hindering growth and yield while contributing to production instability.

These challenges highlight the urgent need for climate-resilient wheat varieties. [10].

Agronomic and chemical strategies include targeted fertilization, micronutrient supplementation, and integrated disease-management practices, all of which help mitigate stress and pest damage. Genetic advances, such as genomic selection, accelerate breeding for drought- and heat-tolerant cultivars. Additionally, seed priming techniques - that is, physical methods involving electromagnetic and laser/light-based treatments - enhance germination and improve stress resistance [11-12]. Therefore, integrated strategies that combine agronomy, breeding, and precision priming technologies are essential for sustainable wheat production.

Laser-assisted seed priming enhances seed metabolism and improves tolerance to environmental stress by reprogramming metabolic pathways within seeds at three interconnected levels: activating primary metabolism, reinforcing redox homeostasis, and stimulating secondary metabolite biosynthesis [13]. Consequently, laser priming boosts energy production, nutrient allocation, and stress adaptation.

The rapid activation of α-amylase is essential for the early response to laser priming by accelerating starch hydrolysis. This process increases glucose availability, enhances mitochondrial activation, raises mitochondrial membrane potential, and boosts ATP synthesis, providing a critical energy source for germination [13]. Additionally, laser priming stimulates cyclic AMP (cAMP) signaling, promoting root elongation and improving nutrient uptake.

Moreover, it affects gene regulation through the mitogen-activated protein kinase (MAPK) signaling cascade. This cascade activates transcription factors that enhance the antioxidant defense enzymatic system, including superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX). Consequently, laser priming improves the detoxification of reactive oxygen species (ROS) and offers cellular protection against lipid peroxidation [14].

Furthermore, laser priming enhances ion transport efficiency and regulates ionic balance, which is vital for achieving an optimal hormonal balance. This entire process supports germination and promotes early seedling growth [15].

Laser irradiation not only stimulates secondary metabolism but also enhances nitrogen assimilation and its conversion into amino acids, which contributes to vigorous early growth. These metabolic changes lead to

structural and molecular modifications, such as increased seed coat permeability, improved water uptake, and the upregulation of heat-shock proteins, metal transporters, and drought-responsive genes.

The current study explored the effects of laser-based seed priming on the physiological, biochemical, and quality characteristics of wheat seeds. The aim was to uncover new mechanistic insights into the stress resilience induced by lasers in wheat and to increase the levels of nutritionally important compounds.

MATERIALS AND METHODS

Plant Material: The study was conducted on the commonly cultivated Georgian wheat variety Red Doly (*Triticum carthlicum* Nevski).

Laser Irradiation of wheat seeds: Seeds were irradiated using a continuous-wave CO_2 laser (Laser 6040ER-100; 10.6 µm, max 100 W) equipped with a 12 mm focusing lens (focal length 50.6 mm). The irradiation was conducted at two angles under low-defocusing conditions (ΔF = 120–210 mm), with power settings of 25–35 W and beam speeds of 25–55 mm/s.

The seeds were placed on the laser platform, and both beam movement and defocusing were controlled by software. After irradiation, both treated and intact (control) seeds were sown, and germinated sprouts were used for subsequent analyses.

Extraction of free and bound phenolic compounds: Phenolic compounds (free and bound) were extracted from dried; ground germinates and sprouts following modified methods of Martín-Diana et al. [16, 17].

Free Phenolics: One gram of sample was extracted with 20 mL of One gram of the sample was extracted using 20 mL of an ethanol/water mixture (80:20, v/v) through magnetic stirring for 10 minutes at room temperature. The mixture was then centrifuged at 2500 × g for 10 minutes at 25 °C. The supernatant was filtered, and the residue was re-extracted under the same conditions. The combined extracts were evaporated at 40 °C, re-dissolved in a methanol/water mixture (80:20), filtered through a 0.22 µm filter, and stored at −20 °C.

Bound Phenolics: The remaining residue was subjected to alkaline hydrolysis with 10 M NaOH overnight at room temperature. Afterwards, the solution was acidified to pH 2 and extracted three times with ethyl acetate. Following centrifugation, acid hydrolysis was carried out with concentrated hydrochloric acid (HCl) at 85 °C for 30 minutes. This was followed by additional extraction with ethyl acetate. The organic phases were combined, evaporated at 40 °C, and then re-dissolved in methanol. The solution was filtered through a 0.22 µm filter and stored at -20 °C.

High-performance liquid chromatograph (HPLC) Analysis of Phenolic Compounds: Free and bound phenolics were analyzed using an Agilent 1260 Infinity HPLC system with a Supelco C18 column (25 cm × 4.6 mm, 5 µm) at 35 °C. Injection volume was 20 µL [18].

Mobile phases:

- A: 1% acetic acid in water
- B: acetonitrile

Flow rate: 1 mL/mi. Gradient: 5%–60% B (37 min), 60%–98% B (3 min), re-equilibration to 5% B (5 min). Compounds were identified at 290 and 320 nm.

Total Antioxidant Capacity (TAC): TAC of free and bound fractions was evaluated using 2,2-diphenyl-1-picrylhydrazyl–(DPPH) radical scavenging and oxygen radical absorbance capacity assays.

DPPH Assay: Antioxidant activity was measured at 515 nm following a modified Brand-Williams method [19]. Extract (1 mL) was mixed with 2 mL DPPH solution and incubated for 30 min.

Radical scavenging activity (%) was calculated as:

$$\%R = [(C - S) / C] \times 100$$

where C is the control absorbance, and S is the sample absorbance.

Cell Culture and Oxidative Stress Model: Jurkat human T leukemia cells (DSMZ) were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 4 mM L-glutamine, penicillin (100 U/mL), and streptomycin (100 U/mL) at 37 °C in 5% CO_2.

Oxidative stress was induced with 50 µM H_2O_2 for 24 h (4×10^5 cells/mL). Extracts were added to intact and stressed cells for 24 h.

Cell Viability (MTT Assay): Cells (2×10^6 cells/mL) were treated as described, centrifuged, washed with PBS, and incubated with MTT (8 mg/mL) for 4 h at 37 °C. Formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm.

Cell viability (%) was calculated as:

$$K = (A_{sample} / A_{control}) \times 100$$

Statistical Analysis: Experiments were conducted in a completely randomized design with three biological replicates. Results are expressed as mean ± SD. Data were analyzed using one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). The relationships between phenolic content and antioxidant activity were assessed using Pearson correlation analysis and linear regression techniques. Statistical significance was set at $p < 0.05$.

RESULTS

Total Free Phenols and Antioxidant Capacity: Laser irradiation significantly increased the total phenolic content (TPC) in "Red Doly" wheat at all developmental stages—grain, germinates, and green sprouts—compared to the respective control samples (Figure 1). In both grains and germinates, the laser-treated samples exhibited significantly higher phenolic levels than the untreated samples ($p < 0.05$). The most pronounced effect occurred at the green-sprout stage, where the laser treatment resulted in the highest TPC among all samples ($p < 0.01$). These findings indicate that phenolic accumulation becomes more responsive to laser stimulation during later developmental stages, especially in metabolically active green tissues.

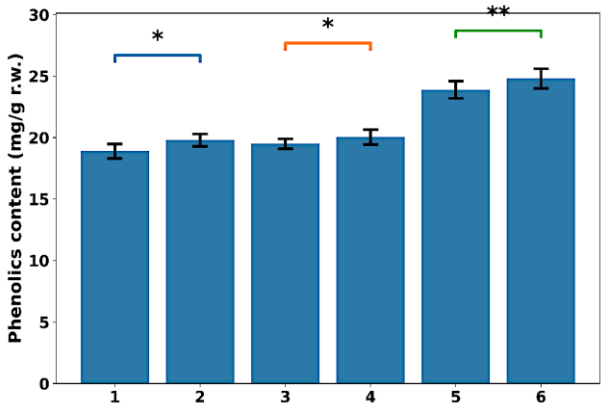


Figure 1. TPC in grains, germinates, and green sprouts of "Red Doly" wheat under control and CO_2 laser-treated conditions. The values are reported as mean ± standard deviation (SD) (n = 3). Asterisks indicate significant differences between the control and laser-treated samples at the same developmental stage (* $p < 0.05$; ** $p < 0.01$). The samples include: (1–2) grains (control, laser-treated), (3–4) germinates (control, laser-treated), (5–6) green sprouts (control, laser-treated).

TAC, measured by DPPH radical scavenging activity, exhibited a similar pattern (Figure 2). Laser-treated grains and germinates showed significant increases in TAC compared with controls ($p < 0.05$), while green sprouts

showed the greatest enhancement ($p < 0.01$). The parallel increase in TPC and TAC indicates that laser treatment enhances antioxidant potential throughout development.

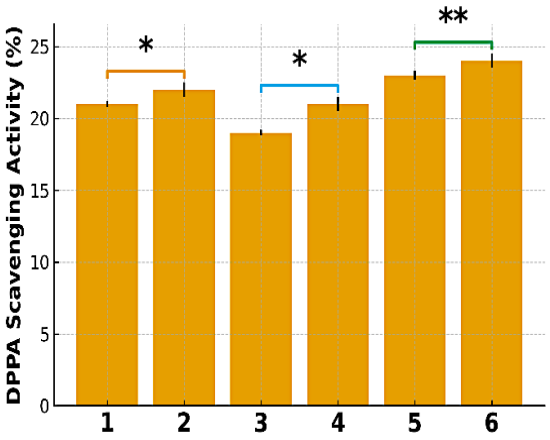


Figure 2. The TAC, measured by DPPH radical-scavenging activity, of extracts from "Red Doly" wheat at various developmental stages, under control and CO_2 laser-treated conditions. The bars represent the mean ± standard deviation (n = 3). Asterisks indicate significant differences between control and laser-treated samples at the same developmental stage (* $p < 0.05$; ** $p < 0.01$). The samples include: (1) control grain, (2) laser-treated grain, (3) control germinates, (4) laser-treated germinates, (5) control green sprouts, and (6) laser-treated green sprouts.

Correlation analysis showed a strong positive link between phenolic content and TAC, with a Pearson correlation coefficient of $r = 0.81$ ($p = 0.0004$; see Figure 3). Antioxidant activity consistently rose with higher levels of phenolics, particularly in green sprouts. This suggests that phenolic compounds significantly contribute to boosting the antioxidant capacity of “Red Doly” wheat.

Overall, these findings demonstrate that laser irradiation progressively stimulates phenolic biosynthesis and antioxidant activity, with the most pronounced effects observed in green sprouts.

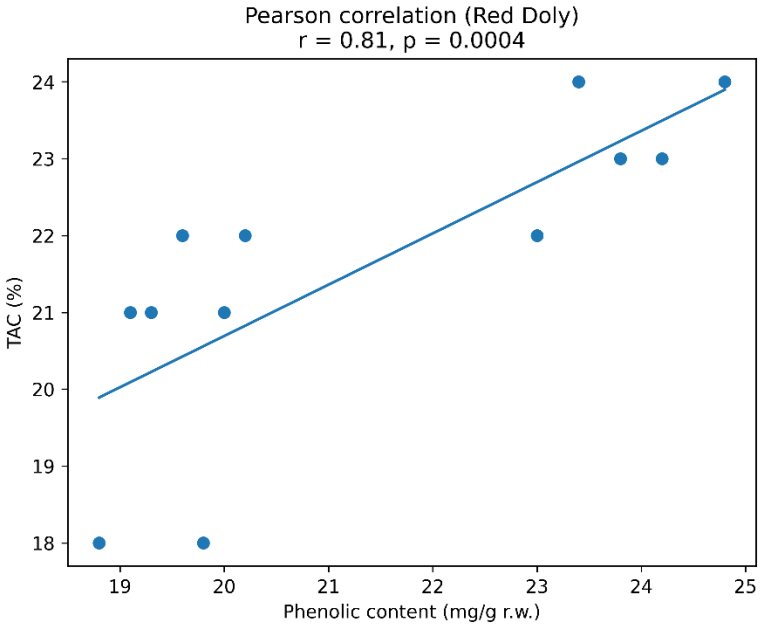


Figure 3. Relationship between TPC and TAC of wheat phenolic fractions. Pearson’s correlation coefficient ® and p-value are shown. The solid line indicates linear regression. A significant positive correlation was observed ($r = 0.81$, $p = 0.0004$).

Phenolic Acid Composition and Quantitative Changes: Chromatographic profiling demonstrated clear qualitative and quantitative differences between the free and bound phenolic fractions in wheat sprouts (Figures 4 and 5). The free phenolic fraction comprised 4-hydroxybenzoic, caffeic, vanillic, syringic, ferulic, sinapic, and *p*-coumaric acids, with caffeic acid identified as the predominant compound.

In contrast, the bound fraction—released following sequential alkaline and acid hydrolysis—displayed a comparable range of phenolic acids but was 79 compartmental by a marked predominance of ferulic acid.

This distribution pattern reflects the well-established compartmentalization of phenolic acids in cereal matrices, where hydroxycinnamic acids, particularly ferulic acid, are predominantly esterified or ether-linked to cell wall polysaccharides and structural components [21, 25-26, 33].

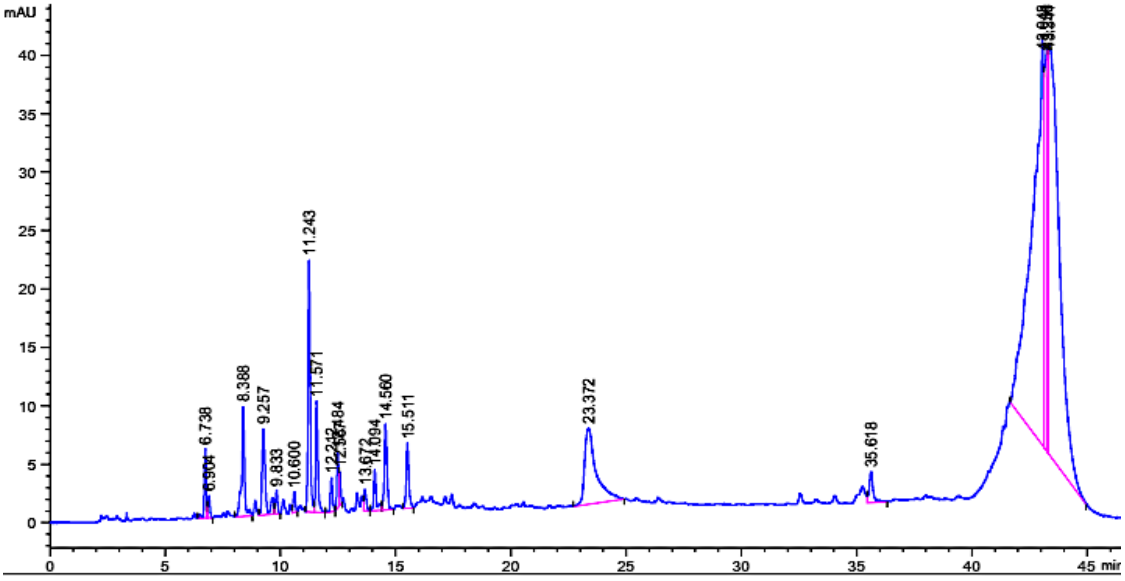


Figure 4. Chromatographic profile of the sprout extract showing free phenolic compounds. Rt (min): 4-hydroxybenzoic acid (8.38), caffeic acid (11.24), vanillic acid (14.25), syringic acid (14.56), ferulic acid (15.51), sinapic acid (16.15), and p-coumaric acid (16.93). Detection at 290 nm.

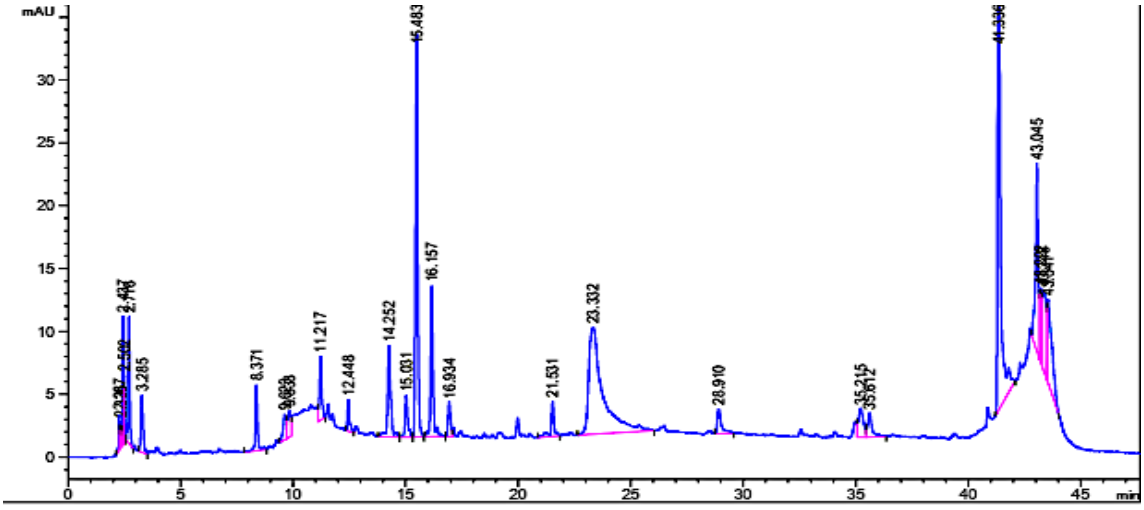


Figure 5. Chromatographic profile of the sprout extract after alkaline and acid hydrolysis. The bound phenolic fraction includes gallic acid (Rt = 3.28), 4-hydroxybenzoic acid (8.37), caffeic acid (11.21), vanillic acid (14.23), syringic acid (14.98), ferulic acid (15.48), sinapic acid (16.15), and p-coumaric acid (16.93). Detection at 290 nm.

Quantitative analysis revealed that laser treatment significantly altered the phenolic acid profile of wheat sprouts (Figure 6). Following irradiation, the total phenolic acid content increased by approximately 11%. The most pronounced relative enhancement was observed in the free phenolic fraction (~17%), with statistically significant increases in gallic, vanillic, ferulic, and sinapic acids ($p < 0.05$). Although certain compounds were present at comparatively low absolute concentrations, their proportional increases were considerable.

The bound phenolic fraction, which represented the predominant portion of total phenolic acids, also exhibited a significant increase after laser exposure. In particular, bound ferulic acid increased by approximately 10%, accompanied by significant elevations in bound vanillic and sinapic acids. These changes contributed substantially to the overall rise in total phenolic content.

Collectively, the results indicate that laser irradiation promotes the accumulation of both soluble and cell wall-associated phenolic compounds [20–22] in wheat sprouts. This effect may reflect stimulation of phenolic biosynthesis and/or enhanced liberation of structurally bound phenolics.

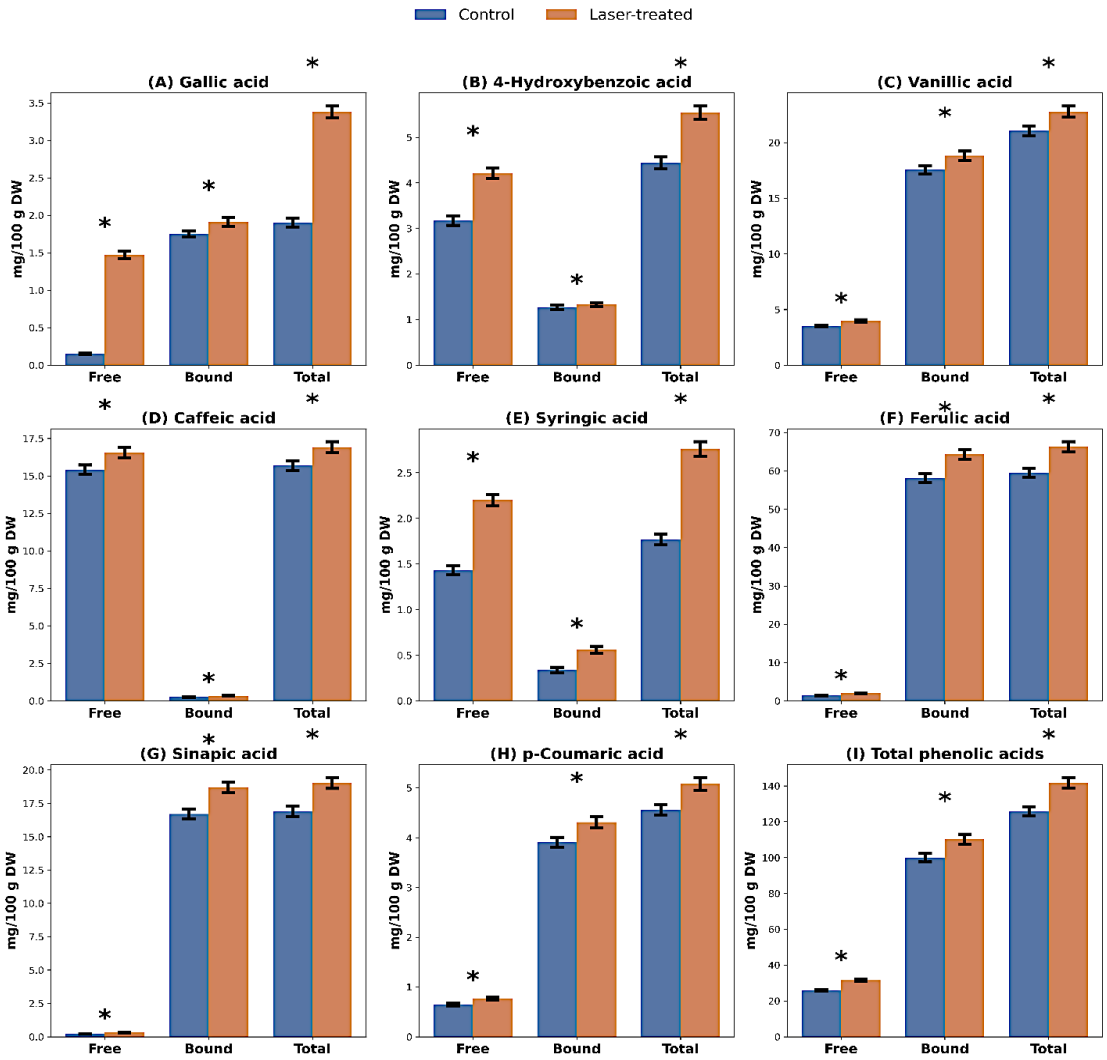


Figure 6. Effect of CO_2 laser treatment on individual phenolic compounds: (A) gallic acid, (B) 4-hydroxybenzoic acid, (C) vanillic acid, (D) caffeic acid, (E) syringic acid, (F) ferulic acid, (G) sinapic acid, (H) p-coumaric acid, and (I) total phenolic acids. Bars represent mean ± SD (n = 3). Asterisks (*) indicate significant differences between control and laser-treated samples ($p < 0.05$).

Effects of Phenolic Fractions on Jurkat Cell Viability: Phenolic fractions derived from "Red Doly" wheat sprouts influenced the viability of Jurkat T lymphocytes under both normal and oxidative stress conditions (Figure 7).

In intact cells, treatment with the total phenolic fraction resulted in approximately 32% viability, whereas fractions enriched in free and bound phenolic compounds increased viability to approximately 58% and 47%, respectively. These findings suggest a stimulatory effect of phenolic fractions—particularly free phenolics—on cell viability in the absence of oxidative stress.

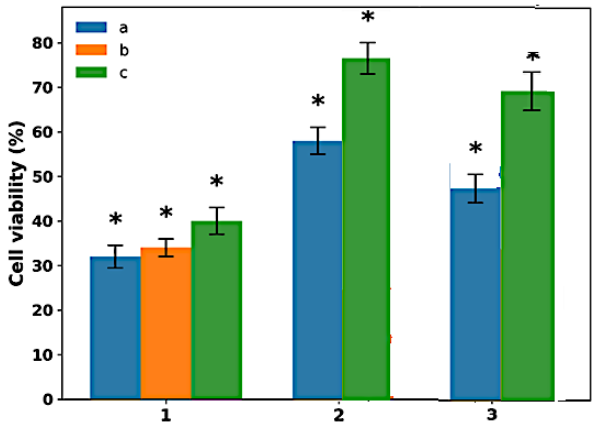


Figure 7. Effects of different phenolic fractions derived from "Red Doly" wheat sprouts on the viability of Jurkat T lymphocytes under oxidative stress.

Experimental groups: (a) untreated (intact) Jurkat cells; (b) Jurkat cells exposed to H_2O_2; (c) Jurkat cells exposed to H_2O_2 and supplemented with phenolic fractions obtained from "Red Doly" wheat sprouts. Phenolic treatments: (1) total phenolic fraction; (2) fraction enriched in free phenolic acids; (3) fraction enriched in bound phenolic compounds. Data are presented as mean ± standard deviation (SD). Asterisks (*) denote statistically significant differences compared with H_2O_2-treated cells ($p < 0.05$). The concentration of H_2O_2 and exposure duration are specified in the Methods section.

Exposure to H_2O_2 significantly reduced Jurkat cell viability, confirming the induction of oxidative damage. However, supplementation with wheat sprout-derived phenolic fractions significantly attenuated H_2O_2-induced cytotoxicity ($p < 0.05$). The total phenolic fraction produced a moderate protective effect, whereas the fractions enriched in free and bound phenolic compounds exhibited more pronounced cytoprotective activity, restoring cell viability toward control levels.

These results demonstrate that phenolic compounds from "Red Doly" wheat sprouts, particularly the free and bound fractions, effectively protect Jurkat T lymphocytes against oxidative stress, thereby supporting their functional antioxidant capacity

DISCUSSION

The current study provides integrated mechanistic, biochemical, and functional evidence that CO_2 laser–based seed priming activates phenylpropanoid metabolism and enhances the antioxidant and cytoprotective capacity of "Red Doly" wheat. Laser irradiation significantly increased phenolic accumulation, antioxidant activity, and cellular protective effects in a developmentally regulated manner, with green sprouts exhibiting the most pronounced responses.

These findings are consistent with the structured functional food development model proposed by

Martirosyan D.M. and Stratton S. [23], particularly through the integration of functional goal definition, mechanistic validation, biomarker assessment, and preclinical cellular efficacy testing. To our knowledge, this study is among the first to associate CO_2 laser priming–induced phenolic enhancement with direct cytoprotective effects demonstrated in a human cell–based model.

Importantly, this study advances the field of laser seed priming beyond its traditional scope. Previous investigations have primarily focused on agronomic performance, germination rate, biomass accumulation, stress tolerance, or general compositional changes in plant tissues [12-13]. Although some studies reported increases in total phenolic content or antioxidant capacity, these findings were largely confined to chemical assays and compositional profiling and do not determine whether such biochemical alterations translated into measurable biological effects in human-relevant experimental systems. In contrast, the present study moves beyond descriptive compositional analysis by establishing a direct functional link between CO_2 laser–induced phenolic enrichment and cytoprotective efficacy in a human T-lymphocyte oxidative stress model. This study is one of the first to show that priming with CO_2 laser stimulation enhances phenolic metabolism, leading to measurable protection of human cells against oxidative stress. By integrating chromatographic profiling, antioxidant biomarker assessment, and cellular validation within a unified experimental framework, this work provides translational evidence of biological relevance that has not previously been demonstrated in laser-priming research. This integrative strategy substantially strengthens the causal continuum between physical seed stimulation, metabolic activation, bioactive compound accumulation, and functional cellular outcomes.

Laser irradiation significantly increased total free phenolic content and antioxidant activity at all developmental stages of “Red Doly” wheat, with the most pronounced effects observed in green sprouts. Wheat sprouts are increasingly recognized as valuable functional food ingredients due to their high concentrations of bioactive phytochemicals, including phenolic acids and antioxidant enzymes [10, 24–26]. The enhanced responsiveness of green sprouts suggests that this developmental stage represents an optimal target for producing phenolic-enriched sprout-based ingredients with enhanced health-promoting potential [27]. Within the structured functional food development framework, this fulfils Step 1, in which a clearly defined functional objective—enhanced antioxidant and cytoprotective performance—is experimentally validated.

At the mechanistic level, the observed increases in free and bound phenolic compounds strongly suggest activation of the phenylpropanoid pathway. Phenolic biosynthesis in cereals is regulated by phenylalanine ammonia-lyase (PAL), cinnamic acid 4-hydroxylase (C4H), and 4-coumarate CoA ligase (4CL), enzymes known to respond to photo stimulation [13,28–31]. Laser-induced photo biomodulation likely alters the cellular redox balance and Ca^{2+} signaling, thereby activating transcriptional regulators of secondary metabolism and consequently enhancing phenolic biosynthesis. Comparable upregulation of phenylpropanoid-related enzymes has been reported under exposure to red and UV radiation [11,31]. Thus, this study provides mechanistic insight into how a precisely controlled physical stimulus can modulate defined metabolic pathways responsible for the production of functional bioactive compounds (Step 5 of the functional food development model).

The preferential enhancement of free phenolic fractions has important functional implications. Free phenolic acids, such as caffeic, gallic, and sinapic acids, exhibit higher solubility and greater radical-scavenging efficiency compared with their bound counterparts

[25,32]. The strong positive correlation between total phenolic content and antioxidant activity (r = 0.81, p = 0.0004) provides direct evidence that the laser-induced increase in antioxidant capacity **is** largely attributable to elevated phenolic concentrations. Similar correlations have been reported in cereal sprouts [21,25,26], confirming phenolics as principal determinants of antioxidant functionality. Within the functional development framework, this corresponds to Step 6, where biomarker-based validation (TAC and cell viability assays) substantiates the biological relevance of the identified bioactive compounds.

From a formulation and quality-control perspective, antioxidant capacity is widely recognized as a functional indicator of nutritional value and shelf-life stability in cereal-based foods [21,33]. The present findings demonstrate that laser priming not only increases phenolic concentrations but also significantly enhances overall antioxidant functionality, thereby supporting phenolic content as a reliable biochemical marker for assessing the functional quality of laser-primed wheat ingredients.

Chromatographic analysis revealed a coordinated enhancement of both free and bound phenolic fractions, with caffeic acid predominating in the free fraction and ferulic acid in the bound fraction [6, 34, 35]. Through detailed compound profiling (Step 2 of the functional development process), specific bioactive molecules responsible for functional activity were characterized, thereby strengthening the scientific rationale for phenolic-enriched wheat sprouts as targeted functional ingredients.

From a nutritional standpoint, the distinction between free and bound phenolics is particularly significant. Free phenolics are generally more bio accessible and readily absorbed in the upper gastrointestinal tract, whereas bound phenolics may reach the colon, where they are released via microbial fermentation and exert localized antioxidant and anti-inflammatory effects [5,10,32,36]. Accordingly, laser priming enhances not only total phenolic levels but also phenolic diversity and potential bioavailability [37]. The observed increase in bound ferulic acid may additionally influence cell wall integrity, lignification, texture, and water-holding capacity, suggesting added technological advantages for cereal processing applications [6, 34, 35]. Collectively, these findings indicate that laser priming can simultaneously improve the nutritional functionality and technological performance of wheat-based ingredients.

Beyond conventional chemical antioxidant assays, this study provides direct cellular validation of functional efficacy. Phenolic fractions derived from laser-treated “Red Doly” wheat significantly protected Jurkat T lymphocytes against H_2O_2-induced oxidative stress. In particular, the free phenolic fractions restored cell viability to approximately 80%, indicating robust cytoprotective activity. The underlying mechanisms likely involve a combination of direct ROS scavenging, stabilization of mitochondrial function, and modulation of redox-sensitive signaling pathways, including NF-κB and MAPK [5,21,38]. Bound phenolic fractions also conferred protection, albeit to a lesser extent, likely reflecting their comparatively lower immediate bio accessibility. These findings correspond to Step 8 (preclinical efficacy validation), providing controlled human cell-based evidence of biological activity before in vivo investigation.

Notably, phenolic fractions also enhanced the viability of intact Jurkat cells in the absence of oxidative stress, particularly the free fractions. This observation suggests a potential trophic or metabolic-supportive effect, possibly mediated through modulation of basal redox homeostasis or improved mitochondrial efficiency. Similar stimulatory effects of cereal-derived phenolics have been documented in immune and epithelial cell systems [5,21,38-39]. From an applied perspective, these results reinforce concept that phenolic-enriched wheat sprouts function not merely as passive antioxidant

sources but as biologically active ingredients capable of enhancing cellular resilience and functional stability.

From a sustainability and agricultural innovation perspective, laser-assisted seed priming represents a chemical-free, energy-efficient, and scalable technology [12]. As climate change intensifies oxidative stress pressures on crops, strategies that stimulate intrinsic antioxidant defense systems without chemical inputs are becoming increasingly valuable [24,31,40,42,43]. Moreover, laser priming aligns with consumer demand for minimally processed, clean-label functional foods enhanced through physical rather than chemical interventions.

Collectively, this study advances multiple stages of structured functional food development by integrating functional objective definition, compound identification, mechanistic elucidation, biomarker validation, and preclinical cellular efficacy testing. It establishes a clear link between laser-induced metabolic activation in plants and observable cytoprotective effects in human cells, marking a significant advancement compared to earlier laser-priming studies.

From a broader sustainability perspective, laser-assisted seed priming offers a strategy to enhance crops' intrinsic antioxidant systems without the need for additional chemical treatments [24,39-40]. By systematically integrating functional goal definition, compound characterization, mechanistic validation, biomarker assessment, and preclinical testing within a unified framework [23], this study offers a comprehensive and translational model for the development of next-generation functional cereal ingredients.

The findings identify laser-primed "Red Doly" wheat sprouts as a promising and functionally enhanced raw material for the development of cereal-based functional foods, sprout powders, nutraceutical formulations, and dietary supplements with improved antioxidant and cytoprotective properties [41-42,44,45].

By demonstrating mechanistic pathway activation, targeted compositional enrichment, and human cell–level functional validation within a unified experimental framework, this work delivers a comprehensive and distinctly novel contribution to both laser-priming research and functional food science [46,47].

CONCLUSIONS

This study demonstrates that CO_2 laser–based seed priming is an effective, non-chemical strategy for enhancing phenylpropanoid metabolism and improving the functional quality of "Red Doly" wheat. Laser irradiation significantly increased total phenolic content, antioxidant capacity, and cytoprotective activity across developmental stages, with the most pronounced effects observed at the green-sprout stage.

Importantly, this work advances laser seed-priming research beyond descriptive compositional characterization by establishing a direct functional relationship between laser-induced phenolic enrichment and quantifiable biological efficacy demonstrated in a human cell-based model. By integrating compound profiling, biomarker-based antioxidant assessment, and preclinical cellular validation within a unified experimental framework, the study provides robust translational evidence supporting the biological relevance of laser-stimulated metabolic modulation.

Within the 17-step Functional Food Development Model [23], the present investigation fulfils several foundational stages, including:

- Functional objective definition (Step 1),
- Bioactive compound identification (Step 2),
- Mechanistic pathway interpretation (Step 5),
- Biomarker-based validation (Step 6),
- Preclinical cellular efficacy testing (Step 8).

Collectively, these findings position laser-primed "Red Doly" wheat sprouts as a phenolic-enriched functional ingredient with experimentally validated

cytoprotective potential. The scalability, sustainability, and chemical-free nature of CO_2 laser priming further support its practical integration into functional cereal production systems, aimed at enhancing both nutritional value and health-promoting properties.

Limitations: Despite demonstrating significant phenolic enrichment and cytoprotective efficacy, several limitations should be acknowledged:

(a) Biological validation was performed in a single human T-lymphocyte model under controlled in vitro conditions. Although this provides preclinical functional evidence (Step 8), it does not fully replicate the complexity of systemic physiological responses in vivo.

(b) Dose–response relationships and potential synergistic interactions among individual phenolic compounds were not systematically investigated. Such analyses would help clarify the relative contributions of specific bioactive constituents versus collective matrix effects.

(c) Activation of the phenylpropanoid pathway was inferred from metabolite accumulation rather than directly verified through gene expression profiling, enzyme activity measurements, or transcriptomic analysis. Future studies incorporating molecular and regulatory pathway assessments would strengthen the mechanistic interpretation.

(d) Subsequent translational stages of the Functional Food Development Model—including gastrointestinal bioavailability assessment, in vivo efficacy studies, safety evaluation, and randomized human clinical trials—remain to be completed to establish clinical functionality and substantiate potential health claims.

Future research integrating molecular pathway analysis, simulated gastrointestinal digestion models, animal studies, and controlled human interventions will be essential to comprehensively validate the health-promoting potential of laser-primed wheat sprouts as a functional food ingredient.

Abbreviations: cAMP: cyclic AMP, MAPK: mitogen-activated protein kinase, SOD: superoxide dismutase, CAT: catalase, APX: ascorbate peroxidase, ROS: reactive oxygen species, HPLC: high-performance liquid chromatograph, TAC: total antioxidant capacity, DPPH: 2,2-diphenyl-1-picrylhydrazyl, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, DMSO: dimethyl sulfoxide, SD: standard deviation, ANOVA: one-way analysis of variance (ANOVA). HSD: honestly significant difference, Rt: retention time, 4CL: 4-coumarate CoA ligase, PAL: phenylalanine ammonia-lyase, TPC: total phenolic content.

Competing Interests: No competing Interests.

Author Contributions: Conceptualization, TS, AB, IC, NB; Methodology, BQ, JAT, ES; Formal analysis, ME, MM, NG; Investigation, MT, LC; Writing—original draft, TS; Writing—review and editing, TS, IC, AS, SK; Visualization, LC; Project administration, TD; Funding acquisition, MT. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: We would like to express our appreciation to all those who cooperated in this research.

Funding: This study was funded by the project "Quantitative and qualitative evaluation of phenolic compounds (grain germinates, and grass seedling) of wheat processed with innovative laser bioagrotechnology to make a functional health-improving drug. (Grant agreement No. FR-22-6966; 09.03.2023) funded by the Shota Rustaveli National Science Foundation of Georgia within the framework of the state scientific grants competition, 2022.

REFERENCES

1. FAO. (2021). *FAOSTAT Statistical Database*. Food and Agriculture Organization of the United Nations
2. Mondal S, Soumya NPP, Mini S, Sivan SK. Bioactive compounds in functional food and their role as therapeutics. *Bioactive Compounds in Health and Disease.* 2021;4(3):24–39. DOI: https://doi.org/10.31989/bchd.v4i3.786

3. Janigashvili G, Chkhikvishvili I, Ratiani L, Maminaishvili T, Chkhikvishvili D, Sanikidze T. Effects and medical application of plant-origin polyphenols: A narrative review. *Bioactive Compounds in Health and Disease*. 2024; 7(8):375-385. DOI: https://doi.org/10.31989/bchd.v7i8.1414
4. Akhtar A, Asghar W, Khalid N. Phytochemical constituents and biological properties of domesticated *Capsicum* species: a review. *Bioactive Compounds in Health and Disease*. 2021;4(9):201–225. DOI: https://doi.org/10.31989/bchd.v4i9.837
5. Fardet A. New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? *Nutr Res Rev.* 2010;23(1):65-134. DOI: https://doi.org/10.1017/S0954422410000041
6. Shewry PR, Hey SJ. The contribution of wheat to human diet and health. *Food Energy Secur.* 2015;4(3):178-202. DOI: https://doi.org/10.1002/fes3.64
7. Aune D, Keum N, Giovannucci E, Fadnes LT, Boffetta P, Greenwood DC, et al. Whole grain consumption and risk of cardiovascular disease, cancer, and all cause and cause specific mortality: systematic review and dose-response meta-analysis of prospective studies. *BMJ.* 2016; 353: i2716. DOI: https://doi.org/10.1136/bmj.i2716
8. Ficco DBM, Petroni K, Mistura L, D'Addezio L. Polyphenols in Cereals: State of the Art of Available Information and Its Potential Use in Epidemiological Studies. *Nutrients.* 2024; 16(13):2155. DOI: https://doi.org/10.3390/nu16132155
9. Abdulrazak E, Jameel Q. Effect of spinach-derived glutathione against carbon tetrachloride-induced oxidative stress in rats. *Functional Foods in Health and Disease*. 2022;12(8):442–454. DOI: https://doi.org/10.31989/ffhd.v12i8.972
10. Günal-Köroğlu D, Esatbeyoglu T, Capanoglu E. Effect of germination on the phenolic compounds: content, bioavailability, food applications, and health benefits. *Food Measure.* 2025;19: 8144–8164. DOI: https://doi.org/10.1007/s11694-025-03392-6
11. Zhang J, Wang C, Fang W, Yang R, Yin Y. Production of High-Quality Wheat Sprouts of Strong Antioxidant Capacity: Process Optimization and Regulation Mechanism of RedLight Treatment. *Foods.* 2024; 13(17):2703. DOI: https://doi.org/10.3390/foods13172703
12. Iglesias-Ganado Á, Martín-García J, Poveda J, López-Sainz MF, Sánchez-Gómez T, Santamaría O. Improvement of Wheat and Barley Cultivation Through Seed Priming with UV, Ozone, and Nutripriming (Fe, Zn, and B). *Applied Sciences.* 2025;15(18):9988. DOI: https://doi.org/10.3390/app15189988
13. Hernández AC, Domínguez PA, Cruz OA, Ivanov R, Carballo CA, Zepeda BR. Laser irradiation effects on field performance of maize seed genotypes. *International Agrophysics.* 2009; 23: 327-332.
14. Perveen R, Jamil Y, Ashraf M, Ali Q, Iqbal M, Ahmad MR. He-Ne laser-induced improvement in biochemical, physiological, growth and yield characteristics in sunflower (Helianthus annuus L.). *Photochem Photobiol.* 2011;87(6):1453-1463. DOI: https://doi.org/10.1111/j.1751-1097.2011.00974.x
15. Shu K, Zhou W, Chen F, Luo X, Yang W. Abscisic acid and gibberellins antagonistically regulate germination and seedling establishment. *Trends in Plant Science*. 2018; 23(3): 177–188. DOI: https://doi.org/10.3389/fpls.2018.00416
16. Martin-Diana AB, Izquierdo N, Albertos I, Sanchez MS, Herrero A, Sanz MA, et al. Valorization of Carob's Germ and Seed Peel as Natural Antioxidant Ingredients in Gluten-Free Crackers: Carob Antioxidant by-Products in Gluten-Free Snack. *Journal of Food Processing and Preservation*. 2017; 41: e12770. DOI: https://doi.org/10.1111/jfpp.12770
17. Martín-Diana AB, García-Casas MJ, Martínez-Villaluenga C, Frías J, Peñas E, Rico D. Wheat and Oat Brans as Sources of Polyphenol Compounds for Development of Antioxidant Nutraceutical Ingredients. *Foods.* 2021;10(1):115. DOI: https://doi.org/10.3390/foods10010115
18. García-Villalba R, Espín JC, Aaby K, Alasalvar C, Heinonen M, Jacobs G et al. Validated Method for the Characterization and Quantification of Extractable and Nonextractable Ellagitannins after Acid Hydrolysis in Pomegranate Fruits, Juices, and Extracts. *J Agric Food Chem.* 2015; 63(29):6555-6566. DOI: https://doi.org/10.1021/acs.jafc.5b02062
19. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT—Food Sci. Technol.* 1995; 28: 25–30. DOI: https://doi.org/10.1016/S0023-6438(95)80008-5
20. Shahidi F, Yeo J. Bioactivities of Phenolics by Focusing on Suppression of Chronic Diseases: A Review. *Int J Mol Sci.* 2018;19(6):1573. DOI: https://doi.org/10.3390/ijms19061573
21. Adom KK, Liu RH. Antioxidant activity of grains. *J Agric Food Chem.* 2002;50(21):6182-6187. DOI: https://doi.org/10.1021/jf0205099
22. Mattila P, Pihlava JM, Hellström J. Contents of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. *J Agric Food Chem*. 2005;53(21):8290-8295. DOI: https://doi.org/10.1021/jf051437z
23. Martirosyan DM, Stratton S. Quantum and temporal theories of functional food science in practice. *Functional Food Science.*2023;(5):55–62. DOI: https://doi.org/10.31989/ffs.v3i5.1122
24. Mittler R. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* 2002;7(9):405-410.

DOI: https://doi.org/10.1016/s1360-1385(02)02312-9

25. Moore J, Hao Z, Zhou K, Luther M, Costa J, Yu LL. Carotenoid, tocopherol, phenolic acid, and antioxidant properties of Maryland-grown soft wheat. *J Agric Food Chem.* 2005;53(17):6649-6657. DOI: https://doi.org/10.1021/jf050481b
26. Zieliński H, Kozłowska H. Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. *J Agric Food Chem*. 2000;48(6):2008-2016. DOI: https://doi.org/10.1021/jf990619o
27. Ahmed WE, Ragab I, Gadallah GEM, Raghad M. Alhomaid, Mona S. Almujaydil, Effect of sprouting whole wheat grain on the sensory quality, physicochemical properties, and antioxidant activity of cupcakes. *Applied Food Research*. 2024; 4(1):100412. DOI: https://doi.org/10.1016/j.afres.2024.100412
28. Wang S, Zhang X, Fan Y, Wang Y, Yang R, Wu J, et al. Effect of magnetic field pretreatment on germination characteristics, phenolic biosynthesis, and antioxidant capacity of quinoa. *Plant Physiol Biochem.* 2024; 212:108734. DOI: https://doi.org/10.1016/j.plaphy.2024.108734
29. Vasilevski GJ. perspectives of the application of biophysical methods in sustainable agriculture. *Bulg. J. Plant Physiol*. 2003;179–186
30. Wu W, Wu H, Liang R, Huang S, Meng L, Zhang M, et al. Light regulates the synthesis and accumulation of plant secondary metabolites. *Frontiers in Plant Science.* 2025; *16*. DOI: https://doi.org/10.3389/fpls.2025.1644472
31. Lan H, Wang C, Yang Z, Zhu J, Fang W, Yin Y. The Impact of Lighting Treatments on the Biosynthesis of Phenolic Acids in Black Wheat Seedlings. *Foods.* 2024; 13(16): 2499. DOI: https://doi.org/10.3390/foods13162499
32. Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr*. 2005;81(1 Suppl):230S-242S. DOI: https://doi.org/10.1093/ajcn/81.1.230S
33. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agric Food Chem.* 2005;53(10):4290-4302. DOI: https://doi.org/10.1021/jf0502698
34. Salawu SO, Bester MJ, Duodu KG. Phenolic Composition and Bioactive Properties of Cell Wall Preparations and Whole Grains of Selected Cereals and Legumes. *J. Food Biochem.* 2014; 38: 62–72. DOI: https://doi.org/10.1111/jfbc.12026
35. Marcotuli I, Vurro F, Mores A, Pasqualone A, Colasuonno P, Cabas-Lühmann P, et al. Genetic Study of Total Phenolic Content and Antioxidant Activity Traits in Tetraploid Wheat via Genome-Wide Association Mapping. *Antioxidants.* 2025;14(9):1048. DOI: https://doi.org/10.3390/antiox14091048
36. Leri M, Scuto M, Ontario ML, Calabrese V, Calabrese EJ, Bucciantini M, et al. Healthy Effects of Plant Polyphenols: Molecular Mechanisms. *Int J Mol Sci.* 2020;21(4):1250. DOI: https://doi.org/10.3390/ijms21041250
37. Hurina V, Nauciene Z, Zukiene R, Degutyte-Fomins L, Tuckute S, Ivanauskas L, et al. Seed Priming with Cold Plasma and Vacuum Increases the Amounts of Phenolic Compounds and Antioxidant Activity in Lavender Herb. *Horticulturae*. 2025; 11(12):1413. DOI: https://doi.org/10.3390/horticulturae11121413
38. Li L, Wang Q, Cao Y, Li J, Wu Y, Hua C, et al. Antioxidant action and potential neuroprotection of polyphenolics extracted from Astragalus membranaceus residue. *Front Nutr.* 2025; 12:1621848. DOI: https://doi.org/10.3389/fnut.2025.1621848
39. Monteiro MK, da Costa E, Schmidt L, Welke J, Augusti PR. Can plant phenolic compounds alleviate toxic effects induced by mycotoxins? A narrative review. *Food Bioscience*. 2025; 71:107339. DOI: https://doi.org/10.1016/j.fbio.2025.107339
40. Lesk C, Rowhani P, Ramankutty N. Influence of extreme weather disasters on global crop production. *Nature*. 2016;529(7584):84-87. DOI: https://doi.org/10.1038/nature16467
41. Nawaz H, Rehman HU, Ihsan MZ, Rizwan MS, Hussain N, Ali B, et al. Organic seed priming with curtailed seed rate compensated wheat grains productivity by upgrading antioxidant status against terminal drought at flowering and milking. *Sci Rep*. 2024;14(1):4941. DOI: https://doi.org/10.1038/s41598-024-54767-6
42. Dixon RA, Paiva NL. Stress-Induced Phenylpropanoid Metabolism. *Plant Cell.* 1995;7(7):1085-1097. DOI: https://doi.org/10.1105/tpc.7.7.1085
43. Li J, Guo X, Zhang S, Zhang Y, Chen L, Zheng W, et al. Effects of light quality on growth, nutritional characteristics, and antioxidant properties of winter wheat seedlings (Triticum aestivum L.). *Frontiers in Plant Science,* 2022;13. DOI: https://doi.org/10.3389/fpls.2022.978468
44. Jatana BS, Grover S, Ram H, Baath GS. Seed Priming: Molecular and Physiological Mechanisms Underlying Biotic and Abiotic Stress Tolerance. *Agronomy*. 2024; 14(12):2901. DOI: https://doi.org/10.3390/agronomy14122901

45. Rhaman MS, Imran S, Rauf F, Khatun M, Baskin CC, Murata Y, et al. Seed Priming with Phytohormones: An Effective Approach for the Mitigation of Abiotic Stress. *Plants (Basel).* 2020;10(1):37.
DOI: https://doi.org/10.3390/plants10010037
46. Veerana M, Poochim B, Intharasuwan P, Saphanthong P, Lim JS, Choi EH, et al. Plasma Seed Priming Can Improve the Early Seedling Establishment and Antioxidant Activity of Water Convolvulus Microgreens. *Plants (Basel).* 2025;14(23):3648.
DOI: https://doi.org/10.3390/plants14233648
47. El-Shazoly RM, Hamed HMA, El-Sayed MM. Individual or successive seed priming with nitric oxide and calcium toward enhancing salt tolerance of wheat crop through early ROS detoxification and activation of antioxidant defense. *BMC Plant Biology*. 2024; 24:730.
DOI: https://doi.org/10.1186/s12870-024-05390-0