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Postharvest application of thiol compounds affects surface browning and antioxidant activity of fresh-cut potatoes

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Abstract

The aim of this study was to compare the effects of sodium metabisulphite and the thiol compounds, glutathione (GSH), L-cysteine (CYS), and *N*-acetylcysteine (NAC), on the enzymatic browning, antioxidant activities, total phenolic, and ascorbic acid content of potatoes after 1, 24, and 48 hr. Three different concentrations (0.5%, 1.0%, and 2.0%) of each thiol compound were tested. While sulphite solution inhibited polyphenol oxidase as expected, NAC and CYS also decreased its activity. CYS-treated samples exhibited the highest residual thiol content, while the amount of residual thiol in GSH-treated samples was the lowest. The 2.0% NAC and 2.0% CYS solutions were the most effective at increasing antioxidant activity and ascorbic acid content; however, the results of total phenolic content assays were complicated. In summary, solutions containing 2.0% NAC, 1.0% CYS, and 2.0% CYS prevented enzymatic browning and increased the residual thiol content, ascorbic acid, and antioxidant activities of fresh-cut potatoes significantly, but GSH did not significantly inhibit browning.

Practical applications

Fresh-cut potatoes are susceptible to enzymatic browning, which significantly reduces their commercial value. In literature, there have been several methods to protect the enzymatic browning of fruits and vegetables. Among these methods, thiols are good inhibitors of enzymatic browning. So, GSH, CYS, and NAC were used in this study. The outcomes of current work may help to inhibit polyphenol oxidase activity and increase the ascorbic acid content, residual thiol content, and antioxidant activity of fresh-cut potatoes. Both CYS and NAC may be useful alternatives to sulphite anti-browning agents, which may have adverse health effects.

KEYWORDS

antioxidant activity, enzymatic browning, polyphenol oxidase, thiols

1 | INTRODUCTION

Enzymatic browning is one of the most important causes of deterioration in foods. It not only negatively affects the sensory properties of fruit and vegetables; it also impacts their nutritional value (Ioannou & Ghoul, 2013; Jang, Sanada, Ushio, Tanaka, & Ohshima, 2002). Peroxidase (POD, 1.11.1.7) and polyphenol oxidase (PPO, EC 1.10.3.1) are two important oxidoreductases enzymes which are responsible for enzymatic browning. POD can oxidize phenols to quinones in the presence of hydrogen peroxide. PPO can also catalyze two reactions: the hydroxylation of monophenols to diphenols and the oxidation of diphenols to quinones. Polymerization of the resulting quinones leads to the

formation of melanins, which are high molecular weight pigments that produce dark colors in tissues (Martinez & Whitaker, 1995; Queiroz, Mendes Lopes, Fialho, & Valente-Mesquita, 2008; Troiani, Tropiani, & Clemente, 2003). Generally, PPO is located in the plastids and phenolic substances are found in vacuoles. When tissue is damaged, the enzyme comes into contact with the phenolic substrates and browning occurs. Enzymatic browning causes undesirable color, odor, and taste formation and significant loss of nutritional value, affecting the acceptability of products by consumers (Yingsanga, Srilaong, Kanlayanarat, Noichinda, & McGlasson, 2008). Various chemicals have been applied for inhibition of PPO activity, but sulphites are the most effective. However, sulphites can induce serious adverse reactions in sensitive individuals, most of whom already suffer from asthma. The majority of sulphite reactions include dermatologic, respiratory, or gastrointestinal signs and symptoms (Lester, 1995). The Food and Drug Administration (FDA) prohibited the use of the sulphites in meats, in food recognized as a source of vitamin B1, and on fruit and vegetables intended to be served or sold as raw. The acceptable daily intake of sulphites has been established as 0.7 mg/kg of body weight (Gabriela, Loey, Smout, & Hendrickx, 2005; U.S. Food & Drug Administration, 2018). Therefore, researchers have investigated alternative PPO inhibitors, like CYS, kojic acid, 4-hexylresorcinol, citric acid, tartaric acid, or ascorbic acid (Ali, Khan, Malik, Nawaz, & Shahid, 2019; Tsouvaltzis & Brecht, 2017; Vasconcelos et al., 2015). Thiols, which are compounds with the sulfhydryl functional group (-SH) (like GSH, CYS, and NAC), are good inhibitors of enzymatic browning. This inhibition may take place via two different mechanisms: The first one is direct inhibition of PPO due to the affinity of the -SH group for the copper ion at the active site of the enzyme, leading to modification of active site, and inactivation of the enzyme. The reaction of CYS with quinone intermediates to form stable colorless products is the second mechanism of inhibition (Demirkol & Ercal, 2011; Nicolas, Richard-Forget, Goupy, Amiot, & Aubert, 1994).

Potatoes are the third largest food crop in the world, but they are prone to enzymatic browning. When freshly cut potatoes are not treated with inhibitors, pink, brown, gray, or black color can appear within minutes (Cacae, Delaquis, & Mazza, 2002; Sapers, Miller, & Choi, 1995; Wang et al., 2015). In the literature, thiol compounds have been used as anti-browning agents in a variety of other fruit and vegetables (Eissa, Fadel, Ibrahim, Hassan, & Elrashid, 2006; Friedman & Bautista, 1995; Wu, 2014). However, the present study represents the comparison of thiol compounds (GSH, CYS, and NAC) as enzymatic browning inhibitors in this essential food crop. Further, to the best of our knowledge, this is the first investigation of residual thiol contents, antioxidant activity, and ascorbic acid contents in thiol-treated fresh-cut potatoes during 48 hr of storage.

2 | MATERIALS AND METHODS

2.1 | Samples

Potato tubers (*Solanum tuberosum* L.) were provided by the Potato Research Institute (Niğde, Turkey) in March 2018. Potatoes were harvested at the maturity stage. "Agria" was the preferred cultivar because it is one of the most frequently used types of potato for French fries. Potatoes were stored at 10°C, but they were kept at room temperature for 15 days for reconditioning before analysis.

2.2 | Reagent and chemicals

N-(1-pyrenyl)-maleimide (NPM), 2,2-diphenyl-1-picrylhydrazyl (DPPH), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), neocuproine, copper(II) chloride, ammonium acetate, TRIS hydrochloride, L-serine, diethylenetriaminepentaacetic acid (DETAPAC), 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ), L-ascorbic acid, pyrocatechol, polyvinylpyrolidone (PVP-40), GSH, CYS, and NAC were supplied from Sigma (St. Louis, MO). Sodium metabisulphite, hydrochloric acid, Folin-Ciocalteu phenol reagent, sodium carbonate, iron(III) chloride hexahydrate, sodium acetate, metaphosphoric acid, formic acid, potassium phosphate dibasic, and Triton X-100 were purchased from Merck (Darmstadt, Germany). Acetonitrile, methanol, acetic acid, ascorbic acid, boric acid, and orthophosphoric acid [all high-performance liquid chromatography (HPLC) grade] were provided from Fisher (St. Louis, MO).

2.3 | Preparation of fresh-cut potatoes and anti-browning solutions

Potato tubers were peeled, washed with tap water, and then, they were cut into sticks (9 mm × 9 mm × 50 mm) by an industrial slicer (Emir, Turkey). A ruler and a knife were used to ensure the resulting sticks were consistent in size. Potatoes were divided into five groups: GSH, CYS, NAC, sodium metabisulphite, and untreated samples. Solutions of each thiol compound were prepared at different concentrations (0.5%, 1.0%, and 2.0%, w/v) at 25°C. Sodium metabisulphite solution (1.0%) was used as a benchmark. Five potatoes were used for each treatment and 100 g (±2 g) of potato sticks were randomly selected, then they immersed in 300 ml of the appropriate solution for 1 min. Untreated samples were left as control. After removal from the solution, the potato sticks were allowed to air dry for 1 hr. The sticks were stored at room temperature for 24 and 48 hr and then, kept at −18°C until analysis. Triplicate experiments were performed.

2.4 | Total color change

The surface color change of fresh-cut potatoes was quantified using a Colorimeter PCE-CSM 7 (PCE Instruments, UK) to measure CIELAB color values (*L*, *a*, and *b*). The total color change (ΔE) was calculated using the CIELAB color difference formula $\Delta E = [(L_t - L_{initial})^2 + (a_t - a_{initial})^2 + (b_t - b_{initial})^2]^{1/2}$ to quantify browning of the cut potato surface after 24 and 48 hr. The subscript *initial* denotes values measured immediately after cutting, *t* denotes values measured after a given time *t*.

2.5 | Enzyme extraction and polyphenol oxidase activity determination

The PPO activities of samples were detected according to the method of Birdal (2011) with some modifications. Two and a half grams of potatoes were homogenized with a tissue-tearor (Wiggen Hauser D130, Germany) in 5 ml of phosphate buffer (0.05 mol/L, pH 6.3) containing 1% (w/v) polyvinylpyrolidone and 0.25% (v/v) Triton X-100 for 30 s. The homogenate was filtered through four layers of cheesecloth, and the filtrate was centrifuged (Hettich Universal 320R, Germany) at 13,130 ×*g* for 30 min. The collected supernatant was used as the crude enzyme extract. The sample cuvette consisted of 2.8 ml of phosphate buffer, 0.1 ml of substrate solution (0.1 mol/L catechol in phosphate buffer), and 0.1 ml of crude extract. Phosphate buffer was used instead of the enzyme extract in the blank cuvette. PPO activity was determined by measuring the change in absorbance at 420 nm with a UV-visible spectrophotometer (Shimadzu UV-1240, USA). Absorbance measurement was immediately started after adding enzyme solution. The alteration in absorbance for 5 min was monitored. One unit (U) of PPO activity was defined as the amount of enzyme that caused an increase in absorbance of 0.001 per minute.

2.6 | Determination of thiol contents

The GSH, CYS, and NAC contents of potatoes were measured using the chromatographic method developed by Winters, Zukowski, Ercal, Matthews, and Spitz (1995) and modified by Demirkol, Adams, and Ercal (2004). One gram of potato was weighed into 15 ml centrifuge tube and homogenized with tissue-tearor in 5 ml of serine borate buffer solution (100 mmol/L Tris–HCl, 10 mmol/L boric acid, 5 mmol/L serine, and 1 mmol/L DETAPAC, pH 7.0) to block γ-glutamyl transferase activity. The mixture was centrifuged at 13,130 ×*g* at 4°C for 20 min. Collected supernatants (250 μl for GSH, 50 μl for CYS, 50 μl for NAC) were diluted with distilled water as necessary to reach 250 μl. The diluted supernatants were derivatized with 750 μl of NPM (1 mmol/L in acetonitrile) which reacts with free sulfhydryl groups to form fluorescent derivatives. The solution was incubated at room temperature for 5 min, after which 10 μl of 2 N HCl was added to stop the reaction. The tubes were placed in a beaker filled with granular ice at all stages of sample preparation. The derivatized samples were filtered through a 0.45 μm nylon disk into a vial and subsequently injected into a Reliasil ODS-1 C_{18} column (Orochem, Naperville, USA) with 5 μm packing material and 250 × 4.6 mm i.d. in a reverse phase HPLC system. The results were expressed as mmol GSH, CYS, or NAC per kg of dry weight.

The HPLC system (Hitachi, Tokyo, Japan) consisted of an L-2130 pump, L-2300 oven, L-2200 auto sampler, and 5,440 fluorescence detector (λ_{ex} = 330 nm, λ_{em} = 376 nm). The analytes were separated isocratically using a mobile phase consisting of 70% acetonitrile and 30% (v/v) water with pH adjusted to 2.5 by addition of 1 ml of acetic acid and 1 ml of o-phosphoric acid per liter. The calibration curves were constructed by preparing mixed standard solutions, each containing a known concentration of GSH, CYS, and NAC in the range of 0 to 2.5 mmol/L. The r^2 values of the curves for each of the thiol compound were 0.994, 0.999, and 0.993, respectively. Limit of detection (LOD) and limit of quantification (LOQ) were determined 0.05 and 0.16 μmol/L for GSH, 0.03 and 0.11 μmol/L for CYS, 0.06 and 0.19 μmol/L for NAC, respectively.

2.7 | Preparation of extracts for total phenolic contents and antioxidant activities

The method of Capanoglu, Beekwilder, Boyacioglu, Hall, and de Vos (2008) was applied to prepare potato extracts for total phenolic content and antioxidant activity analyses. For each extract, 2 g of potato was added to 3 ml of methanol:water (75:25, v/v) solution homogenized with tissue tearor for 3 min. After sonication (Bandelin Sonorex, RK 100H, Germany) for 15 min and centrifugation (13,130 ×*g*, 4°C) for 10 min, the supernatant was collected. Another 3 ml of methanol solution was added to each pellet, and all of the above steps were repeated. The supernatants from the first and second water:methanol extractions were then combined, and the final volume was adjusted to 10 ml.

2.8 | Determination of antioxidant activities and total phenolic contents

DPPH radical scavenging activities of samples were determined according to the procedure of Brand-Williams, Cuvelier, and Berset (1995) with some modifications. Samples were prepared by adding 200 μL of extract to 3 ml of 0.051 mmol/L DPPH solution, followed by mixing and incubation at room temperature for 30 min. The change in absorbance was measured at 517 nm, spectrophotometrically. A standard curve, (r^2 = 0.991) was constructed by plotting the absorbance values versus the concentration of standard Trolox solutions. The results were presented in g Trolox equivalence per kilogram in dry weight.

Ferric reducing antioxidant power (FRAP) values were determined using the method described previously by Benzie and Strain (1996). Briefly, 100 μL of extract, 1.2 ml of distilled water, and 1.8 ml of FRAP reagent were mixed and incubated at 37°C for 15 min. FRAP reagent consisted of 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ, and 20 mmol/L $FeCl_3.6H_2O$ mixed in a ratio of 10:1:1 (v/v), respectively. The absorbance at 593 nm was recorded with the spectrophotometer. The calibration graph was constructed using standard $FeSO_4$ solutions (r^2 = 0.995). The results were given as $FeSO_4$ equivalent in g/kg dry weight.

The cupric ion reducing antioxidant capacity (CUPRAC) assay was performed according to the method developed by Apak, Güçlü, Ozyürek, and Karademir (2004). In a test tube, 1 ml each of 10^{-2} mol/L copper(II) chloride (in distilled water), 7.5 × 10^{-2} mol/L neocuproine (in 96% ethanol), and ammonium acetate buffer solution (in distilled water, pH 7.0) were mixed. Then, a 1.1 ml aliquot of extract diluted in distilled water was added to give a final volume of 4.1 ml. Samples

were incubated at room temperature for 1 hr. The absorbance was determined spectrophotometrically at 450 nm, and the results were expressed as Trolox equivalent in g/kg dry weight ($r^2 = 0.998$).

For total phenolic content, 100 μL of sample extract was mixed with 0.2 ml of Folin–Ciocalteu reagent and 2 ml of distilled water, and then, the mixture was incubated at room temperature for 3 min. After this, 1 ml of 20% (w/v) sodium carbonate solution was added to the mixture, and total phenolic content was determined after incubation at room temperature for 1 hr. The absorbance was measured at 765 nm with a spectrophotometer (Wojdyło, Oszmiański, & Czemerys, 2007). Quantification was done with respect to the standard curve of gallic acid ($r^2 = 0.991$). The results were expressed as g/kg in dry weight.

2.9 | Determination of ascorbic acid content

Ascorbic acid content (AA) was determined using chromatographic method Lee and Coates, (1999). Two grams of potato sticks were homogenized with tissue tearor in 10 ml of metaphosphoric acid solution (2.5%) for 2 min. After homogenization, the tubes were centrifuged at 13,130 ×*g* at 4°C for 15 min. Two milliliters of supernatant were taken to Eppendorf tubes, and they were centrifuged once again at 36,220 ×*g*. Samples were filtrated with 0.45 μm nylon filters. They were injected into a 5-μm C18 column in a reverse phase HPLC system. The HPLC system (Hitachi, Tokyo, Japan), which consists of L-2130 pump, L-2200 autosampler, L-2455 diode array detector, L-2300 oven, was used to determine the ascorbic acid concentration. The diode array detector was operated at wavelength of 244 nm. A reversed-phase Reliasil ODS-1 C18 column (5 μm, 250 × 4.6 mm) (Orochem, Naperville, USA) was utilized for separation. The analytes were separated isocratically using a mobile phase consisting of 0.1% formic acid solution. Calibration curve was plotted with AA standard ($r^2 = 0.999$). Results were expressed as g/kg in dry weight. LOD and LOQ were found 0.62 and 1.86 mg/kg, respectively.

2.10 | Statistical analysis

The results are expressed as mean ± standard deviation. The statistical analyses were performed using SPSS (version 11.5, SPSS Inc., USA). Significant differences were determined using one-way analysis of variance ANOVA followed by Duncan's multiple range test with a significance level of 0.05.

3 | RESULTS AND DISCUSSION

3.1 | Total color change and polyphenol oxidase activity

Appearance is an important factor in consumers' perception of food quality, and the appearance of fresh-cut produce is negatively affected by the color of compounds formed during enzymatic browning. The total color change (ΔE) represents the overall color variations during storage. ΔE resulting from enzymatic browning of potatoes in the presence of GSH, CYS, NAC, and sulphite after 24 and 48 hr are given in Figure 1. The results show that ΔE values were highest for untreated samples at both time points, followed by the sulphite-treated samples. The high ΔE of the sulphite-treated samples may be explained by the brightening effect of sulphites, which was also reported by Cárcel, García-Pérez, Sanjuán, and Mulet (2010). With similar measurements for $L_{initial}$, higher L_t values than those of thiol-treated samples (data not shown here) could result in higher calculated ΔE in the sulphite-treated group. In the CIELAB color scale, ΔE value is considered to be perceptible when it reaches the range from 4 to 6 (Cabezas-Serrano, Amodio, Cornacchia, Rinaldi, & Colelli, 2009). In this study, while all of the ΔE values were higher than 4, fresh-cut potatoes treated with NAC and CYS (except 0.5% NAC) showed the least color change at the end of the storage. However, GSH-treated samples exhibited higher ΔE values than the other thiol-treated potatoes during entire storage period. Richard-Forget, Rouet-Mayer, Goupy, Philippon, and Nicolas (1992) explained that effects of thiol compound (CYS) on color change depended on concentration. CYS in sufficient amounts (i.e., CYS-to-phenol ratios greater than 1) prevented color development by trapping *o*-quinones as colorless CYS-quinone products. However, if CYS-to-phenol ratios are less than 1, excess *o*-quinones are formed, which can cooxidize CYS-quinone products. This reaction leads to phenol regeneration and deep color formation. In this research, thiol solutions were prepared at the same weight by volume (w/v). Because of its higher molecular weight, the molar concentrations of GSH were lower than those of the other thiol compounds and may have been low to be effective. Thus, another contributing factor to the increased browning observed in the GSH-treated samples may be the thiol concentrations chosen.

Potatoes are a good source of phenolic compounds, which are synthetized by plants as a protective response to bacteria, fungi, viruses, and insects. Phenolics like chlorogenic acid may serve as substrates for oxidation by PPO to *o*-quinone and other compounds, resulting in browning (Akyol, Riciputi, Capanoglu, Caboni, & Verardo, 2016). As shown in Figure 1, the PPO activity of untreated potato samples increased throughout 48 hr of storage, but sulphite treatment totally inhibited the PPO activity as expected. Thiol-treated potatoes were found to have significantly lower enzyme activity than untreated samples during storage (except GSH 0.5% and 2.0% after 24 hr). It is apparent that when the effects on PPO activity were compared, GSH solutions showed the least inhibition. In contrast, 1.0% CYS and 2.0% CYS completely inhibited PPO activity after 48 hr. The 2.0% NAC treatment significantly inhibited the PPO activity after 24 hr and, to a lesser extent, after 48 hr. One of the mechanism of CYS to prevent enzymatic browning is direct inhibition of PPO due to the affinity of the -SH group for the copper ion which is located in the active site of the enzyme. In addition, CYS, which traps the quinone formed as a result of enzymatic oxidation, forms colorless CYS-quinone compounds. These cysteinyl adducts have also been proved to be competitive inhibitors of PPO

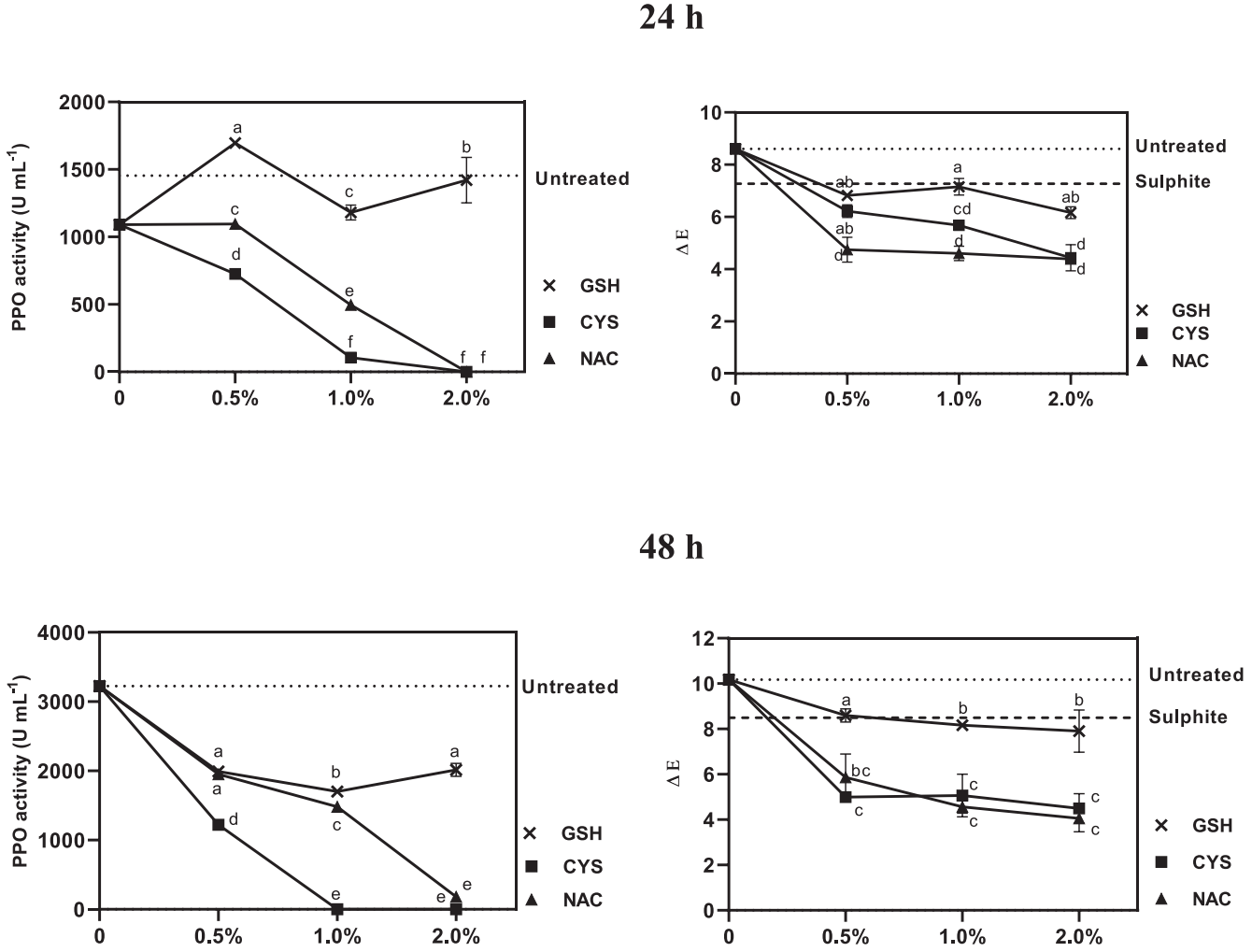

FIGURE 1 PPO activity (U ml^{-1}) and total color change (ΔE) of potato samples after 24 and 48 hr. Values followed by different letters in the same chart are significantly different ($p < .05$). There was no PPO activity in sulphite-treated samples for 24 and 48 hr. Data presented are the means and standard deviations (error bars) of three samples ($n = 3$)

(Altunkaya & Gökmen, 2008; Nicolas et al., 1994). Friedman and Bautista (1995) examined the concentration of GSH, CYS, NAC and sodium bisulphite necessary to inactivate 50% of endogenous PPO in Russet potato suspensions and found them to be 0.18, 0.28, 0.29 and 0.52 mmol/L for sodium bisulphite, CYS, NAC, and GSH, respectively. The relative effectiveness of thiol PPO inhibitors is similar to that reported here. Eissa et al. (2006) reported that CYS and GSH at low concentrations exerted the same inhibitory activity in apple juice at storage temperature 25°C; however, increasing concentration resulted in significant improvement in effectiveness of GSH. Unlike this study, our results showed that CYS in any concentration had higher effect on PPO inhibition. Here, the difference in food matrix may have contributed to the discrepancy between our results and theirs. Each type of food may need to be evaluated separately before drawing general conclusions about PPO inhibition by thiol compounds. In addition, the reaction of thiols with dehydroascorbic acid and carbohydrates and the binding of PPO to potato starch granules may also contribute to the observed differences in inhibition of enzymatic browning (Friedman & Bautista, 1995).

3.2 | Thiol contents

The sulfhydryl (-SH) functional group of thiol compounds contains the most reactive and reduced form of biological sulphur. Also known as "mercaptans," thiol compounds protect cells from many kinds of oxidative damage. GSH, CYS, and NAC are important thiol compounds in a variety of biological applications. They have been used to increase the antioxidant capacity of foods and as mucolytic agents (Mokhtari, Afsharian, Shahhoseini, Kalantar, & Moini, 2017; Öztürk, Cerit, Mutlu, & Demirkol, 2017). In this study, thiol compounds were used as anti-browning agents: fresh-cut potatoes were dipped in GSH, CYS, and NAC solutions at three different concentrations (0.5%, 1.0% and 2.0%). Figure 2 shows sample chromatograms from a mixed standard (GSH, CYS, and NAC) and 2.0% CYS-treated potato. The separation and detection of thiol compounds via HPLC was facilitated by derivatization with NPM (Demirkol et al., 2004). The residual thiol contents of samples after 1, 24, and 48 hr are given in Table 1. Although no CYS was detected in untreated sample, GSH and NAC were present at 0.01 and 0.02 mmol/kg, respectively.

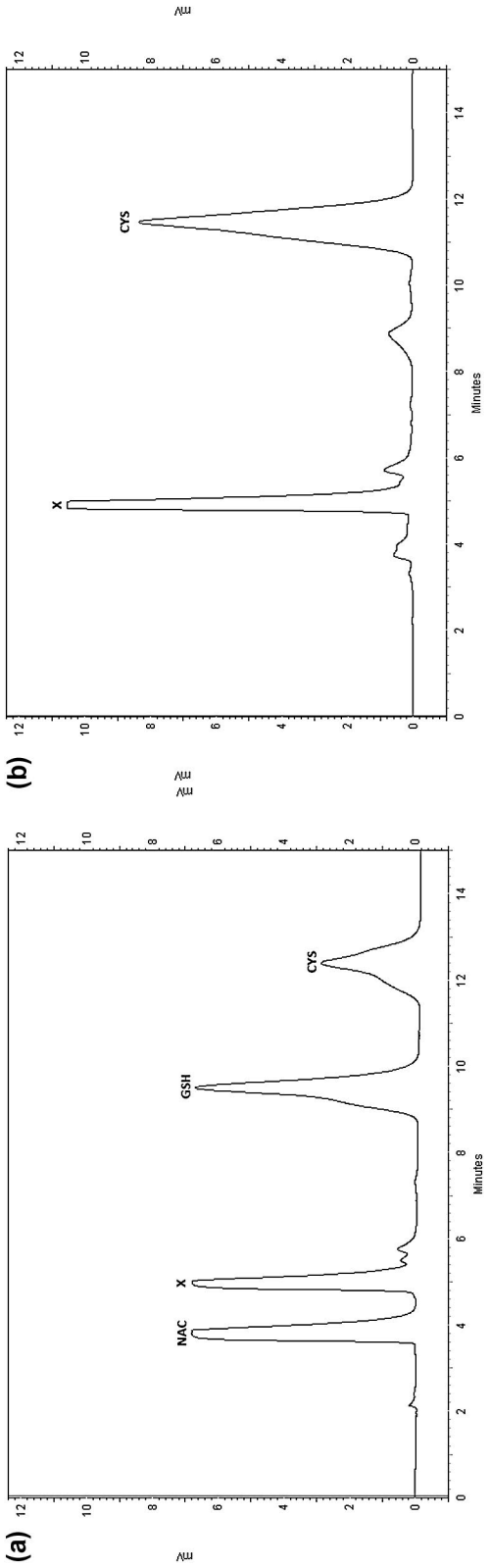

FIGURE 2 Chromatogram of NPM-derivatized mix standard showing GSH, CYS, NAC (a) and CYS 2.0%-treated sample (b). X represents the NPM hydrolysis peak

Stiller, Dancs, Hesge, Hoefgen, and Bánfalvi (2007) detected both CYS and GSH in potatoes. However, they used freshly harvested tubers, which may exhibit higher levels of GSH than the stored potatoes used in this study. In addition, the variety of potato could also affect thiol levels. One hour after treatment, samples contained 0.27 mmol/kg GSH, 21.88 mmol/kg CYS, or 13.56 mmol/kg NAC, respectively. Statistical analysis was applied separately for each thiol compound due to difference in chemical structures. The residual thiol contents throughout storage were found to be directly proportional to applied concentration in all groups. However, the GSH concentration after all time points was lower than those of CYS and NAC. Similar results were observed by El Hosry, Auezova, Sakr, and Hajj-Moussa (2009), when GSH was added to white wine at different concentrations, there was a considerable reduction (up to 73%) in GSH levels even on day 0. GSH is sensitive to oxygen and is easily oxidized during storage, preparation, or processing. It was reported that the significant losses in GSH content were observed in the thermal processing of tomato and ginger samples, and the storage of white wine during 8 months of bottle aging (Demirkol & Ercal, 2011; Ferreira-Lima, Burin, Caliari, & Bordignon-Luiz, 2016; Gümüşay, Borazan, Ercal, & Demirkol, 2015).

3.3 | Antioxidant activities, total phenolic, and ascorbic acid contents

Antioxidant activities of potato extracts were determined with three different spectrophotometric assays: DPPH scavenging activity, FRAP, and CUPRAC. According to the results shown in Table 2, all treated samples were significantly higher DPPH scavenging activity compared to control after 24 hr. In addition, higher-concentration thiol treatments tended to correspond to more DPPH scavenged, except for GSH-treated samples after 48 hr. The extract from the 2.0% NAC solution scavenged the most DPPH, (1.89 g/kg trolox), even more than the sulphite after 24 hr. The FRAP values of all samples after 1, 24, and 48 hr ranged 1.20–13.54, 0.95–5.91, and 0.62–1.83 g/kg $FeSO_4$, respectively (Table 2). The highest FRAP value was determined in the 2.0% NAC-treated sample for all storage periods. After 24 hr, untreated samples had the lowest FRAP values, as well as the 1.0% and 2.0% GSH and 0.5% CYS after 48 hr. Because the residual amount of GSH was so low, antioxidant activities of GSH-treated samples were significantly lower than that of CYS and NAC. Gacche, Shete, Dhole, and Ghole (2006) investigated the affinity of CYS toward DPPH in order to evaluate its antioxidant potential. They demonstrated that CYS had a high affinity toward DPPH and antioxidant activity increased with the increase in CYS concentration similar to that reported here. In general, the relative antioxidant activities determined by DPPH scavenging and FRAP are in agreement. The 2.0% CYS, 2.0% NAC, and 1.0% CYS-treated samples yielded the highest CUPRAC values among all samples (Table 2). Correspondingly, these samples also had the highest thiol content. Güngör, Özyürek, Güçlü, Çekiç, and Apak (2011) compared the

TABLE 1 Total phenolic, ascorbic acid, and thiol content of samples

	Total phenolic content (g/kg GAE)			Ascorbic acid content (g/kg)			Thiol content (mmol/kg)		
Treatments	**1 hr**	**24 hr**	**48 hr**	**1 hr**	**24 hr**	**48 hr**	**1 hr**	**24 hr**	**48 hr**
GSH 0.5%	2.17 ± 0.16^{g}	1.48 ± 0.02^{gh}	1.55 ± 0.08^{b}	0.38 ± 0.03^{de}	0.09 ± 0.01^{g}	nd	0.01 ± 0.00^{b}	0.02 ± 0.00^{c}	0.01 ± 0.00^{b}
GSH 1.0%	3.05 ± 0.04^{bcd}	1.56 ± 0.06^{g}	1.10 ± 0.06^{ef}	0.49 ± 0.01^{d}	0.27 ± 0.01^{f}	nd	0.02 ± 0.00^{b}	0.03 ± 0.00^{b}	0.01 ± 0.00^{b}
GSH 2.0%	2.74 ± 0.14^{de}	1.95 ± 0.03^{d}	1.25 ± 0.08^{d}	0.60 ± 0.04^{c}	0.59 ± 0.01^{cd}	0.14 ± 0.02^{e}	0.27 ± 0.00^{a}	0.08 ± 0.00^{a}	0.02 ± 0.00^{a}
CYS 0.5%	2.35 ± 0.34^{fg}	2.31 ± 0.06^{b}	0.98 ± 0.01^{g}	0.74 ± 0.07^{ab}	0.25 ± 0.02^{f}	0.05 ± 0.01^{e}	4.18 ± 0.13^{c}	0.14 ± 0.01^{c}	0.02 ± 0.00^{c}
CYS 1.0%	2.81 ± 0.08^{cde}	1.84 ± 0.03^{de}	1.30 ± 0.03^{cd}	0.82 ± 0.04^{ab}	0.66 ± 0.02^{bcd}	0.64 ± 0.04^{c}	13.20 ± 0.34^{b}	3.78 ± 0.42^{b}	1.17 ± 0.03^{b}
CYS 2.0%	3.13 ± 0.21^{bc}	1.76 ± 0.08^{ef}	1.03 ± 0.07^{fg}	0.76 ± 0.00^{ab}	0.76 ± 0.00^{a}	0.95 ± 0.00^{b}	21.88 ± 0.49^{a}	8.19 ± 0.67^{a}	3.15 ± 0.08^{a}
NAC 0.5%	2.68 ± 0.11^{ef}	1.67 ± 0.11^{f}	1.27 ± 0.07^{d}	0.61 ± 0.07^{c}	0.47 ± 0.00^{e}	0.36 ± 0.06^{d}	2.09 ± 0.17^{c}	0.37 ± 0.06^{c}	0.02 ± 0.00^{b}
NAC 1.0%	2.57 ± 0.16^{ef}	1.43 ± 0.10^{h}	1.75 ± 0.06^{a}	0.75 ± 0.02^{ab}	0.58 ± 0.09^{d}	0.74 ± 0.05^{c}	6.73 ± 0.08^{b}	0.76 ± 0.02^{b}	0.05 ± 0.01^{b}
NAC 2.0%	5.13 ± 0.06^{a}	2.50 ± 0.08^{a}	1.37 ± 0.07^{c}	0.71 ± 0.03^{bc}	0.69 ± 0.02^{ab}	1.17 ± 0.02^{a}	13.56 ± 0.20^{a}	6.40 ± 0.41^{a}	1.72 ± 0.10^{a}
Sulphite	3.29 ± 0.15^{b}	2.14 ± 0.04^{c}	1.62 ± 0.04^{b}	0.86 ± 0.04^{a}	0.67 ± 0.03^{abc}	1.10 ± 0.06^{ab}	nd	nd	nd
Untreated	2.65 ± 0.031^{ef}	1.84 ± 0.05^{de}	1.14 ± 0.03^{e}	0.32 ± 0.08^{e}	0.24 ± 0.01^{f}	nd	0.01 ± 0.00; 0.02 ± 0.00	nd	nd

Note: Values followed by the different letter are significantly different for each storage period ($p < .05$). Data presented are the means and ± standard deviation of three samples ($n = 3$). nd: not detected. Statistical analysis was applied separately for each thiol compound. Untreated sample contained 0.01 μmol/kg GSH and 0.02 μmol/kg NAC, respectively.

TABLE 2 Antioxidant activities of samples

	DPPH scavenging activity (g/kg Trolox)			FRAP values (g/kg $FeSO_4$)			CUPRAC values (g/kg Trolox)		
Treatments	**1 hr**	**24 hr**	**48 hr**	**1 hr**	**24 hr**	**48 hr**	**1 hr**	**24 hr**	**48 hr**
GSH 0.5%	0.33 ± 0.04^{fg}	0.32 ± 0.00^{f}	0.25 ± 0.01^{d}	1.45 ± 0.08^{g}	1.02 ± 0.02^{gh}	0.80 ± 0.03^{g}	8.42 ± 0.10^{ab}	4.33 ± 0.07^{ef}	2.69 ± 0.01^{e}
GSH 1.0%	0.42 ± 0.05^{e}	0.43 ± 0.00^{e}	0.19 ± 0.01^{f}	1.68 ± 0.09^{fg}	1.28 ± 0.04^{f}	0.62 ± 0.04^{h}	7.27 ± 0.05^{cde}	4.46 ± 0.07^{e}	2.45 ± 0.08^{f}
GSH 2.0%	0.55 ± 0.06^{d}	0.70 ± 0.03^{c}	0.16 ± 0.01^{h}	2.36 ± 0.35^{ef}	1.89 ± 0.10^{d}	0.68 ± 0.01^{h}	8.96 ± 0.08^{a}	4.84 ± 0.09^{d}	2.39 ± 0.11^{f}
CYS 0.5%	0.41 ± 0.04^{ef}	0.34 ± 0.00^{f}	0.21 ± 0.01^{ef}	1.20 ± 0.11^{efg}	1.31 ± 0.07^{f}	0.66 ± 0.04^{h}	6.79 ± 0.05^{ef}	5.55 ± 0.05^{d}	2.88 ± 0.06^{d}
CYS 1.0%	0.43 ± 0.01^{e}	0.44 ± 0.01^{e}	0.31 ± 0.02^{c}	2.41 ± 0.19^{e}	1.32 ± 0.08^{f}	1.04 ± 0.06^{e}	8.07 ± 0.29^{bc}	6.03 ± 0.09^{b}	3.75 ± 0.10^{a}
CYS 2.0%	0.53 ± 0.02^{d}	0.72 ± 0.00^{c}	0.42 ± 0.00^{b}	2.23 ± 0.40^{ef}	2.10 ± 0.06^{c}	1.16 ± 0.09^{d}	7.22 ± 0.44^{cde}	6.58 ± 0.07^{a}	3.84 ± 0.05^{a}
NAC 0.5%	0.65 ± 0.05^{c}	0.34 ± 0.02^{f}	0.23 ± 0.01^{de}	3.30 ± 10.93^{d}	1.17 ± 0.03^{fg}	0.91 ± 0.05^{f}	7.41 ± 0.94^{cd}	4.20 ± 0.14^{f}	2.70 ± 0.02^{e}
NAC 1.0%	1.50 ± 0.08^{b}	0.52 ± 0.01^{d}	0.31 ± 0.02^{c}	7.04 ± 0.70^{a}	1.57 ± 0.01^{e}	1.24 ± 0.03^{c}	6.69 ± 0.20^{efg}	4.70 ± 0.06^{d}	3.28 ± 0.09^{c}
NAC 2.0%	2.03 ± 0.05^{a}	1.89 ± 0.06^{a}	0.71 ± 0.03^{a}	13.54 ± 0.45^{a}	5.91 ± 0.06^{a}	1.83 ± 0.04^{a}	5.99 ± 0.05^{g}	5.74 ± 0.10^{c}	3.50 ± 0.15^{b}
Sulphite	1.49 ± 0.03^{b}	0.92 ± 0.01^{b}	0.71 ± 0.02^{a}	5.34 ± 0.21^{c}	2.35 ± 0.02^{b}	1.73 ± 0.08^{b}	6.18 ± 0.20^{fg}	3.60 ± 0.01^{g}	2.53 ± 0.05^{ef}
Untreated	$0.28.\pm 0.00^{g}$	0.22 ± 0.00^{g}	0.22 ± 0.01^{e}	1.86 ± 0.13^{efg}	0.95 ± 0.03^{h}	0.87 ± 0.02^{fg}	8.37 ± 0.17^{ab}	3.44 ± 0.08^{g}	2.64 ± 0.04^{e}

Note: Values followed by the different letter are significantly different for each storage period ($p < .05$). Data presented are the means and ± standard deviation of three samples ($n = 3$).

TEAC (trolox equivalent antioxidant capacity) values of individual thiol compounds by FRAP and CUPRAC methods. The $TEAC_{FRAP}$ and $TEAC_{CUPRAC}$ values of GSH, CYS, NAC were found to be 0.07, 0.14, 0.48 and 0.57, 0.39, 0.43, respectively. As a result, the study reported that the most appropriate method was CUPRAC, which is sensitive to thiol-type antioxidants. Moreover, Mazor, Greenberg, Shamir, Meyerstein, and Meyerstein (2006) noted that the FRAP assay is insensitive to GSH because GSH cannot reduce Fe^{+3}. They suggested that the -SH group of GSH was a somewhat weaker reductant than that of NAC. Therefore, the highest FRAP and CUPRAC values belonged to different types of thiol solutions in this study. When the residual thiol concentration decreased, the correlation between thiol levels and CUPRAC values disappeared due to the relatively greater amount of other antioxidants like phenolics or ascorbic acid.

Changes in total phenolic content among thiol-treated, sulphite-treated, and untreated samples were investigated and shown in Table 1. Total phenolic content showed a decreasing trend with time. After 1 hr, the highest thiol content belonged to 2.0% CYS-treated sample and the lowest one was in untreated sample. In addition, it was seen that amount of total phenols increased with the concentration of thiol treatments. This may be due to synergism of the thiol compounds with phenols which was explained in the study of Altunkaya and Gökmen, (2008). However, the same trend could not be said for 24 hr and 48 hr of storage. It was expected that the total phenolic content would increase with increasing thiol concentration, but it did not. The action of thiol compounds such as CYS against polyphenol degradation is explained with different mechanisms. The first one is competitive inhibition of enzyme in the presence of CYS. The other one is that coupled oxidation by quinone leads to phenol regeneration. In addition, in the presence of excess CYS, phenolic compounds are fully degraded to their corresponding CYS adducts. Altunkaya and Gökmen, (2009) also reported that although CYS prevented browning in lettuce it had no positive effect for prevention of the degradation of phenolic compounds. Similarly, in this study, although 2.0% CYS-treated samples completely inhibited PPO activity after 48 hr, it did not increase total phenol contents, significantly.

Potato is one of the most widely consumed vegetable in many countries and it is a significant food source for AA. So, the protection of AA of potatoes is important. In this study, the AA content of fresh-cut potato sticks was examined throughout 48 hr of storage. As shown in Table 1, while the AA content of sulphite-treated sample was 0.86 g/kg, it was 0.32 g/kg in untreated sample which means that AA began to decrease as soon as it was sliced. After 24 hr, thiol-treated samples contained up to three times more AA than the untreated sample. At the end of storage, AA totally disappeared in 0.5% and 1.0% GSH-treated potatoes similar to untreated ones. The reason of this may be that the residual thiol amount in GSH-immersed potatoes were significantly lower than those of CYS and NAC. There have been several studies in the literature that thiol compounds protect the loss of ascorbic acid during storage. Sonni, Clark, Prenzler, Riponi, and Scollary (2011) examined the antioxidant action of GSH and AA/GSH pair in a model white wine. High concentration of GSH (860 mg/L) delayed the decay of AA, but on depletion, it again induced the formation of different polymeric pigments. Albrecht, Schafer, and Zottola (1990) analyzed selected cruciferous (including six cultivars of broccoli) and noncruciferous vegetables for AA content after 3 weeks storage at 2°C. It was emphasized that vegetables, which could protect AA better, had high sulfur and GSH content. "Gola" litchi fruit was treated with different concentrations (0.0%, 0.25%, 0.50%, 0.75%, and 1.0%) of CYS and stored at 5°C for 28 d. CYS-treated Gola litchi fruit had 2.25-fold higher AA than control sample. The reason was explained that CYS might suppress the oxidative-degradation of ascorbic acid during storage. In this study, an unexpected increase was observed in the AA of samples treated with CYS 2.0%, NAC 2.0%, and sulphite solution after 48 hr of storage. Cánovas et al. (2020) found a strong and moderately strong relationship between CYS and AA during shelf life of fruit-based baby foods. They explained that CYS reduced the AA to DHA quicker than degradation of DHA to 2,3-diketogulonic acid. It was also demonstrated that sulfhydryl compounds such as GSH, CYS and homocysteine (HCY) have the potential to reduce DHA to AA by Park (2001). Therefore, a possible explanation of our results is that high residual thiol concentration might increase AA in potatoes.

4 | CONCLUSION

In summary, using thiol compounds as anti-browning agents has been reported in the literature, but antioxidant activity, ascorbic acid, and residual thiol content in the potato samples during 48 hr of storage was examined for the first time in the research described here. Overall, results showed that CYS (1.0%, 2.0%) and NAC (2.0%) were the most effective treatments for inhibiting the PPO activity and significantly increasing the thiol contents, ascorbic acid, and antioxidant activity. However, GSH solutions in any concentration were not suitable for preventing the enzymatic browning of potatoes. CYS and NAC which are commercially accessible and inexpensive, can be used as potential sulphite substitutes to reduce enzymatic browning in fruit and vegetables, since sulphites can induce serious adverse reactions in sulphite-sensitive individuals.

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CONFLICT OF INTEREST

There is no conflict of interest for this manuscript.

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