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Research article

# Effect of L-glutathione treatment on biochemical properties, antioxidant capacity and antioxidant enzymes activity in strawberry fruits during storage

Karim Manda-Hakki, Hamid Hassanpour *

*Department of Horticultural Sciences, Faculty of Agriculture, Urmia University, Urmia, Iran*

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ABSTRACT

The potential of L-glutathione (GSH) (0, 4, 16, 32 and 64 mM) to improve the post-harvest quality and antioxidant capacity of strawberries was investigated during storage (0, 5, 10, and 15 days) in this study. Results showed that weight loss in fruits treated with 64 mM GSH was significantly lower than the control. GSH treatments resulted in higher levels of total phenol content and antioxidant capacity in treated fruits of strawberry. Based on the results, GSH 64 mM significantly increased the levels of total flavonoid, anthocyanin, ascorbic acid, total soluble protein, antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), guaiacol peroxidase (POD), ascorbate peroxidase (APX) and Phenylalanine ammonia-lyase (PAL). In addition, GSH 64 mM decreased Malondialdehyde (MDA) levels and prevented cell membrane lipid peroxidation. In conclusion, the results of the present study showed that the use of GSH 64 mM may be a promising strategy to improve the marketability, quality and antioxidant capacity of strawberry fruits during storage.

## 1. Introduction

Strawberry (*Fragaria* × *ananassa*) was cultivated as one of the important fruit crops throughout the world. This product has high bioactive compounds such as vitamins, minerals, flavonoids, etc. And is highly consumed due to its health-promoting properties [1]. Also, strawberry fruits have attractive colour, soft texture, and excellent and unique taste and are widely consumed as rich sources of natural antioxidants [2]. However, strawberry fruits rot very quickly after harvest and are subject to water loss during storage and become susceptible to various diseases and consequently, a huge economic loss is imposed on the strawberry industry [1,3]. Also, fruits such as strawberries are very susceptible and vulnerable to diseases, which become more severe post-harvest period. After harvest, rot induced by fungi such as Botrytis cinerea and Rhizopus stolonifer results in huge economic losses for this crop [4]. Therefore, using an appropriate postharvest treatment is important to delay respiration rate, remove physical damage and desiccation, and the limitation of fungal decay for increasing shelf life [5].

It was determined that due to the perishable nature of strawberries, it is not suitable to store strawberries at a low temperature without using other protective compounds Therefore, it is necessary to use other post-harvest techniques to extend the shelf-life of fruits during cold storage. One of these techniques is the use of chemical compounds [6]. Amino acids are one of the chemical compounds used to increase storage life, and farmers have used them to enhance performance and different crop growth. The use of

* Corresponding author.
*E-mail addresses:* ha.hassanpour@urmia.ac.ir, phhassanpour@gmail.com (H. Hassanpour).

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amino acids has many advantages, including the availability of certain minerals and nutrients and positive and constructive interaction [7]. Glutathione (γ-glutamylcysteinylglycine, GSH) is the most important group of non-protein sulfur compounds in organisms and an abundant low molecular weight polypeptide which consists of glutamic acid, cysteine, and glycine and is the most abundant intracellular thiol found in almost all cells and protects them from oxidation and electrophiles. Its redoxactive thiol group (-SH) of cysteine residue mediates the GSH antioxidant function that oxidizes when glutathione reduces the target molecule [8,9].

In most horticultural crops, especially fruits, the use of glutathione in different concentrations leads to a positive effect on fruit yield and quality [10]. In previous studies, it has been shown that the application of exogenous GSH is beneficial to various plants under environmental stresses to increase the antioxidant compounds. It was also observed that exogenous GSH reduces the toxicity of cadmium lead, copper and chromium in higher plants [11,12]. Previously, it has been reported that the antioxidant capacity of strawberry fruits increased by GSH application via improving antioxidant compounds [13]. Also, in sweet pepper fruits, it was shown that reactive oxygen species (ROS) and malondialdehyde (MDA) content were reduced by GSH post-harvest treatment and led to an increased trend in the activity of glutathione reductase (GR), ascorbate peroxidase (APX), and Monodehydroasorbate reductase (MDHAR), and improved self-life of sweet pepper fruits during storage [14].

Considering the high production of strawberries in greenhouses and to maintain the quality of this product as well as to reduce post-harvest losses and maintain the marketability of strawberries, healthy and inexpensive methods should be used in post-harvest technology. However, glutathione application to preserve the nutritional quality of strawberry fruits during cold storage has not yet been reported elsewhere. Therefore, we investigated the efficacy of glutathione to understand the impact on the modulation of antioxidant and phenolic compounds in 'Sabina' strawberry during cold storage to resolve this research gap. In addition, the antioxidant enzyme activities including CAT, SOD, POD, APX and PAL and as well as MDA and soluble protein content of treated strawberry fruits with different GSH treatments during storage were analyzed. Therefore, a technical reference for the post-harvest preservation of strawberry fruits was provided by conducting the present study in short and long-term storage.

## 2. Materials and methods

### 2.1. Plant materials and treatment

Strawberry fruits (*Fragaria* × *ananassa* cv. Sabrina) were harvested in October 2023 at the full red stage from a commercial-covered greenhouse with a moderate climate by hydroponic method in Urmia, Iran. The harvested strawberry fruits were transferred to the lab with the necessary precautions to prevent mechanical damage. Fruits were selected with the same colour, uniform size, and without pests and diseases, and divided into 5 groups of 15. One group was considered as a control (or L-glutathione 0) and was sprayed with distilled water and 4 other groups were sprayed with L-glutathione (4, 16, 32 and 64 mM). After 2 h at room temperature (22 ± 2 °C) and surface drying, the treated fruits were placed in zipped bags and closed to prevent the growth of fungal spores, and maintained at 4 °C and 90–95 % RH for 15 days. Finally, different traits were determined after 5, 10 and 15 days of storage using the juice and compared with the control (0 day). Also, three biological replicates with 10 fruits per replicate were used for each treatment.

### 2.2. Weight loss

The weight loss (%) was calculated by the following formula: % weight loss = (M1−M2/M1) × 100, where M1 represents the weight of the sample at 0 d in gram and M2 is the weight of the final fruits in gram which measured by digital scale.

### 2.3. Total phenol (TP), total flavonoids (TF), total anthocyanin (TA) content and antioxidant activity

One ml of the juice of strawberry fruits was added to 10 ml of methanol containing HCl (1 %, v/v) and held at 0 °C for 10 min. The slurry was centrifuged at 10,000×*g* at 4 °C for 15 min, then the supernatant was used. According to the Folin-Ciocalteu method, the 50 μl extract was added to 4.2 mL of distillate water, followed by 10 % (v/v) diluted Folin's reagent (250 μl) and vortexed for 10 s to mix. Then 20 % Na2CO3 (500 μl) was added and mixed well. The mixtures were kept in a dark room for 30 min to react. The absorbance was spectrophotometrically read at 765 nm against a blank. To measure the TP content of samples, the calibration curve of standard gallic acid (10–250 mg/L) was provided and expressed as mg gallic acid equivalent (GAE) per 100 g FW [15].

For the determination of TF, the Shin et al. [16] method was used with little modifications. First, 500 μl of the extract was mixed with 150 μl of 5 % sodium nitrite, and 300 μl of 10 % aluminium chloride was added after 5 min. Then, 1 ml of 1 M sodium hydroxide was added after 5 min, and finally, the volume of the solution reached 5 ml with distillate water. The absorbance of samples was measured spectrophotometrically at 510 nm. Catechin (CAT) was used as to make a standard calibration curve and TF content was expressed in mg of CAT per 100 g FW.

To measure TA content, the pH differential method was used. For this purpose, two buffers with pH 1 and 4.5 were prepared, then 100 μL of the supernatant solution were added to 2.5 ml of buffer with pH = 1, and the amount of absorbance at two wavelengths of 530 and 700 nm was read by spectrophotometric method, and then the same method It was also repeated with pH = 4.5. The content of TA was calculated as mg cyanide 3 glucoside equivalent per L using the following formula [17].

$$TA = (A \times MW \times DF \times 100)/(\varepsilon \times 100)$$

where, A = absorption, MW = molecular weight of cyanidin 3-glucoside (449.2), DF = dilution factor, ε = molar extinction coefficient

of cyanidin 3-glucoside (29,600).

For the determination of antioxidant capacity, ferric reducing antioxidant property (FRAP) method was used. Briefly, acetate buffer (300 mM), 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ) solution (10 mM) in HCl (40 mM), and FeCl3 × 6 H2O solution (10 mM) were freshly prepared and mixed with 10:1:1 ratio. Sample extracts were diluted (1:10, sample: acetate buffer) and 50 μl of each sample was allowed to react with 3.5 ml of the FRAP solution for 10 min at 37 °C. The absorbance of the ferrous tripyridyltriazine complex was spectrophotometrically read at 593 nm against blanks. The antioxidant activity was expressed as mM of iron per 100 g FW [18].

### 2.4. Ascorbic acid content

The ascorbic acid content was spectrophotometrically measured. Briefly, 100 μl of fruit extract was mixed with 10 ml of 1 % metaphosphoric acid and 1 ml of the obtained solution was mixed with 9 ml of 2,6-Dichloroindophenol 50 μM and vortexed for a few seconds, then the absorbance was read at 515. Ascorbic acid content was measured based on a calibration curve of standard ascorbic acid (10–100 mg/L) and was calculated as mg of ascorbic acid in 100 g FW [19].

### 2.5. Malondialdehyde (MDA) content

The method of thiobarbituric acid (TBA) was used to measure the content of MDA based on the Hu et al. [20]. The absorbance of samples was spectrophotometrically read at 450, 532, and 600 nm, respectively. Also, the blank was 10 % of TAC and MDA content was expressed in mM/kg FW.

### 2.6. Enzyme activities assay and soluble protein content

The 0.5 ml sample was added in 2 ml of potassium phosphate buffer (50 mM, pH 7.5) consisting of 1 % Polyvinylpyrrolidone (PVP), 50 mM Tris and 1 mM EDTA. Then the obtained extracts were centrifuged at 10,000×*g* for 30 min at 4 °C, and the collected supernatant as an enzyme extract was applied for enzyme activities assay and soluble protein content.

#### 2.6.1. Soluble protein content

The Bradford method [21] was applied to measure the soluble protein of extracts. After extraction, 50 μL of the extract with 2950 μL of Bradford solution was mixed thoroughly, and the absorbance of the mixture was read at 595 nm after 10 min. Bovine serum albumin (BSA) as a standard (100 μl of standard samples mixed with 200 μl of diluted dye (acid solution Coomassie Brilliant Blue G 250)) was applied to determine the content of protein (mg/g FW).

#### 2.6.2. Superoxide dismutase (SOD) activity

The Beauchamp and Fridovich [22] method was applied to determine SOD activity (EC 1.15.1.1). According to this method, 3 ml reaction mixture was made of 13 mM methionine, 2 μM riboflavin, 50 mM phosphate buffer (pH 7.5), 0.1 μM EDTA, 75 μM nitro blue tetrazolium (NBT) and the ability of the enzyme extract to inhibit the photochemical reduction of NBT was determined by reading absorbance at 560 nm. Then, SOD activity was determined by subtracting the reduction of NBT in the presence of light and without the presence of enzyme extract from the reduction of NBT with enzyme extract ($E = 2.8\ mM^{-1}\ cm^{-1}$) and was expressed in U $kg^{-1}$ FW.

#### 2.6.3. Catalase (CAT) activity

CAT enzyme activity (EC 1.11.1.6) was evaluated using the Aebi [23] method based on the decomposition of $H_2O_2$ at 240 nm within 1 min. After mixing 2.8 ml of 50 mM phosphate buffer (pH 7.0, containing 2 mM EDTA), 100 μl of $H_2O_2$ (300 mM), and 100 μl of enzyme extract, a reaction mixture (3 mL) was obtained. CAT activity was calculated using E = 39.4 mM-1 cm-1 as the extinction coefficient and was calculated in U $kg^{-1}$ FW.

#### 2.6.4. Ascorbate peroxidase (APX) activity

The method of Nakano and Asada [24], according to the $H_2O_2$-dependent oxidation of ascorbate at 290 nm, was applied to determine the activity of APX (EC 1.11.1.11). Briefly, a reaction assay of 100 μl ascorbate (7.5 mM), 2.6 ml 25 mM phosphate buffer (pH 7.0), 100 μl $H_2O_2$ (300 mM), 2 mM EDTA, and 100 μl of enzyme extract was prepared. APX activity was measured using the extinction coefficient (2.8 mM L-1cm-1) and was calculated in U $kg^{-1}$ FW.

#### 2.6.5. Guaiacol peroxidase (POD) activity

The method of Nakano and Asada [24] was used to measure the POD activity with a little modification. For this purpose, the reaction mixture containing 3 μl of 50 mM phosphate buffer (pH = 7) was mixed with 50 μl of pure guaiacol with 50 μl of 3 μM hydrogen peroxide in an ice bath and then, 50 μl of enzyme extract was added. The activity of POD was spectrophotometrically recorded as an increase in absorption within 1 min at 420 nm. One enzyme unit of POD is equal to the decomposition of one μM of tetraguaiacol in 1 min and was calculated in U $kg^{-1}$ FW.

#### 2.6.6. Phenylalanine ammonialyase (PAL) activity

To prepare the enzyme extract, 5 ml strawberry juice was homogenized in an ice bath with 5 mL 0.1 mM cold acetic acid sodium acetate buffer (pH 5.5) containing 4 % (w/v) insoluble PVP and extracted (4 °C for 1 h). The extracts were centrifuged at 10,000×*g* for

30 min at 4 °C and the collected supernatant was used as an enzyme extract. After that, to determine the PAL activity, 1 ml of 50 mM potassium phosphate buffer (pH = 7), 0.5 ml of 10 mM phenylalanine, 0.4 ml of twice distilled water and 100 μl of enzyme extract were mixed and kept in a 37 °C bath for 1 h and then 0.5 ml of 6 M hydrochloric acid was added for stopping the reaction. The absorbance of the samples was spectrophotometrically read at 260 nm [25]. The amount of activity was calculated as nmol/g/min FW.

### 2.7. Statistical analysis

The study was carried out in factorial CRD (completely randomized design) with 3 replications. The first factor included different concentrations of glutathione (0, 4, 16, 32, and 64 mM) and the second factor was storage time (5, 10, and 15 days). The data normality was checked through Kolmogorov-Smirnov and Shapiro-Wilk test. Then, the obtained data were analyzed using software SAS version 9.4 (SAS Institute, Cary, NC). Duncan's multiple range test was applied to compare the means and a difference was considered statistically significant when the p-value was less than 0.05 ($p \leq 0.05$).

## 3. Result

### 3.1. Weight loss

The effect of storage time on fruit weight loss was significant at 1 % level. Our results showed the lowest weight loss was observed in 5 storage days and the highest weight loss was seen in 15 storage days. Based on the results, an increasing trend in weight loss was revealed during storage (Fig. 1a).

Also, the effect of GSH treatment on fruit weight loss was significant at 1 % level and the results indicated that the highest weight loss was seen in the 4 mM GSH treatment and the lowest weight loss was seen in 64 mM GSH treatment compared to the control (Fig. 1b).

### 3.2. Total phenol (TP), total flavonoids (TF), total anthocyanin (TA) content and antioxidant activity

The effect of storage time on TP content was statistically significant at the level of 5 %. The results showed the lowest and highest TP was observed in 5 and 15 storage days, respectively and an increasing trend during storage was revealed (Fig. 2 a). Also, The GSH treatment had a statistically significant effect on TP content ($P < 0.01$). The results showed that with the increase of GSH concentration, the content of TP increases compared to the control, so that the highest and lowest TP content was seen in 64 and 4 mM GSH treatments, respectively (Fig. 2b).

The interaction effect of storage time and GSH treatment was statistically significant on TF content ($P < 0.05$). Based on the results, the highest content of TF was seen in the 16 mM GSH treatment at 10 storage days, whereas the 4 mM GSH treatment at 5 storage days had the lowest TF content (Fig. 3). Our results revealed that the GSH treatments did not maintain TF content at 5 storage days

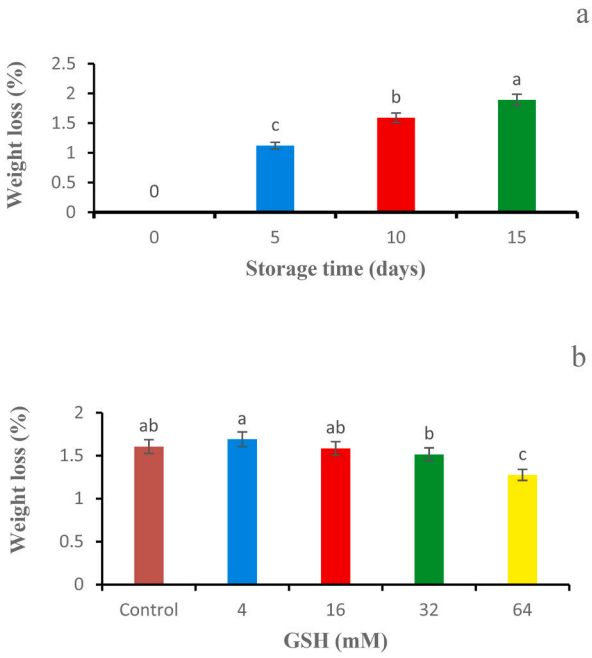


**Fig. 1.** The effect of storage time (a) and L-glutathione treatment (b) on weight loss of Sabrina strawberry fruits.

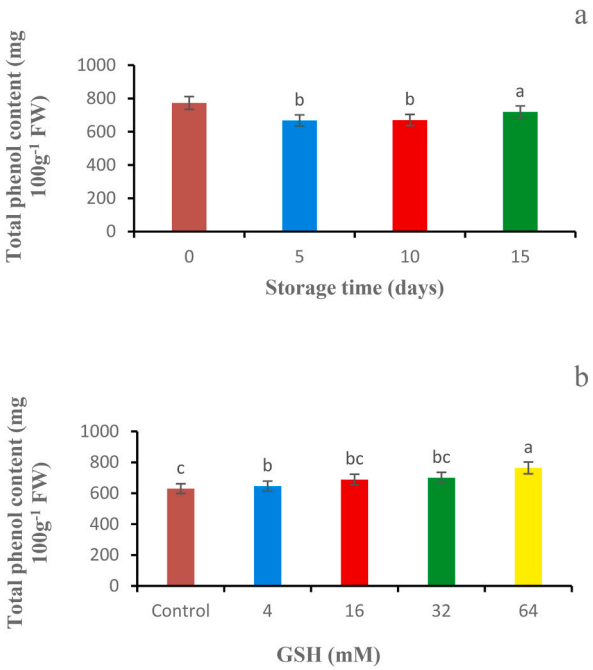


**Fig. 2.** The effect of storage time (a) and L-glutathione treatment (b) on total phenol content of Sabrina strawberry fruits.

compared to the control, however, the effect of the 64 mM treatment was greater. At 10 storage days, TF showed an increasing trend in the 4 and 16 mM treatments. Whereas, at 15 storage days, this trend decreased and the treatments did not maintain TF content, however, the 16 mM treatment had a greater effect compared to other treatments.

Storage time had no statistically significant effect on antioxidant activity. Whereas, the GSH treatments had a statistically significant effect on antioxidant activity ($P < 0.05$). According to the results, all GSH treatments increased the antioxidant activity. However, our results showed that the 32 mM GSH treatment had the lowest antioxidant activity, whereas the highest antioxidant activity was seen in the 16 mM GSH treatment compared to the control during storage (Fig. 4 a).

According on the results, storage time had no statistically significant effect on content of TA. Whereas, the effect of GSH treatment on TA content was statistically significant at 5 % level. The results of the present study revealed that the highest TA content was observed in 64 mM GSH treatment whereas the lowest TA content was seen in 16 mM GSH treatment compared to control during storage (Fig. 4 b).

### 3.3. Ascorbic acid content

The effect of storage time on ascorbic acid content was not statistically significant, whereas GSH treatments had a statistically significant effect on ascorbic acid content ($P < 0.05$). The obtained results showed that treated fruits with 4 mM GSH had the lowest content of ascorbic acid. Whereas the highest content of ascorbic acid was seen in 64 mM GSH treatment (Fig. 5 a).

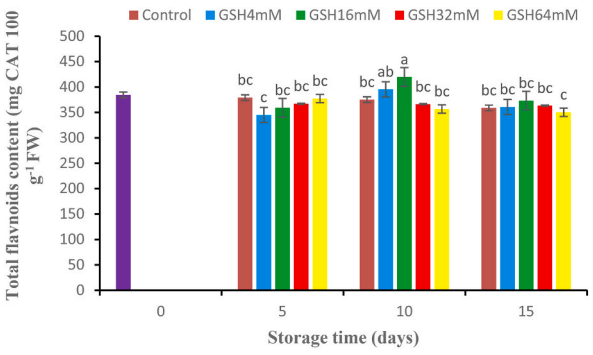


**Fig. 3.** Interaction effect of storage time and L-glutathione treatment on total flavonoid content.

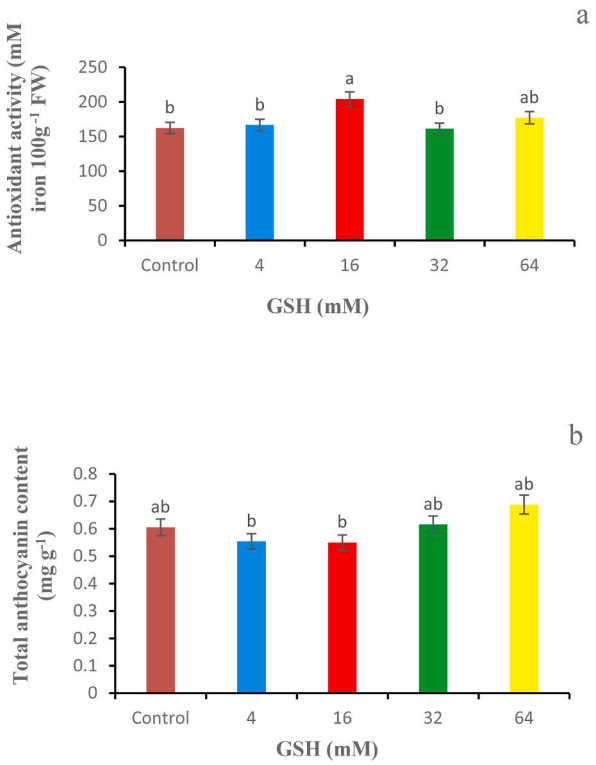


**Fig. 4.** Changes in antioxidant activity (a) and anthocyanin content (b) in Sabrina strawberry fruits treated with L-glutathione during post-harvest storage.

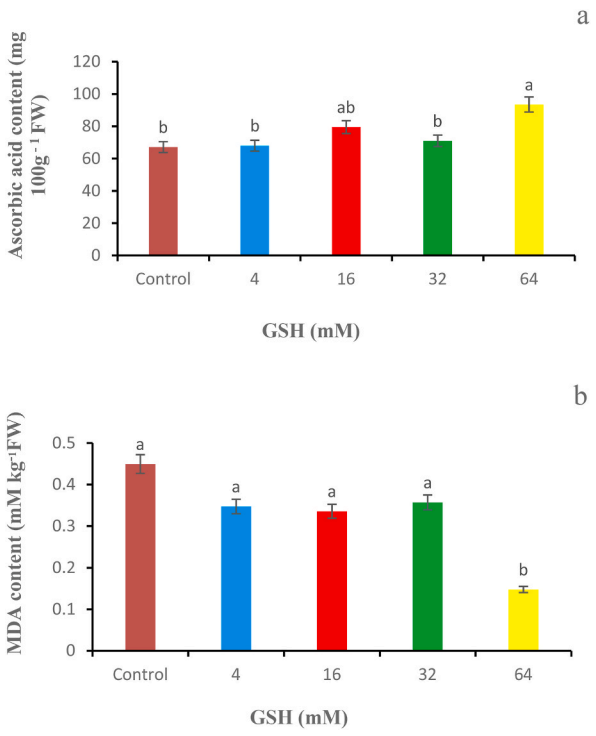


**Fig. 5.** Changes in ascorbic acid (a) and MDA content (b) in Sabrina strawberry fruits treated with L-glutathione during postharvest storage.

### 3.4. MDA and soluble protein content

Based on the obtained results, the effect of GSH treatment on content of MDA was statistically significant at 5 %. Our results revealed that all GSH treatments decrease the level of MDA content and lipid peroxidation. Our results showed that 32 mM GSH treatment had the lowest effect and 64 mM GSH treatment had the highest effect on content MDA in strawberry fruits compared to control during storage (Fig. 5 b).

The interaction effect of storage time and GSH treatment on soluble protein content was statistically significant at 5 %. The obtained results showed that the highest soluble protein content of strawberry fruits was seen in the 16 mM treatment at 10 storage days. The lowest soluble protein content was revealed in 4 mM GSH treatment at 5 storage days compared to the control. Based on results an increasing trend in soluble protein content was observed during storage and under GSH treatments (Fig. 6).

### 3.5. CAT enzyme activity

The interaction of storage time and GSH treatment had a statistically significant on CAT enzyme activity ($P < 0.01$). According to the results, 4 mM GSH treatment at 10 storage days had the lowest and 64 mM GSH treatment at 10 storage days had the highest effect on CAT enzyme activity (Fig. 7). Our results revealed that the strawberry fruits treated with different GSH concentrations, except 4 mM, showed an increasing trend in the CAT activity compared to the control during storage.

### 3.6. SOD enzyme activity

The effect of GSH treatment on the activity of SOD enzyme was significant at 1 % level. Our results indicated that the highest and lowest SOD enzyme activity was seen in 64 and 16 mM GSH treatments, respectively (Table 1). The results showed that treated strawberry fruits with GSH treatments revealed an increasing trend in SOD enzyme activity compared to control during storage (Table 1).

### 3.7. APX enzyme activity

GSH treatment had a statistically significant effect on APX enzyme activity ($P < 0.01$). The obtained data revealed the highest APX enzyme activity of strawberry fruits was observed in 64 mM GSH treatment, whereas, the lowest APX enzyme activity was seen in 32 mM GSH treatment compared to the control during storage (Table 1). Based on the results, the strawberry fruits treated with different concentrations of GSH, except 32 mM, showed an increasing trend in the APX enzyme activity compared to the control during storage (Table 1).

### 3.8. POD enzyme activity

The effect of GSH treatment on the activity of POD enzyme was statistically significant at 1 % level. The obtained data revealed that the highest and lowest POD enzyme activity of strawberry fruits was seen in 64 and 32 mM GSH treatments, respectively compared to the control during storage (Table 1). The results showed that POD enzyme activity of treated fruits had an increasing trend in the 4 and 64 mM GSH treatments, whereas its activity in the 16 and 32 mM GSH treatments decreased compared to the control. It should be noted that 4, 16 and 32 GSH treatments had no statistically significant difference with control (Table 1).

### 3.9. PAL enzyme activity

GSH treatment had a statistically significant effect on PAL enzyme activity ($P < 0.01$). Our results showed that 4 mM GSH treatment had the lowest effect and 64 mM treatment had the highest effect on PAL enzyme activity of strawberry fruits compared to the control

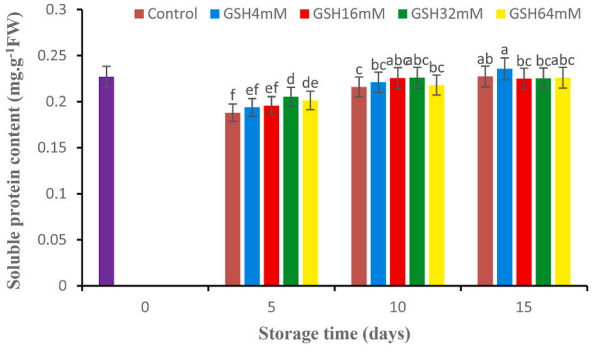


**Fig. 6.** Interaction effect of storage time and L-glutathione treatment on soluble protein content of Sabrina strawberry fruits.

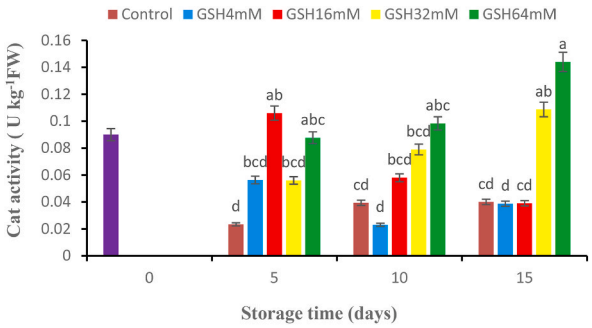


**Fig. 7.** Interaction effect of storage time and L-glutathione treatment on CAT activity of Sabrina strawberry fruits.

**Table 1**
Changes in SOD, APX, POD, and PAL activity of Sabrina strawberry fruits treated with L-glutathione during storage time.

| | Control (0) | 4 | 16 | 32 | 64 (mM) |
|---|---|---|---|---|---|
| SOD activity (U kg-1) | $0.463 \pm 0.93^{bc}$ | $0.488 \pm 0.11^{abc}$ | $0.440 \pm 0.65^{c}$ | $0.524 \pm 0.34^{ab}$ | $0.55 \pm 1.5^{a}$ |
| APX activity (U kg-1) | $0.016 \pm 0.48^{b}$ | $0.028 \pm 0.23^{b}$ | $0.036 \pm 1.03^{b}$ | $0.028 \pm 0.09^{b}$ | $0.065 \pm 0.08^{a}$ |
| POD activity (U kg-1) | $0.175 \pm 0.87^{b}$ | $0.203 \pm 0.27^{b}$ | $0.155 \pm 1.1^{b}$ | $0.100 \pm 0.87^{b}$ | $0.328 \pm 0.45^{a}$ |
| PAL activity (nmol/g/min) | $5289.6 \pm 0.67^{d}$ | $5474.9 \pm 0.97^{cd}$ | $6118.2 \pm 0.69^{b}$ | $6070.3 \pm 1.2^{bc}$ | $6865.2 \pm 0.87^{a}$ |

Means in a row with different letters are significantly different from one another ($p < 0.05$).

during storage (Table 1) According to the results, with increasing GSH concentration, the PAL activity was increased in treated fruits compared to the control (Table 1).

## 4. Discussion

Glutathione (GSH) plays an important role in cells including signal transduction of hormones or molecules and protection of intracellular protein thiols. GSH also plays a role in redox reactions, amino acid membrane transport, and ROS scavenging [9,26,27]. Postharvest application of GSH on the strawberry fruits acts as a barrier and reduces transpiration losses. The softening of the cell walls polysaccharides in strawberry fruits occurred via degradation of the middle layer and reduction of pectin during postharvest storage. According to our findings, strawberry fruits stored for a long time show more weight loss. Therefore, storage time has a direct relationship with the weight loss of the fruits, which can be caused by increasing respiration rate and higher water loss. Fruit weight loss is a main indicator that reflects the respiration rate and evaporation of moisture between the fruit and the surrounding air. Previous studies showed that fruits treated with various compounds and kept in cold storage have a lower weight loss due to the reduction of the stomata opening and the resistance to water vapour diffusion [28]. GSH treatment also prevents water vapour diffusion by reducing cell membrane permeability and stomata opening, consequently decreasing the weight loss of the fruits. It has been previously reported that GSH prevented further weight loss in okra [29], which is consistent with our results. The decrease in fruit weight loss by GSH can be due to the removal of free radicals and the decrease in respiration.

Antioxidant compounds have a synergistic relationship with each other, for example, thiol group compounds such as glutathione contribute to regenerating phenolic compounds by losing their electrons and causing an increase in these antioxidant compounds [30]. The data of our research showed that the storage time had no significant effect on the antioxidant activity. As in previous studies, such a result was obtained [26]. In recent studies, it has been shown that the application of exogenous GSH is beneficial to higher plants under different environmental stresses because it increases the physiological properties and antioxidant defence system [11,12]. The obtained results revealed that the application of GSH on strawberry fruits increased the TP and TF content. As it has been found in previous studies the use of GSH on fruits increases the post-harvest life and leads to improvement of TP and TF [13], which is consistent with our results. Also, Li et al. [29] reported that the postharvest treatment of GSH in okra improved the content of total phenol.

GSH is found in different plant tissues in concentrations of 2–3 mM and plays an important role in enzyme regulation, cell differentiation, cell signalling and cell death, and is considered an antioxidant [31]. Postharvest GSH application on strawberry fruits through enhancing antioxidant compounds leads to increased antioxidant capacity [13]. On the other hand, GSH through a direct effect on antioxidants of fruits and consequently human nutrition leads to increases in human health. Our results also showed that postharvest GSH application increases antioxidant activity and TA content. It has been determined that GSH scavenges ROS in plant cells, which is a substrate for AsA-GSH cycle enzymes, and produces AsA as a water-soluble antioxidant [32]. GSH is reduced by affecting the glutathione-ascorbate cycle and increases and regenerates ascorbate, thus contributing to conserving ascorbic acid [33]. Also, ascorbic acid, as a non-enzymatic antioxidant, along with antioxidant enzymes, has a defensive and antioxidant role in cells. In addition, a positive correlation between them has been observed and a change in each may cause a change in the content of the other [33]. Also, ascorbic acid is a main index to measure the nutritional quality of strawberry fruits and a main antioxidant compound in strawberries, which postharvest GSH application of strawberry fruits maintains ascorbic acid content [13]. Our results also showed

that GSH treatment preserves the content of ascorbic acid during storage.

One of the basic processes of plant secondary metabolism is phenylpropanoid which produces alkaloids, phenols, flavonoids, and lignin, all of which play an important role in many plant functions such as disease resistance [34]. Among the most important natural antioxidants are Phenolic compounds, which are produced directly through the phenylpropanoid [35]. The antioxidant properties of phenolic compounds are related to their chemical structure, which can act as electron or proton donors [36]. One of the key regulatory enzymes in the Phenylpropanoid pathway for the synthesis of phenolic compounds is phenylalanine ammonia lyase (PAL) [16]. The first step in the biosynthesis of phenylpropanoids is the conversion of phenylalanine to trans-cinnamic acid, which is catalyzed by the PAL enzyme and results in the production of secondary metabolites such as lignin, phenols, and flavonoids [37]. Our results revealed that GSH increased the activity of PAL in treated strawberry fruits during storage, and its activity correlated positively with the enhancement of total phenol, flavonoid and anthocyanin content in treated fruits compared to control. Also, several studies have reported that phenolic compounds and ascorbic acid are the main antioxidant compounds in fruits because they improve fruit's antioxidant capacity through the non-enzymatic system and play an important role in eliminating ROS [38,39].

ROS and MDA levels are well-known indicators to determine the oxidative stress rate, it has been suggested that plants enhance stress tolerance partly by modulation of the level of MDA and H2O2 and O2 formation rate [40,41]. Our results showed that GSH treatment reduces the level of MDA in strawberry fruits during storage. It has been observed that the use of GSH in okra reduces the level of ROS and MDA and leads to increased postharvest life in cold storage [27]. GSH can moderate some other antioxidants. Also, GSH acts as a cofactor, interacts with hormones and redox molecules, participates in stress-induced signal transduction, and reduces MDA levels [42,43]. According to the results, postharvest GSH application increases the soluble protein content of strawberry fruits during storage. GSH is effective in the production of defence proteins and different types of kinases. Also, it affects the synthetic pathway enzymes or protein breakdown and plays a main role in protein biosynthesis [14]. The enhancement of soluble protein content under the postharvest treatment of GSH seems to be related to its role in removing ROS to decrease protein degradation and as a result, improve protein biosynthesis [14]. Usually, soluble protein may decrease over time. Glutathione plays a vital role in preserving total soluble protein and preventing the reduction of its content which subsequently leads to an increase in the longevity of fruits during storage [43].

Antioxidant enzymes are considered an important part of the antioxidant capacity in fruits and an increase in their activity leads to the preservation of fruit quality and increased antioxidant activity [44]. The antioxidant defence systems include enzymes that contribute to the ascorbate glutathione cycle such as dehydroascorbate reductase (DHAR), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR) and glutathione reductase (GR), which together with other enzymes such as superoxide dismutase (SOD) and catalase (CAT) play a main role in the reduction of ROS production [44]. SOD provides a frontline of defense against ROS by inhibiting O2 [45]. In this study, it was also observed that antioxidant activity has been improved with the enhancement of SOD activity. In addition, APX plays the main role in the scavenging of $H_2O_2$ in the glutathione ascorbate cycle [46]. Previous studies showed that GSH through acting on the glutathione-ascorbate cycle causes the production of antioxidant enzymes such as CAT, SOD, and APX, which play an important role in protecting cells against free radicals [33]. In line with previous studies, our results also showed that GSH treatments could increase the antioxidant enzymes activities such as CAT, SOD, POD and APX. In a previous study, postharvest application of GSH on peppers was done and the results revealed that the GSH treatments enhance the antioxidant enzymes activity including APX, POD, CAT, and SOD and lead to increased postharvest life of peppers [47]. It seems that antioxidant enzymes have synergistic effects and improve the quality of fruits. Our results are in line with this study. Overall, our findings showed that GSH treatment increases defence systems and antioxidant compounds and can be used as a suitable treatment to improve the storage life of Sabrina strawberry fruits.

## 5. Conclusion

This research investigated the biochemical and antioxidant compounds of strawberry fruits treated with GSH during cold storage. The results showed that GSH treatments reduced MDA accumulation and prevented fruit weight loss. The process of fruit degradation was slowed down by maintaining and increasing the content of soluble protein, antioxidant capacity, TP, TF, TA content and ascorbic acid. On the other hand, the increase of postharvest life and antioxidant capacity of strawberry fruits treated with GSH was maintained by increasing the activity of PAL, CAT, SOD, APX and POD enzymes. Therefore, GSH treatment was effective in maintaining strawberry fruit quality and increasing postharvest life.

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## Data availability statement

Data will be made available on request.

## Additional information

No additional information is available for this paper.

## CRediT authorship contribution statement

**Karim Manda-Hakki:** Investigation, Data curation, Conceptualization. **Hamid Hassanpour:** Writing – review & editing, Validation, Project administration, Methodology, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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