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Kinetic Analysis of Guaiacol Peroxidase in Solanum lycopersicum under Immature and Post-Harvest Conditions in the Presence of Potential Amino Acid Inhibitors

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A B S T R A C T

Objective: Tomato (*Solanum lycopersicum*), a climacteric fruit, is highly susceptible to oxidative degradation during ripening and postharvest storage, which compromises shelf life and visual quality. Guaiacol peroxidase (GPX), a key enzyme in oxidative processes, catalyzes the oxidation of phenolic compounds, contributing to enzymatic browning. This study aimed to characterize the kinetic properties and inhibition patterns of GPX across three physiological stages immature, ripe, and stored and to evaluate the modulatory effects of two natural amino acids, glycine and cysteine.

Methods: GPX activity was quantified using spectrophotometric assays. Kinetic parameters (Km and Vmax) were determined via Michaelis–Menten and Lineweaver Burk analyses. Enzyme activity was assessed under varying substrate (guaiacol and hydrogen peroxide) concentrations and pH conditions to establish optimal catalytic parameters.

Results: GPX activity was optimal at pH 6.5, with a consistent Km of 5 mM across all physiological stages. Vmax values increased progressively during ripening and storage (0.019, 0.027, and 0.033 µmol/min, respectively). Substrate inhibition occurred at guaiacol concentrations above 10 mM, with maximal activity observed at 10 mM hydrogen peroxide (100 µL). Both cysteine and glycine inhibited GPX, with cysteine showing greater potency across all stages, as reflected by lower IC50 values: 0.8 vs. 1.0 mM (immature), 0.9 vs. 1.3 mM (ripe), and 1.2 vs. 1.5 mM (stored). Lineweaver–Burk analysis indicated non-competitive inhibition in immature fruit, shifting to competitive inhibition in ripe and stored stages, suggesting developmental changes in enzyme structure or isoform composition.

Conclusion: GPX activity in tomato is developmentally regulated and can be effectively suppressed by natural amino acids, particularly cysteine. These findings provide promising bio-based strategies to reduce enzymatic browning and enhance oxidative stability during postharvest handling of tomatoes.

Keywords: Antioxidant, Free Radical, Inhibitor, Guaiacol peroxidase, Glycine, Tomato

➢ **How to cite this paper**

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Introduction

Peroxidase (EC 1.11.1.x) is a prominent member of the oxidoreductase enzyme class, playing a pivotal role in the antioxidant defense systems of plants, animals, and humans. This enzyme catalyzes redox reactions by utilizing peroxides most notably hydrogen peroxide (H_2O_2) as electron acceptors. Through the decomposition of reactive oxygen species (ROS), peroxidase contributes to the mitigation of oxidative damage to cellular structures. The general reaction can be summarized as $RH_2 + H_2O_2 \rightarrow R + 2H_2O$, wherein phenolic or amine substrates are oxidized and H_2O_2 is reduced to water [1,2]. In plant physiology, peroxidase is implicated in a variety of essential processes, including the detoxification of H_2O_2, lignin polymerization, cell wall fortification, wound healing, and responses to abiotic stressors such as cold, drought, and salinity [3]. Moreover, it plays a central role in pathogen resistance. Peroxidase activity is also recognized as a major contributor to enzymatic browning in fruits and vegetables, a phenomenon involving the oxidation of phenolic compounds in the presence of H_2O_2, resulting in the formation of brown pigments that adversely affect visual quality and marketability [4].

The biochemical characteristics of peroxidase are source-dependent, although its optimal pH generally ranges between 5 and 7, with a temperature optimum typically observed between 30°C and 50°C. Key kinetic parameters such as the Michaelis-Menten constant (Km), which reflects substrate affinity, and the maximum reaction velocity (Vmax), are critical for evaluating enzyme behavior under varying physiological and postharvest conditions [5].

Peroxidase activity can be inhibited by several biochemical agents. For instance, L-cysteine, a sulfur-containing amino acid with a thiol functional group, has been identified as an effective inhibitor [6]. Its inhibitory mechanism involves direct interaction with the enzyme's active site or metal cofactors. In applied biosciences, horseradish peroxidase (HRP) has been extensively utilized in diagnostic immunoassays such as ELISA and Western blotting due to its capacity to generate colorimetric signals. In food technology, peroxidase activity serves as a thermal processing indicator during procedures such as blanching. In agricultural sciences, the modulation of peroxidase activity is considered a biomarker for plant resistance to stress conditions and pathogen attack [8,9]. Conversely, excessive peroxidase activity in fresh produce may necessitate inhibition to prevent enzymatic browning and to enhance postharvest shelf life [10].

Overall, peroxidase represents a dual-function enzyme with both protective and degradative roles in plant-based food systems. A comprehensive understanding of its biochemical behavior, optimal conditions for activity, and regulatory mechanisms is essential for the development of sustainable strategies aimed at postharvest quality management, reduction of spoilage, and extension of shelf life in horticultural commodities [11]. The application of antioxidant compounds has been increasingly recognized as an effective strategy in the reduction of postharvest deterioration and the extension of shelf life in fruits and vegetables. These compounds function by suppressing oxidative pathways, inhibiting the activity of browning-related enzymes such as peroxidase and polyphenol oxidase, and enhancing endogenous cellular antioxidant defenses, thereby maintaining tissue integrity and sensory quality [12].

Solanum lycopersicum (tomato), classified as a climacteric fruit, undergoes postharvest ripening processes that require precise control. Harvesting at the optimal maturity stage and ensuring appropriate storage conditions are critical for maintaining commercial quality [13].

Antioxidants such as glycine betaine, L-cysteine, ascorbic acid (vitamin C), tocopherol (vitamin E), natural polyphenolic compounds, chitosan, and glutathione have demonstrated efficacy in preserving quality and reducing oxidative deterioration. These agents function through radical scavenging, upregulation of antioxidant enzyme activities, inhibition of lipid peroxidation, suppression of enzymatic browning, and stabilization of cellular membranes under abiotic and biotic stress conditions [14]. Their application via immersion, spraying, edible coatings, or active packaging systems has shown promise in the development of sustainable postharvest technologies. When used in combination with natural antimicrobial and antifungal agents, synergistic effects may be observed, further enhancing product safety and longevity [15].

Glycine betaine, a quaternary ammonium compound, is widely recognized as a compatible solute that accumulates under drought stress. It is predominantly localized in chloroplasts, where it

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stabilizes thylakoid membranes and sustains photosynthetic efficiency. Functioning as a cytoplasmic osmoprotectant, it safeguards proteins and membranes under osmotic stress.

L-cysteine has been extensively utilized in postharvest applications due to its potent antioxidant properties and capacity to inhibit enzymatic browning. Competitive inhibition of polyphenol oxidase and peroxidase, along with the formation of copper-binding complexes, has been reported in commodities such as apple, potato, and pear. Experimental data have also demonstrated enhanced antioxidant capacity, delayed senescence, and reduced oxidative damage in commodities such as longan, lettuce, and litchi. In Solanum melongena (eggplant), L-cysteine treatments have been associated with improved firmness, reduced chilling injury, and extended storage life. This evidence supports the utility of antioxidant-based interventions in integrated postharvest systems aimed at minimizing losses and ensuring high-quality fresh produce. Further research on dosage optimization, application methods, and interactions with other preservation technologies remains essential for broader implementation [16,17].

The primary objective of this investigation was to characterize the kinetic behavior and inhibition mechanisms of GPX in Solanum lycopersicum (tomato) fruit at three distinct physiological stages: immature, ripe, and postharvest-stored. Furthermore, the modulatory effects of two endogenous amino acids glycine and cysteine on GPX activity were systematically assessed. To achieve this, spectrophotometric assays were employed to determine key enzyme kinetic parameters, including the Michaelis-Menten constant (Km) and maximum reaction velocity (Vmax). The enzyme's activity was evaluated under varying substrate concentrations specifically guaiacol and hydrogen peroxide as well as across a range of pH values, in order to establish optimal catalytic conditions. The type and extent of enzymatic inhibition induced by glycine and cysteine were analyzed using Lineweaver-Burk double reciprocal plots, allowing for the classification of inhibition as competitive or non-competitive, dependent on the developmental stage of the fruit. This study aims to advance the understanding of oxidative enzyme regulation in climacteric fruits and to propose sustainable, biologically derived interventions for mitigating enzymatic browning. The findings are intended to support the application of natural antioxidant agents particularly sulfur-containing amino acids such as cysteine for the enhancement of postharvest quality and oxidative stability in horticultural produce.

Materials and Methods

This investigation was conducted at the research laboratory of Payame Noor University. Cherry tomatoes (*Solanum lycopersicum* L.) sourced from local farms in Kurdistan province were obtained at two physiological stages: immature and ripe. Tomatoes were procured in October 2024 from farms located in Baneh County, Kurdistan Province. Immediately after harvest, fruits were categorized into two ripening stages immature and fully ripe and homogenates were prepared from each group. To assess the impact of cold storage, a subset of samples was stored at 4 °C for two weeks. Homogenates were then prepared from these stored fruits for further analysis.

Enzyme Extraction

Following procurement, the tomatoes were washed, and extraneous parts were removed. The fleshy tissues were disinfected using a 2% benomyl solution to inhibit fungal activity. Crude enzyme extracts were prepared by homogenizing the tomato tissue in a blender with distilled water, 0.1 M phosphate buffer (pH 7.0), and 0.02 M phenylmethylsulfonyl fluoride (PMSF) as a protease inhibitor. The homogenate was subjected to centrifugation at 3000 × g for 10 minutes. The resulting supernatant was further centrifuged at 15,000 × g for 30 minutes, and the clear supernatant obtained was utilized as the crude extract for subsequent enzymatic assays, including the determination of catecholase and cresolase activities. To minimize human error, the enzyme homogenate was prepared from all samples in a single batch and stored in 2 mL vials at -20°C until analysis. At the time of assay, the vials were equilibrated to laboratory temperature prior to use. To ensure the precision and reliability of data derived from enzymatic assays, all experiments were performed by a single trained operator under standardized environmental and operational conditions, including a controlled laboratory temperature of 25 ± 1°C, regulated relative

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humidity, and utilization of calibrated instrumentation. The spectrophotometer (specific model, where applicable) was calibrated prior to each experimental series to guarantee the accuracy of absorbance measurements. Data exhibiting a linear absorbance increase, characterized by a coefficient of determination (R^2) exceeding 0.95 during the initial two minutes of the reaction, were exclusively selected for statistical analysis. These criteria were established to minimize errors arising from human, instrumental, and environmental sources, thereby ensuring the robustness of results in the assessment of GPX activity. Potential sources of variability, such as inconsistencies in sample preparation, chemical interferences, and instrumental deviations, were mitigated through the implementation of standardized protocols and a minimum of three experimental replicates per sample.

Protein concentration was quantified using the Bradford method [24], based on the binding of Coomassie Brilliant Blue G-250 to proteins in an acidic medium, with absorbance measured at 595 nm. Protein concentration was calculated using a bovine serum albumin standard curve and expressed as mg/g fresh weight. The absorbance at 595 nm was found to be directly proportional to protein concentration. To evaluate the effects of glycine and cysteine on enzymatic activity, the crude extract was treated in 3 mL vials in triplicate and divided into distinct groups. The control group was treated with distilled water alone. Various concentrations of cysteine and glycine (in mM) were applied, and treatments were performed at ambient temperature (20–25°C) under natural laboratory lighting for 10 minutes.

Peroxidase Activity Assay: The activity of GPX was quantified using a spectrophotometric method [18], based on the oxidation of guaiacol in the presence of hydrogen peroxide (H_2O_2), resulting in the formation of tetraguaiacol, a colored product with absorbance at 470 nm. Materials and equipment utilized included 0.1 M potassium phosphate buffer (pH 6.5), 20 mM guaiacol, 10 mM freshly prepared hydrogen peroxide, enzyme extracts derived from tomato (*Solanum lycopersicum* L.) tissue at various physiological stages (immature, ripe, and stored), and a spectrophotometer configured to measure absorbance at 470 nm. The reaction mixture was prepared in a cuvette by combining 2.8 mL of potassium phosphate buffer, 100 µL of guaiacol, 100 µL of hydrogen peroxide, and 100 µL of enzyme extract. Following gentle mixing, the reaction was initiated, and absorbance at 470 nm was recorded continuously or at 30-second intervals for 2–3 minutes. To ensure the validity of linear absorbance in the measurements conducted, only data exhibiting a linear increase in absorbance with a coefficient of determination (R^2) greater than 0.95 during the first two minutes of the reaction were analyzed. This criterion was applied to guarantee the accuracy and reliability of the results in assessing enzymatic activity. An increase in absorbance was indicative of tetraguaiacol formation and enzymatic activity. A control sample, devoid of enzyme extract, was prepared to determine background absorbance. Enzymatic activity was expressed in units (U), defined as the amount of enzyme required to oxidize 1 µmol of guaiacol per minute. Key experimental considerations included maintaining an optimal pH of 6.5, using freshly prepared hydrogen peroxide due to its instability, conducting assays at 25°C, noting substrate inhibition at guaiacol concentrations exceeding 10 mM, and calibrating the spectrophotometer with the control sample. A linear increase in absorbance at 470 nm was observed to confirm enzymatic activity.

Treatment Conditions (Cysteine and Glycine): The inhibitory effects of cysteine and glycine on GPX activity were evaluated by incorporating these compounds into the reaction mixture at concentrations of 0 mM (control, absence of cysteine and glycine), 0.4 mM, 0.8 mM, and 1.2 mM. The reduction in enzymatic activity was quantified using standard spectrophotometric methods. Lineweaver-Burk plots were constructed to determine the inhibition mechanism (competitive or non-competitive) and to calculate the Michaelis-Menten constant (Km) and maximum reaction velocity (Vmax). This methodology is routinely applied to assess GPX activity in plant tissues, such as those derived from *Solanum lycopersicum* (tomato), facilitating the examination of factors affecting enzyme function, including inhibitors and storage conditions. It should be noted that enzymatic reactions were quantified at guaiacol concentrations of 6, 7, 8, 9, and 10 mM, as well as cysteine and glycine concentrations of 0, 0.2, 0.4, 0.8, and 1.2 mM. The corresponding graphs were generated using Microsoft Excel.

To enhance the accuracy and reliability of absorbance measurements obtained using a

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spectrophotometer, both cuvettes were prepared with identical concentrations of guaiacol substrate and the amino acids cysteine or glycine. The only difference between the two cuvettes was the enzymatic homogenate used to initiate the reaction. Furthermore, in assays conducted in the presence of varying concentrations of cysteine or glycine, measurements without these amino acids were employed as controls to accurately assess their inhibitory effects

Statistical Analysis: The effects of the amino acid's cysteine and glycine on the enzymatic activity of GPX as well as the influence of varying concentrations of the guaiacol substrate, were investigated. Following confirmation of normal distribution using appropriate statistical tests, the obtained data were subjected to statistical analysis. Data were compiled in Microsoft Excel spreadsheets and analyzed using SAS software (version 2000). Analysis of variance (ANOVA) was conducted to assess statistically significant differences among groups. Post-hoc comparisons of means were performed using Duncan's multiple range test at a significance level of $P < 0.05$. All biochemical parameters evaluated in the study were expressed as mean ± standard error of the mean (Mean ± SEM) to ensure precision and consistency in reporting. Graphs were generated using Microsoft Excel.

Results

Determination of Optimal pH for GPX Activity in Solanum lycopersicum:

The optimal pH for GPX activity in tomato (*Solanum lycopersicum* L.) extracts at immature, ripe, and stored physiological stages was determined by quantifying enzyme activity using guaiacol as the substrate across a pH range of 3.0 to 10.0. The maximum absorbance of the quinone product, tetraguaiacol, was recorded at 420 nm, with the GPX reaction conducted for one minute. Initial reaction rates were measured across the specified pH range, with enzyme extract and substrate concentrations maintained constant while the buffer pH was varied. It was observed that GPX activity was absent at acidic pH values below 4.0 and alkaline pH values above 8.0 across all three stages. However, activity was detected within the pH range of 5.0 to 8.0, confirming that GPX activity is exhibited in *Solanum lycopersicum* extracts at all three physiological stages. High concentrations of $[H^+]$ and $[OH^-]$ were found to catalytically inactivate the enzyme. The GPX activity curve, illustrated in Figure 1, was generated across the pH range of 3.0 to 10.0, revealing peak activity at pH 6.5 for all stages. The highest GPX activity was recorded in the stored stage, while the lowest was observed in the immature stage. Consequently, a pH of 6.5 was selected for subsequent enzymatic activity measurements.

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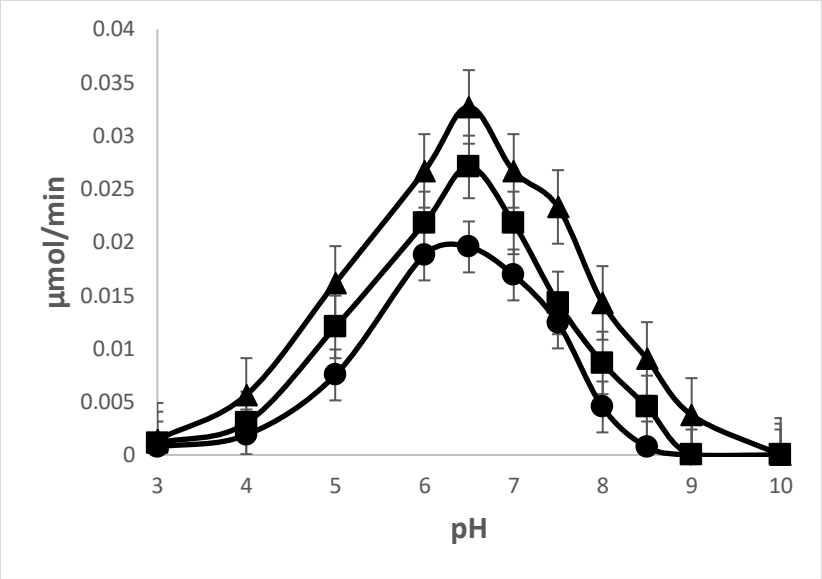

Figure 1: Determination of the optimal pH for GPX activity in Solanum lycopersicum under immature (●), ripe (■), and stored (▲) conditions using guaiacol as the substrate

Determination of Kinetic Parameters of GPX Activity in Solanum lycopersicum Extract (Immature Stage) in the Presence of Guaiacol Substrate: The Km and Vmax values for GPX activity in tomato (*Solanum lycopersicum* L.) extracts were determined at the optimal pH of 6.5 by quantifying initial reaction rates at varying guaiacol concentrations. Initial reaction rates were measured using a spectrophotometric assay. The Michaelis-Menten curve for GPX activity in the immature stage extract (●) across a range of guaiacol concentrations was generated, as illustrated in Figure 2. As the concentration of guaiacol, a phenolic substrate, was increased from 0 to 20 mM, a gradual increase in the initial reaction rate was observed ($p < 0.01$). At a guaiacol concentration of 10 mM, the initial reaction rate was found to reach its maximum. However, as the substrate concentration was further increased from 10 to 20 mM, a sharp decrease in the initial reaction rate was noted, indicative of substrate inhibition. At 20 mM guaiacol, peroxidase activity in the immature stage extract was reduced to 28% of its maximum ($p < 0.01$).

Determination of Kinetic Parameters of GPX Activity in Solanum lycopersicum Extract (Ripe Stage) in the Presence of Guaiacol Substrate: The kinetic parameters, Michaelis constant (Km), and maximum reaction rate (Vmax) of GPX activity were determined for the ripe stage extract of *Solanum lycopersicum* in the presence of guaiacol as the substrate. Initial reaction rates were quantified at an optimal pH of 6.5 across a range of guaiacol concentrations. The Michaelis-Menten kinetics, as illustrated in Figure 2, were characterized for GPX activity in the ripe stage extract (■) with varying guaiacol concentrations. Reaction rates were observed to increase progressively with guaiacol concentrations ranging from 0 to 20 mM. Maximal enzymatic activity was attained at a guaiacol concentration of 10 mM and was maintained up to 12 mM. Beyond 12 mM, substrate inhibition was observed, as evidenced by a decline in the initial reaction rate with a steeper gradient compared to the immature stage extract ($p < 0.001$). At a guaiacol concentration of 20 mM, GPX activity was reduced to 30% of its maximum, corresponding to 0.009 µmol/min ($p < 0.001$). The Vmax was determined to be 0.027 µmol/min, and the Km for guaiacol was calculated as 5 mM. The catalytic efficiency, expressed as the ratio of Vmax to Km, was established at 0.005 µmol/min per mM for the ripe stage extract.

Determination of Kinetic Parameters of GPX Activity in Solanum lycopersicum Extract (Stored Stage) in the Presence of Guaiacol Substrate: The kinetic

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parameters of GPX activity were evaluated in the stored stage extract of *Solanum lycopersicum* using guaiacol as the substrate. Initial reaction rates were measured at an optimal pH of 6.5 across a range of guaiacol concentrations. The Michaelis-Menten kinetics, as depicted in Figure 2, were characterized for GPX activity in the stored stage extract (▲) with varying guaiacol concentrations. A progressive increase in the initial reaction rate was observed as guaiacol concentrations increased from 0 to 20 mM ($p < 0.01$). Maximal enzymatic activity was achieved at a guaiacol concentration of 10 mM and was sustained up to 12 mM. Beyond 12 mM, substrate inhibition was evident, with the initial reaction rate decreasing at a gentler slope compared to the immature and ripe stage extracts ($p < 0.05$). Unlike the immature and ripe stages, the GPX activity in the stored stage extract exhibited a gradual decline following the maximum at 10 mM ($p < 0.05$). At 20 mM guaiacol, the GPX activity was reduced to 48% of its maximum, corresponding to 0.016 µmol/min ($p < 0.01$). The maximum reaction rate (Vmax) was determined to be 0.033 µmol/min. The Michaelis constant (Km) for guaiacol was calculated as 5 mM. The catalytic efficiency, expressed as the ratio of Vmax to Km, was established at 0.0064 µmol/min per mM for the stored stage extract.

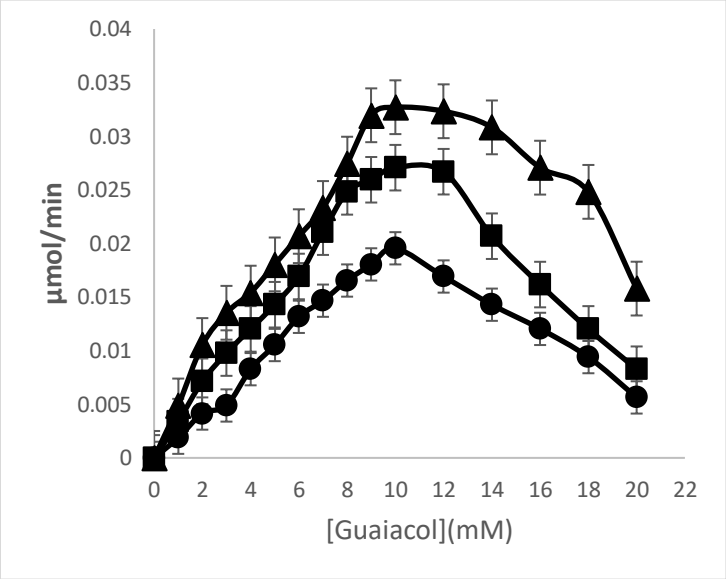

Figure 2: Determination of kinetic parameters of GPX activity in Solanum lycopersicum extract under immature (●), ripe (■), and stored (▲) conditions in the presence of guaiacol substrate

Determination of the Optimal Hydrogen Peroxide Concentration for GPX Activity in Solanum lycopersicum Extracts under Immature, Ripe, and Stored Conditions: The optimal concentration of hydrogen peroxide (H_2O_2) as the secondary substrate for GPX activity, with guaiacol as the primary substrate, was investigated in extracts from *Solanum lycopersicum* at immature, ripe, and stored stages. GPX activity was quantified as µmol/min across a range of H_2O_2 volumes (0 to 300 µL of 10 mM H_2O_2). The relationship between GPX activity and H_2O_2 volume was depicted in Figure 3. Activity was observed to increase with increasing H_2O_2 volumes in extracts from all three stages, with maximal activity recorded at 100 µL of 10 mM H_2O_2 ($p < 0.01$). Beyond this volume, further increases in H_2O_2 did not enhance activity and were associated with a decline in GPX activity ($p < 0.05$). Accordingly, 100 µL of 10 mM H_2O_2 was established as the fixed volume for subsequent enzymatic assays.

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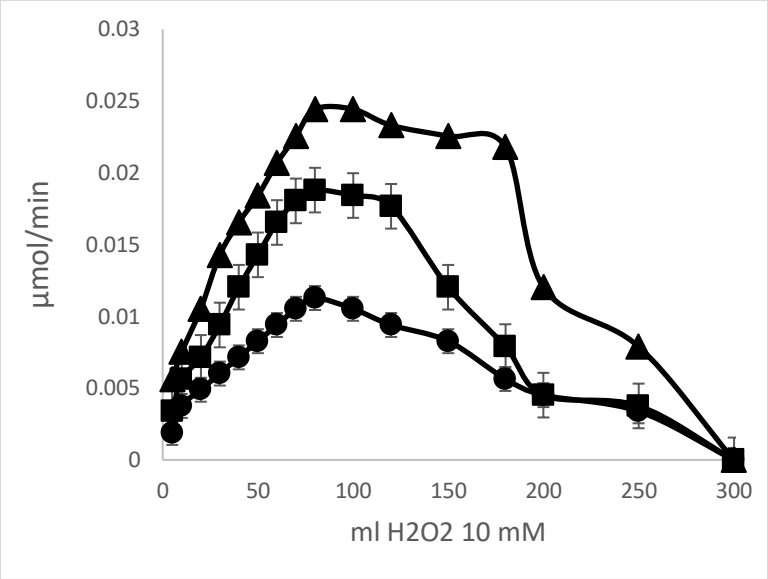

Figure 3: Determination of the optimal hydrogen peroxide concentration for GPX activity in Solanum lycopersicum extracts under immature (●), ripe (■), and stored (▲) conditions in the presence of guaiacol substrate

Determination of Kinetic Parameters of GPX Activity in Solanum lycopersicum Extract (Immature Stage) in the Presence of Guaiacol Substrate and Glycine Amino Acid: The influence of glycine on GPX activity in the immature stage extract of *Solanum lycopersicum* was investigated, alongside the determination of kinetic parameters (Michaelis constant, Km, and maximum reaction rate, Vmax) using guaiacol as the primary substrate. Initial reaction rates were quantified at an optimal pH of 6.5 across varying guaiacol concentrations (0 to 20 mM) in the presence of glycine at concentrations of 0, 0.4, 0.8, and 1.2 mM. The Michaelis-Menten kinetics, as depicted in Figure 4, were characterized for GPX activity in the immature extract under these conditions. In the absence of glycine, a progressive increase in the initial reaction rate was observed with guaiacol concentrations increasing from 0 to 10 mM, ($p < 0.01$), reaching maximal enzymatic activity at 10 mM. Beyond this, substrate inhibition was evident, with the reaction rate decreasing at guaiacol concentrations up to 20 mM, ($p < 0.01$), resulting in a 70% reduction in GPX activity (0.0086 µmol/min). The Vmax was determined to be 0.02 µmol/min, with a Km of 5 mM for guaiacol, and the catalytic efficiency (Vmax/Km) was calculated as 0.0036 µmol/min per mM. In the presence of glycine, GPX activity was consistently reduced across all tested concentrations ($p < 0.05$). At 0.4 mM glycine, the Vmax was 0.018 µmol/min, the Km was 5.2 mM, and the catalytic efficiency was 0.0033 µmol/min per mM. At 0.8 mM glycine, the Vmax decreased to 0.015 µmol/min, with a Km of 5.3 mM and a catalytic efficiency of 0.003 µmol/min per mM. At 1.2 mM glycine, the Vmax was further reduced to 0.013 µmol/min, with a Km of 5.5 mM and a catalytic efficiency of 0.0022 µmol/min per mM. At 20 mM guaiacol, the combined effects of substrate inhibition and glycine inhibition resulted in near-complete suppression of GPX activity across all glycine concentrations ($p < 0.01$).

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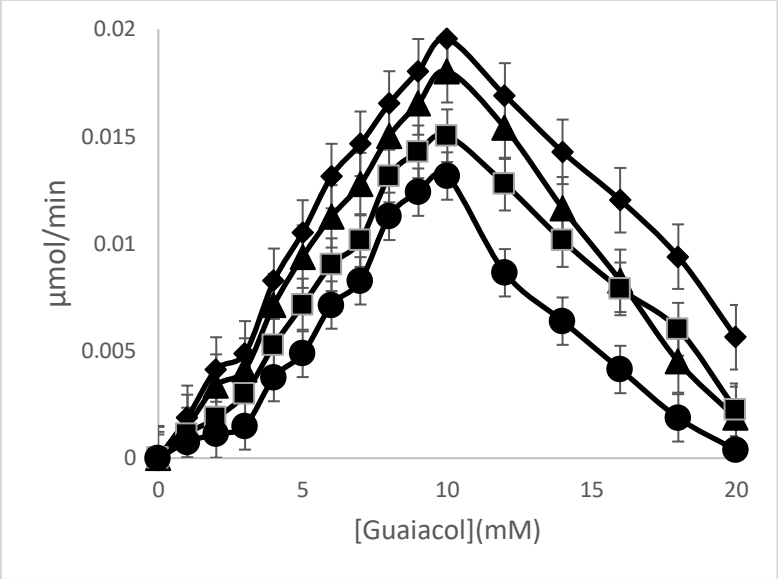

Figure 4: Determination of the kinetic parameters of GPX activity in Solanum lycopersicum extract (unripe stage) in the presence of guaiacol as substrate. Absence of Gly (♦), 0.4 mM Gly (▲), 0.8 mM Gly (■), 1.2 mM Gly (●)

Determination of Kinetic Parameters of GPX Activity in Solanum lycopersicum Extract (Unripe Stage) in the Presence of Guaiacol and the Amino Acid Cysteine: The effect of cysteine on GPX activity in the unripe stage extract of *Solanum lycopersicum* was investigated, alongside the determination of kinetic parameters (Michaelis constant, Km, and maximum reaction rate, Vmax) using guaiacol as the substrate. Initial reaction rates were quantified at an optimal pH of 6.5 across varying guaiacol concentrations (0 to 20 mM) in the absence and presence of cysteine at concentrations of 0, 0.4, 0.8, and 1.2 mM. The Michaelis-Menten kinetics, as depicted in Figure 5, were characterized for GPX activity in the unripe extract under these conditions. In the absence of cysteine, a progressive increase in the initial reaction rate was observed as guaiacol concentrations increased from 0 to 10 mM, ($p < 0.01$), with maximal enzymatic activity achieved at 10 mM. Beyond this concentration, substrate inhibition was evident, with the reaction rate decreasing up to 20 mM guaiacol, ($p < 0.001$), resulting in a 70% reduction in GPX activity (0.006 µmol/min) ($p < 0.001$). The Vmax was determined to be 0.0196 µmol/min, with a Km of 5 mM for guaiacol, and the catalytic efficiency (Vmax/Km) was calculated as 0.0038 µmol/min per mM. In the presence of cysteine, GPX activity was consistently reduced across all tested concentrations ($p < 0.01$). At 0.4 mM cysteine, the Vmax was 0.015 µmol/min, the Km was 5.1 mM, and the catalytic efficiency was 0.003 µmol/min per mM. At 0.8 mM cysteine, the Vmax decreased to 0.012 µmol/min, with a Km of 5.3 mM and a catalytic efficiency of 0.0022 µmol/min per mM. At 1.2 mM cysteine, the Vmax was further reduced to 0.0097 µmol/min, with a Km of 5.9 mM and a catalytic efficiency of 0.0015 µmol/min per mM. At 20 mM guaiacol, the combined effects of substrate inhibition and cysteine inhibition resulted in near-complete suppression of GPX activity across all cysteine concentrations ($p < 0.01$).

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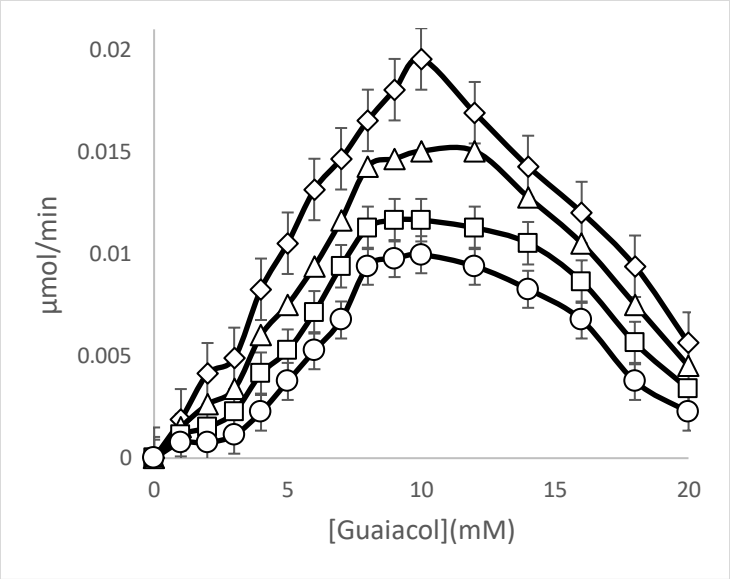

Figure 5: Determination of kinetic parameters of GPX activity in S. lycopersicum extract (unripe stage) in the presence of guaiacol. Absence of Cys (◊), 0.4 mM Cys (Δ), 0.8 mM Cys (□), 1.2 mM Cys (○)

Determination of Kinetic Parameters of GPX Activity in Solanum lycopersicum Extract (Ripe Stage) in the Presence of Guaiacol and the Amino Acid Glycine: The effect of glycine on GPX activity in the ripe stage extract of *Solanum lycopersicum* was evaluated, alongside the determination of kinetic parameters (Michaelis constant, Km, and maximum reaction rate, Vmax) using guaiacol as the substrate. Initial reaction rates were quantified at an optimal pH of 6.5 across varying guaiacol concentrations (0 to 20 mM) in the presence of glycine at concentrations of 0, 0.4, 0.8, and 1.2 mM. The Michaelis-Menten kinetics, as illustrated in Figure 6, were characterized for GPX activity in the ripe extract under these conditions. In the absence of glycine, a progressive increase in the initial reaction rate was observed as guaiacol concentrations increased from 0 to 10 mM, ($p < 0.01$), with maximal enzymatic activity achieved between 8 and 10 mM guaiacol. Beyond this range, substrate inhibition was evident, with the reaction rate decreasing at guaiacol concentrations up to 20 mM, ($p < 0.01$), resulting in a 70% reduction in GPX activity (0.008 µmol/min). The Vmax was determined to be 0.027 µmol/min, with a Km of 4.8 mM for guaiacol, and the catalytic efficiency (Vmax/Km) was calculated as 0.006 µmol/min per mM. In the presence of glycine, GPX activity was progressively reduced across all tested concentrations ($p < 0.01$). At 0.4 mM glycine, the Vmax was 0.023 µmol/min, the Km was 4.9 mM, and the catalytic efficiency was 0.0045 µmol/min per mM. At 0.8 mM glycine, the Vmax decreased to 0.0195 µmol/min, with a Km of 4.8 mM and a catalytic efficiency of 0.0038 µmol/min per mM. At 1.2 mM glycine, the Vmax was further reduced to 0.016 µmol/min, with a Km of 4.8 mM and a catalytic efficiency of 0.003 µmol/min per mM. At 20 mM guaiacol, the combined effects of substrate inhibition and glycine inhibition resulted in near-complete suppression of GPX activity across all glycine concentrations.

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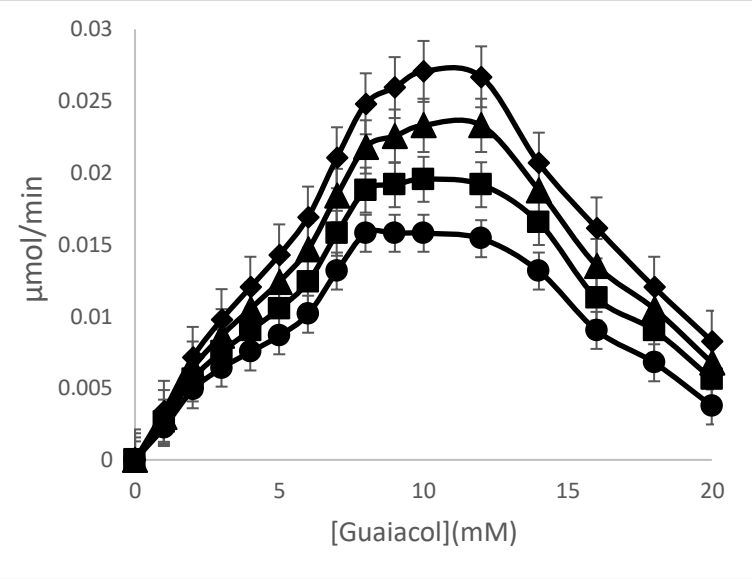

Figure 6: Determination of kinetic parameters of GPX activity in S. lycopersicum extract (ripe stage) in the presence of guaiacol. Absence of Gly (♦), 0.4 mM Gly (▲), 0.8 mM Gly (■), 1.2 mM Gly (●)

Determination of Kinetic Parameters of GPX Activity in Solanum lycopersicum Extract (Ripe Stage) in the Presence of Guaiacol and the Amino Acid Cysteine: The effect of cysteine on GPX in the ripe stage extract of *Solanum lycopersicum* was evaluated, alongside the determination of kinetic parameters (Michaelis constant, Km, and maximum reaction rate, Vmax) using guaiacol as the substrate. Initial reaction rates were quantified at an optimal pH of 6.5 across varying guaiacol concentrations (0 to 20 mM) in the absence and presence of cysteine at concentrations of 0, 0.4, 0.8, and 1.2 mM. The Michaelis-Menten kinetics, as illustrated in Figure 7, were characterized for GPX activity in the ripe extract under these conditions. In the absence of cysteine, a progressive increase in the initial reaction rate was observed as guaiacol concentrations increased from 0 to 10 mM, ($p < 0.01$), with maximal enzymatic activity achieved at 10 mM. Beyond this concentration, substrate inhibition was evident, with the reaction rate decreasing at guaiacol concentrations up to 20 mM, ($p < 0.01$), resulting in a 72% reduction in GPX activity (0.0076 µmol/min). The Vmax was determined to be 0.027 µmol/min, with a Km of 4.8 mM for guaiacol, and the catalytic efficiency (Vmax/Km) was calculated as 0.0056 µmol/min per mM. In the presence of cysteine, GPX activity was progressively reduced across all tested concentrations. At 0.4 mM cysteine, the Vmax was 0.0195 µmol/min, the Km was 5.1 mM, and the catalytic efficiency was 0.0038 µmol/min per mM. At 0.8 mM cysteine, the Vmax decreased to 0.0165 µmol/min, with a Km of 5.5 mM and a catalytic efficiency of 0.003 µmol/min per mM. At 1.2 mM cysteine, the Vmax was further reduced to 0.0124 µmol/min, with a Km of 6.0 mM and a catalytic efficiency of 0.0019 µmol/min per mM. At 20 mM guaiacol, the combined effects of substrate inhibition and cysteine-mediated inhibition resulted in near-complete suppression of GPX activity across all cysteine concentrations.

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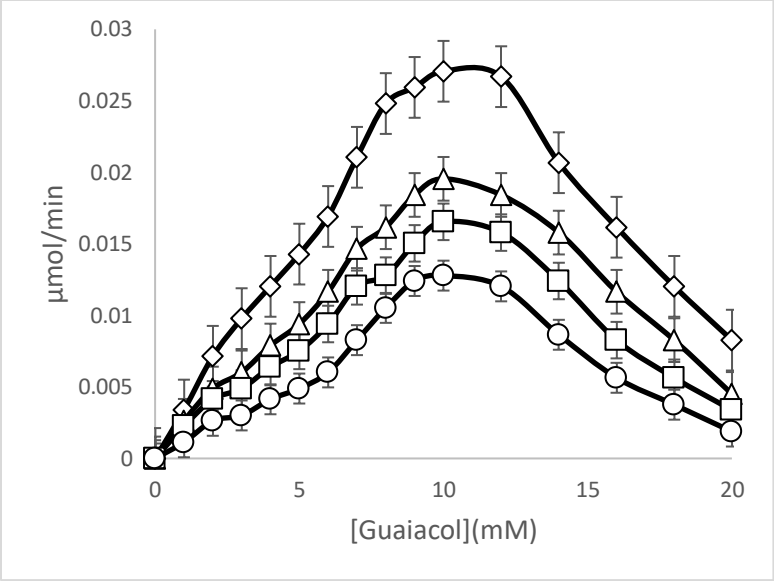

Figure 7: Determination of kinetic parameters of GPX activity in S. lycopersicum extract (ripe stage) in the presence of guaiacol. Absence of Cys (◊), 0.4 mM Cys (Δ), 0.8 mM Cys (□), 1.2 mM Cys (○)

Determination of Kinetic Parameters of GPX Activity in Solanum lycopersicum Extract (Storage Condition) in the Presence of Guaiacol and the Amino Acid Glycine: The influence of glycine on GPX activity in the stored stage extract of *Solanum lycopersicum* was assessed, alongside the determination of kinetic parameters (Michaelis constant, Km, and maximum reaction rate, Vmax) using guaiacol as the substrate. Initial reaction rates were quantified at an optimal pH of 6.5 across varying guaiacol concentrations (0 to 20 mM) in the absence and presence of glycine at concentrations of 0, 0.4, 0.8, and 1.2 mM. The Michaelis-Menten kinetics, as illustrated in Figure 8, were characterized for GPX activity in the stored extract under these conditions. In the absence of glycine, a progressive increase in the initial reaction rate was observed as guaiacol concentrations increased from 0 to 11 mM, ($p < 0.01$), with maximal enzymatic activity achieved between 9 and 11 mM. Beyond this range, substrate inhibition was evident, with the reaction rate decreasing at guaiacol concentrations up to 20 mM, ($p < 0.01$), resulting in a 50% reduction in GPX activity (0.0163 µmol/min). The Vmax was determined to be 0.033 µmol/min, with a Km of 4.0 mM for guaiacol, and the catalytic efficiency (Vmax / Km) was calculated as 0.008 µmol/min per mM. In the presence of glycine, GPX activity was progressively reduced across all tested concentrations ($p < 0.01$). At 0.4 mM glycine, the Vmax was 0.0282 µmol/min, the Km was 5.0 mM, and the catalytic efficiency was 0.0056 µmol/min per mM. At 0.8 mM glycine, the Vmax decreased to 0.024 µmol/min, with a Km of 5.5 mM and a catalytic efficiency of 0.0045 µmol/min per mM. At 1.2 mM glycine, the Vmax was further reduced to 0.02 µmol/min, with a Km of 6.0 mM and a catalytic efficiency of 0.0033 µmol/min per mM. At 20 mM guaiacol, the combined effects of substrate inhibition and glycine-mediated inhibition resulted in near-complete suppression of GPX activity across all glycine concentrations.

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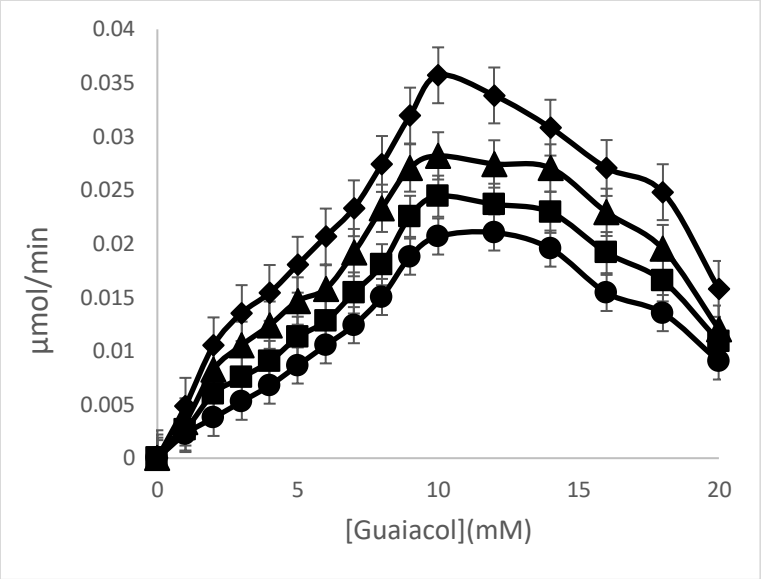

Figure 8: Determination of kinetic parameters of GPX activity in S. lycopersicum extract (storage condition) in the presence of guaiacol. Absence of Gly (♦), 0.4 mM Gly (▲), 0.8 mM Gly (■), 1.2 mM Gly (●)

Determination of Kinetic Parameters of GPX Activity in Solanum lycopersicum Extract (Ripe Stage) in the Presence of Guaiacol and the Amino Acid Cysteine: The effect of cysteine on GPX activity in the ripe stage extract of *Solanum lycopersicum* was investigated, alongside the determination of kinetic parameters (Michaelis constant, Km, and maximum reaction rate, Vmax) using guaiacol as the substrate. Initial reaction rates were quantified at an optimal pH of 6.5 across varying guaiacol concentrations (0 to 20 mM) in the absence and presence of cysteine at concentrations of 0, 0.4, 0.8, and 1.2 mM. The Michaelis-Menten kinetics, as depicted in Figure 9, were characterized for GPX activity in the ripe extract under these conditions. In the absence of cysteine, a progressive increase in the initial reaction rate was observed as guaiacol concentrations increased from 0 to 10 mM, ($p < 0.01$), with maximal enzymatic activity achieved at 10 mM. Beyond this concentration, substrate inhibition was evident, with the reaction rate decreasing at guaiacol concentrations up to 20 mM, resulting in a 60% reduction in GPX activity (0.014 µmol/min). The Vmax was determined to be 0.036 µmol/min, with a Km of 5.0 mM for guaiacol, and the catalytic efficiency (Vmax / Km) was calculated as 0.034 µmol/min per mM. In the presence of cysteine, GPX activity was progressively reduced across all tested concentrations ($p < 0.01$). At 0.4 mM cysteine, the Vmax was 0.026 µmol/min, the Km was 5.4 mM, and the catalytic efficiency was 0.005 µmol/min per mM. At 0.8 mM cysteine, the Vmax decreased to 0.022 µmol/min, with a Km of 6.3 mM and a catalytic efficiency of 0.0037 µmol/min per mM. At 1.2 mM cysteine, the Vmax was further reduced to 0.0154 µmol/min, with a Km of 6.5 mM and a catalytic efficiency of 0.0022 µmol/min per mM. At 20 mM guaiacol, the combined effects of substrate inhibition and cysteine-mediated inhibition resulted in near-complete inactivation of GPX activity across all cysteine concentrations.

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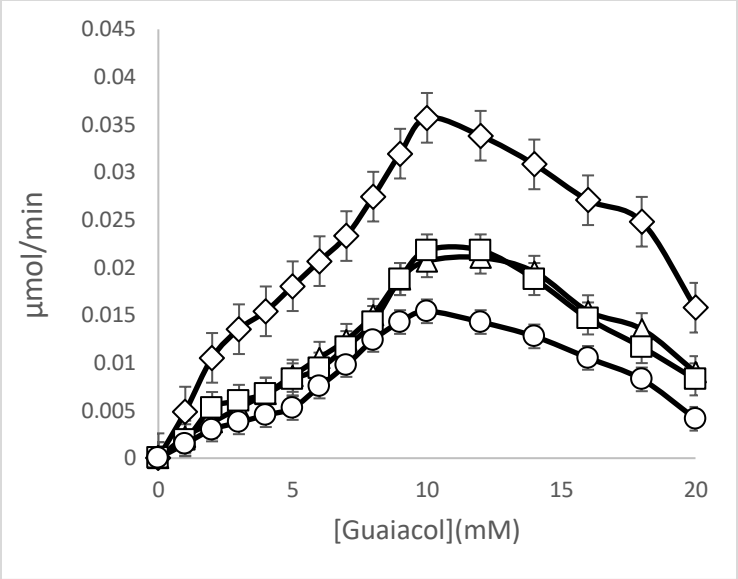

Figure 9: Determination of kinetic parameters of GPX activity in S. lycopersicum extract (ripe stage) in the presence of guaiacol. Absence of Cys (◊), 0.4 mM Cys (Δ), 0.8 mM Cys (□), 1.2 mM Cys (○)

Effect of Glycine and Cysteine on Guaiacol GPX Activity in Solanum lycopersicum Extract under Immature Conditions:

The inhibitory effects of glycine and cysteine on guaiacol glutathione peroxidase (GPX) activity were evaluated in Solanum lycopersicum extracts under immature, mature, and stored conditions. The inhibitor concentration resulting in 50% inhibition of guaiacol GPX activity (IC50) was determined for each condition. In the immature extract, the IC50 values were calculated as 1.0 mM for glycine and 0.8 mM for cysteine, with cysteine exhibiting significantly greater inhibitory potency ($p < 0.05$). In the mature extract, the IC50 values were determined to be 1.3 mM for glycine and 0.9 mM for cysteine, with a significant difference in inhibitory potency between the two inhibitors ($p < 0.05$). For the stored extract, the IC50 values were established as 1.5 mM for glycine and 1.2 mM for cysteine, with cysteine again showing significantly higher inhibitory potency ($p < 0.05$). A significant increase in IC50 values for both glycine ($p < 0.01$) and cysteine ($p < 0.01$) were observed from the immature to the stored stage, indicating reduced sensitivity to inhibition in the stored condition.

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Table 1: IC50 Parameters of GPX Enzyme in Solanum lycopersicum Extract in the Presence of Glycine and Cysteine Inhibitors

IC50 (mM)	Substrate	Inhibitors	S. Lycipersicum
1	Guaiacol	Gly	Immature
0.8		Cys	
1.3		Gly	Mature
0.9		Cys	
1.5		Gly	Storage
1.2		Cys	

Determination of the Type of GPX Inhibition in Solanum lycopersicum under Immature, Mature, and Storage Conditions in the Presence of Glycine and Cysteine:

Lineweaver-Burk plots were constructed to characterize the inhibition of glutathione peroxidase (GPX) activity with guaiacol as the substrate in Solanum lycopersicum extracts under immature, mature, and stored conditions, in the absence and presence of glycine and cysteine as inhibitors. For the immature stage, Lineweaver-Burk plots of guaiacol oxidation at varying glycine concentrations were linear and intersected on the x-axis, indicative of non-competitive inhibition ($p < 0.01$). Similarly, plots at different cysteine concentrations were linear and intersected on the x-axis, confirming non-competitive inhibition ($p < 0.01$). In the mature stage, Lineweaver-Burk plots for guaiacol oxidation at varying glycine concentrations were linear and intersected on the y-axis, consistent with competitive inhibition ($p < 0.01$). An identical pattern was observed for cysteine at different concentrations, also indicating competitive inhibition ($p < 0.01$). Under storage conditions, Lineweaver-Burk plots for guaiacol oxidation in the presence of varying concentrations of glycine and cysteine were linear and intersected on the y-axis, demonstrating competitive inhibition ($p < 0.01$). In summary, guaiacol oxidation by GPX exhibited non-competitive inhibition with glycine and cysteine in the immature stage, whereas competitive inhibition was observed in both the mature and stored stages, with a significant shift in inhibition mechanism between the immature and mature stages ($p < 0.001$).

Table 2: Summary of Kinetic Parameters and IC50 Values for GPX Activity in Solanum lycopersicum Extract under Immature, Mature, and Storage Conditions with Guaiacol Substrate

Type of Inhibition	IC50 (mM)	Inhibitors	S. Lycopersicum	Substrate
Non-Competetive	1	Glycine	Immature	
Non-Competetive	0.8	Cystein		

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Competetive	1.3	Glycine	Mature	Guaiacol
Competetive	0.9	Cystein		
Competetive	1.5	Glycine	Storage	
Competetive	1.2	Cystein		

Discussion

The kinetic properties and activity profile of GPX in *Solanum lycopersicum* fruit extracts were characterized across immature, mature, and stored stages to elucidate its role in plant physiology. GPX, a heme-containing enzyme, is critical for catalyzing the oxidation of phenolic compounds, contributing to metabolic processes, oxidative stress defense, and responses to environmental stressors. This investigation was designed to determine the optimal pH, kinetic parameters (Michaelis constant, Km, and maximum reaction rate, Vmax), the effects of hydrogen peroxide (H_2O_2) as a secondary substrate, and the inhibitory effects of glycine and cysteine on GPX activity with guaiacol as the primary substrate.

GPX activity was observed to be stable within a pH range of 5–8, with maximal activity recorded at pH 6.5 across all developmental stages, aligning with findings reported for apple and carrot GPX [18-21]. Investigations have been conducted to determine the optimal pH for glutathione peroxidase (GPX) activity, revealing a peak in enzymatic activity under callus, ripened, and storage conditions. This peak was consistently observed at a pH of 6.5 across all conditions. Optimal pH values for peroxidase activity have been documented as 6.0 for *Solanum tuberosum* (potato) [25] and *Brassica rapa* (turnip) [26], 7.0 for *Mangifera indica* (mango) [27] and *Crocus sativus* corm [28], and 5.6 for *Prunus domestica* (plum) [29] and *Anethum graveolens* (dill) [30]. These findings indicate variability in peroxidase isozymes across different plant species. Guaiacol peroxidase activity was found to be highest under storage conditions and lowest in unripe conditions, suggesting sustained enzymatic activity throughout plant development, with a progressive increase from the unripe to storage stages. In a comparable study, *Linum usitatissimum* (flax) treated with zinc exhibited enhanced peroxidase isozyme activity during growth, interpreted as a defensive response to oxidative stress [31]. Consequently, stress conditions induced during storage are proposed to activate the plant's antioxidant defense system, particularly through the upregulation of enzymes such as peroxidases, thereby mitigating oxidative stress levels.

This stability at pH 6.5 is attributed to the enzyme's structural properties, where key functional groups, such as histidine or cysteine in the active site, are optimally ionized, facilitating substrate binding (e.g., guaiacol or H_2O_2) and enhancing catalytic efficiency [32]. Deviations from pH 6.5 were found to alter the enzyme's tertiary structure, thereby reducing activity. The pH of 6.5 corresponds to the physiological conditions of the cytoplasm or apoplast in plant cells, enabling optimal GPX function in tissues such as tomato, apple, and carrot fruits. GPX s are known to mediate biological processes, including reactive oxygen species (ROS) neutralization, phenolic compound metabolism, and stress responses. The optimal pH of 6.5 allows GPX to effectively oxidize phenolic compounds or neutralize ROS without compromising its structural integrity or affecting cellular components. Multiple GPX isoforms, often expressed in plants, may exhibit varying pH optima, but pH 6.5 is frequently cited as an average optimum for isoforms in tomato, apple, and carrot [18-21].

Kinetic analyses using guaiacol (0–20 mM) revealed stage-specific Michaelis-Menten kinetics. In the immature stage, Vmax was determined to be 0.0195 µmol/min, Km was 5 mM, and catalytic efficiency (Vmax / Km) was 0.0038 µmol/min/mM, with

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maximal activity at 10 mM guaiacol and a 28% reduction at 20 mM due to substrate inhibition. In the mature stage, Vmax increased to 0.027 µmol/min, Km remained at 5 mM, and catalytic efficiency rose to 0.005 µmol/min/mM, with maximal activity at 12 mM and a 30% reduction at 20 mM. In the stored stage, Vmax was 0.0327 µmol/min, Km was 5 mM, and catalytic efficiency reached 0.0263 µmol/min/mM, with a 48% reduction at 20 mM. The consistent Km across stages indicates stable substrate affinity, while the progressive increase in Vmax suggests enhanced enzyme expression or substrate availability during maturation and storage, consistent with horseradish GPX kinetics, though unit differences complicate direct comparisons [18-21].

The optimal H_2O_2 concentration (100 µL of 10 mM) was found to elicit maximal activity across all stages, with higher concentrations leading to reduced activity, likely due to substrate inhibition or oxidative damage to the enzyme. These findings underscore the dynamic kinetic behavior of GPX in regulating fruit metabolism in response to developmental and environmental cues. Comparative studies on *Cucurbita pepo* (zucchini) peroxidase reported maximal activity at pH 6.5 and 50°C, with a Km of 6.25 mM and Vmax of 0.1 mM/min for H_2O_2, aligning with the present results [22]. In contrast, *Gundelia tournefortii* root peroxidase exhibited maximal activity at pH 5.5–6, with activity increasing with varied phenolic substrates [23].

The inhibitory effects of glycine and cysteine (0.4, 0.8, 1.2 mM) were assessed. In the immature stage, non-competitive inhibition was observed, with IC50 values of 1.0 mM for glycine and 0.8 mM for cysteine. Glycine reduced Vmax from 0.0195 to 0.0131 µmol/min and increased Km from 5 to 5.5 mM, while cysteine reduced Vmax to 0.0376 µmol/min and increased Km to 5.9 mM. In the mature and stored stages, competitive inhibition was observed, with IC50 values of 1.3 mM (glycine) and 0.9 mM (cysteine) for the mature stage, and 1.5 mM (glycine) and 1.2 mM (cysteine) for the stored stage, indicating competition with guaiacol at the active site. Cysteine's stronger inhibition is attributed to its thiol group, enhancing interaction with the enzyme's active site. At 20 mM guaiacol, the combined effects of substrate inhibition and amino acid inhibition nearly abolished GPX activity. The shift from non-competitive to competitive inhibition across stages is likely due to changes in isoform composition or active site structure during fruit maturation. In a study conducted by Gulnur in 2015, which corroborates and supports the findings of the current investigation, the effects of several amino acids, including glycine and cysteine, on guaiacol peroxidase activity in dill were reported, with cysteine identified as the most potent inhibitor of guaiacol peroxidase [30]. These findings align with the results of the present study. It was demonstrated that the enzymatic structure of guaiacol peroxidase becomes increasingly resistant from the unripe stage to the storage phase, requiring a higher concentration of inhibitors to reduce enzymatic activity. In other words, the peroxidase structure becomes more robust against environmental stressors, enabling it to perform its defensive role more effectively.

The study's strengths include its comprehensive evaluation across three developmental stages, rigorous kinetic analyses using Michaelis-Menten and Lineweaver-Burk plots, and detailed assessment of H_2O_2 and inhibitor effects. However, limitations were identified, including the absence of precise numerical data for graphical results, lack of isoform characterization, unspecified storage conditions, use of non-standard units for Vmax, and limited mechanistic analysis of inhibition. Future investigations are recommended to include isoform characterization via electrophoresis or chromatography, gene expression analysis using qPCR or RNA-Seq, evaluation of storage condition impacts (e.g., temperature, humidity), testing of additional substrates, and mechanistic studies of inhibition using spectroscopic or molecular modeling approaches.

From an applied perspective, the synergistic inhibition by glycine, cysteine, and high guaiacol concentrations offers significant potential for controlling enzymatic browning in the food industry, a process driven by GPX and polyphenol oxidase activity that oxidizes phenolic compounds to quinones, forming brown pigments. The observed inhibition, particularly amplified at 20 mM guaiacol, suggests that glycine and cysteine can serve as natural, safe alternatives to chemical inhibitors like sulfites, reducing GPX activity and enhancing shelf life. The elevated Vmax in the stored stage (0.0327 µmol/min) compared to the

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immature (0.0195) and mature (0.027) stages likely reflects activation of defensive pathways against environmental stresses, such as oxidative stress or temperature fluctuations during storage. These findings highlight the modulation of GPX activity by internal factors (e.g., gene expression, isoform composition) and external factors (e.g., inhibitors).

The consistent Km (5 mM) and increasing Vmax across stages provide valuable data for optimizing post-harvest strategies. Incorporation of glycine and cysteine into preservation solutions, active packaging, or edible coatings could minimize GPX activity, reducing browning and extending shelf life. Integration with technologies such as modified atmosphere packaging (MAP) could enhance these effects, preserving sensory and nutritional quality. Further studies on isoform-specific mechanisms and molecular modeling are warranted to refine these applications, contributing to sustainable methods for improving agricultural product quality and reducing food waste.

Valuable insights were obtained regarding the kinetic properties and inhibition patterns of GPX in Solanum lycopersicum fruit at various developmental stages, and the modulatory effects of glycine and cysteine were explored. However, several limitations were identified within the study. Notably, isoform-specific characterization of the enzyme was not performed, the spectrum of inhibitors evaluated was limited, and experiments were conducted exclusively under in vitro conditions. Furthermore, sensory and quality-related attributes of the fruit were not assessed, and molecular analyses, including gene expression profiling, were omitted. To address these deficiencies, it is recommended that future investigations focus on the identification and characterization of specific GPX isoforms, the expansion of natural inhibitor screening, and the implementation of in vivo experimental models. Additionally, integration of physicochemical quality assessments alongside molecular-level evaluations is essential to elucidate enzyme regulation mechanisms and their practical applications in postharvest management strategies.

Conclusion

This study presents a detailed kinetic characterization of GPX activity in *Solanum lycopersicum* fruit across three distinct developmental stages: immature, mature, and postharvest storage. The kinetic parameters revealed a relatively constant Michaelis-Menten constant (Km) alongside a progressive increase in maximum reaction velocity (Vmax), suggesting the differential expression of isoenzymes or enhanced substrate bioavailability during fruit maturation. Optimal enzymatic activity was observed at pH 6.5 and under specific concentrations of hydrogen peroxide, consistent with reported profiles in other horticultural species, thereby confirming the enzyme's biochemical stability and its physiological role in reactive oxygen species (ROS) detoxification during oxidative stress.

Importantly, stage-dependent inhibitory effects were demonstrated for glycine and cysteine, with non-competitive inhibition predominating in immature fruits and competitive inhibition observed in mature and stored fruits. Cysteine exerted a more pronounced inhibitory effect, attributable to the reactivity of its thiol (-SH) group, which may interact with essential catalytic residues or alter the enzyme's redox state. These observations indicate dynamic interactions between GPX and endogenous amino acid inhibitors, offering mechanistic insights into the modulation of enzymatic browning.

From a translational perspective, the synergistic inhibitory effects and substrate-dependent modulation support the potential application of glycine and cysteine in bio-based active packaging systems and edible coatings. Such interventions may serve to attenuate GPX mediated oxidative discoloration, enhance postharvest quality, and extend the shelf life of climacteric fruits, particularly within temperature-sensitive supply chains. Further investigations incorporating isoform-specific profiling, molecular docking, and mechanistic enzyme modeling are warranted to elucidate the structural basis of inhibition and to inform targeted strategies in food preservation and postharvest biotechnology.

Acknowledgment

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Conflicts of interest

There are no conflicts of interest to declare regarding this study.

Ethical considerations

This study adhered to strict ethical guidelines, ensuring integrity, transparency, and responsibility in all phases. Experimental protocols were carefully designed to guarantee responsible resource use, and results were honestly analyzed and reported, aligning with scientific ethical standards.

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