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Gallotannin and *Annona muricata* extract inhibit polyphenol oxidase activity and mitigate browning in *Malus domestica*

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Abstract

Background: The prevention of browning in fruits remains a great concern in the food industry.

Objective: In the present study, we evaluated the anti-browning potentials of gallotannin and *Annona muricata* extract in red apple (*Malus domestica*).

Materials and Methods: Apple slices were made and dipped in the different solutions; distilled water (control), 1 % gallotannin, 1 % *Annona muricata* extract or 1 % ascorbic acid. The treated apple slices were stored at 4 °C for 0, 7 and 14 days and used for the determination of the browning index, polyphenolic content, total protein, polyphenol oxidase and peroxidase activities.

Results: The treatment with gallotannin, *A. muricata* and ascorbic acid reduced browning of apple for storage days 7 and 14. However, only gallotannin treatment preserved the polyphenolic content of the apple slices when compared to the control as well as the other treatment groups. Furthermore, all treatments reduced the activity of the polyphenol oxidase for days 0 and 7 storage, relative to the control. In contrast, the treatments had no effect on the peroxidase activity when compared to the control.

Conclusion: Data support the anti-browning potential of gallotannin, *A. muricata* and ascorbic acid. Further, anti-browning potential of these naturally derived materials may be linked with their inhibitory actions against polyphenol oxidase.

Keywords: enzymatic browning, food processing, natural products, polyphenols

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INTRODUCTION

Apples (*Malus domestica*) are a valuable source of phenolic compounds in the human diet and represent a classic example of fruit susceptibility to enzymatic browning, which is a major problem in the fruit processing industry (Holderbaum et al. 2010, Nenko et al. 2018, Taranto et al. 2017).

The second largest cause of quality loss in fruits and vegetables is enzymatic browning (Ali et al. 2015a, Oms-Oliu et al. 2010). The prevention of browning in fruits remains a great concern in the food industry because the reaction negatively affects the attributes of color, taste, flavor, and nutritional value. It is estimated that more than 50% of fruit market losses is attributable to enzymatic browning (Ali et al. 2015a, 2015b, Whitaker and Lee 1995).

In enzymatic reactions, phenolic compounds are oxidized by polyphenol oxidase (PPO), causing the darkening pigments that scar fresh fruits and vegetables. The browning reaction requires the presence of oxygen, phenolic compounds and polyphenol oxidase (PPO) and is usually initiated by the enzymatic oxidation of monophenols into o-diphenols and quinines. The quinines undergo further non-enzymatic polymerization leading to the formation of pigments. Although enzymatic reaction is beneficial to the color and flavor development of certain food items such as coffee, cocoa and tea, it also impairs the nutritional and appearance quality of fresh-cut produce (Holderbaum et al. 2010, Toivonen and Brummell 2008).

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Several studies have been carried out in the last decades to prevent enzymatic browning (Ali et al. 2015a, Landi et al. 2013, Oms-Oliu et al. 2010). Methods such as use of chemicals, coating, controlled atmosphere and freezing have been used over the decade (Singh and Walker 2006). However, the inadequacy of the control methods necessitates the search for more effective control measures including use of natural anti-browning agents.

Annona muricata is amongst the member of the family of custard apple tree referred to as *annonaceae* and a species of the genus *Annona*. *Annona* is recognized for its edible fruit. The leaf extract of *A. muricata* has been implicated for several biological activities (Adeyemi and Elebiyo 2014, Adeyemi et al. 2017, Pearson et al. 2014). Gallotannin is a polymer of gallic acid molecules and glucose. Gallotannin has potential for several biological activities among which is enzyme inhibition, chelation of substrate metal ions and mineral deprivation (Goel et al. 2005). The present study evaluated the anti-browning potential of gallotannin and *A. muricata* in fresh-cut red apple (*M. domestica*) stored at 4 °C for 0, 7, and 14 days.

MATERIALS AND METHODS

Chemical Reagents

All chemical reagents used were of analytical grade and products of Sigma-Aldrich (St Louis, MO, USA).

Apples

Red apples (*M. domestica*) were purchased at the fruit section of a commercial store at Ilorin in Nigeria.

Anti-browning Agents

Annona muricata

A. muricata leaves were collected from a private residence at Ilorin, Kwara state. They were identified and authenticated at the Herbarium unit of the Department of Plant Biology, University of Ilorin, Nigeria. Voucher number is UIH001/1106.

Preparation of *A. muricata* Extract

A. muricata leaves were air dried and blended to powder using an electric blender. The powdered material (167.32 g) was soaked in 1 L of ethanol for 24 hours after which it was filtered using Whitman's filter paper. The filtrate was freeze dried.

Gallotannin

Gallotannin was a product of Sigma (St Louis, MO, USA).

Experimental Design

The apples (*M. domestica*) were treated with different anti-browning agents between 0 and 14 days. Further details are as follows:

Control group: dipped in distilled water for 2 minutes and stored at 4 °C for 0, 7 and 14 days

Gallotannin group: dipped in 1 % gallotannin for 2 minutes and stored at 4 °C for 0, 7 and 14 days

A. muricata group: dipped in 1 % *A. muricata* for 2 minutes and stored at 4 °C for 0, 7 and 14 days

Ascorbic acid group: dipped in 1 % ascorbic acids for 2 minutes and stored at 4 °C for 0, 7 and 14 days.

Preparation of Apple Homogenates

Apples were manually homogenized using pestle and mortar in potassium phosphate buffer. The homogenized samples were centrifuged at 5000 x g for 20 minutes using a refrigerated centrifuge (Anke TDL-5000B, Shanghai, China).

Determination of Enzymatic Browning

Browning index was determined according to the method described by Jeong et al. (2008) using the supernatants obtained after centrifuging the apple homogenates at 5000 x g for 20 minutes in refrigerated centrifuge (Anke TDL-5000B, Shanghai, China). Briefly 1mL of the supernatant was taken and diluted in 4mL of potassium phosphate buffer. The absorbance of the diluted supernatant was read at 420 nm (Jenway UV spectrophotometer, Staffordshire, UK) against buffer blank. High absorbance values correspond to a greater tissue browning. The experiments were done in triplicate.

Protein Determination

The protein determination was carried out according to Lowry method (Lowry et al. 1951) using a UV spectrophotometer (Jenway, Staffordshire, UK) for absorbance reading at 750 nm.

Assay of Polyphenol Oxidase Activity (PPO; EC 1.14.18.1)

Assay was as described by Furomo and Furutani (2008). Briefly, the assay mixture contained 1.35 mL of 100 mM potassium phosphate buffer, pH 6.8 (assay buffer) and 0.50 mL of 100 mM 4-methylcatechol (4-MC). The assay mixture was incubated for 3 minutes at room temperature in a disposable polystyrene cuvette. 0.15 mL of the apple supernatant was then added to the cuvette containing the assay mixture. The reaction was monitored at 412 nm in a UV spectrophotometer for 2 minutes (Jenway, Staffordshire, UK). Enzyme activity reported as enzyme units (U), was as the quantity of enzyme required to produce 1 micromole of product per minute. A molar absorptivity of 1010 M⁻¹ cm⁻¹ for 4-methyl-o-quinone (Waite 1976) was used.

Assay of Peroxidase Activity (POD; EC 1.11.1.7)

The assay was as described by Chance and Maehly (1951). The assay mixture contained 2.42 mL of assay buffer (2.1 mL of deionized water and 0.32 mL of 100 mM potassium phosphate buffer, pH 6.8), 0.16 mL of 5% hydrogen peroxide, and 0.32 mL of 5% pyrogallol (freshly prepared). The assay mixture was incubated for 3 min at room temperature. After incubation, 0.1 mL of the apple supernatant was added and the POD activity was monitored at 420 nm for two minutes at room

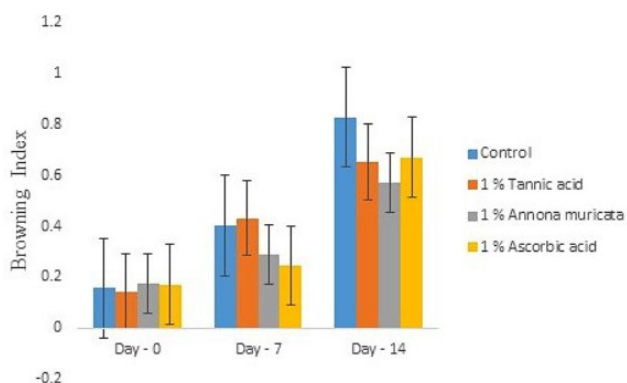


Fig. 1. Browning index of apple (*Malus domestica*) after treatment and storage at 4 °C for 0, 7 and 14 days. Data are presented as mean \pm standard error of mean (SEM), n = 3

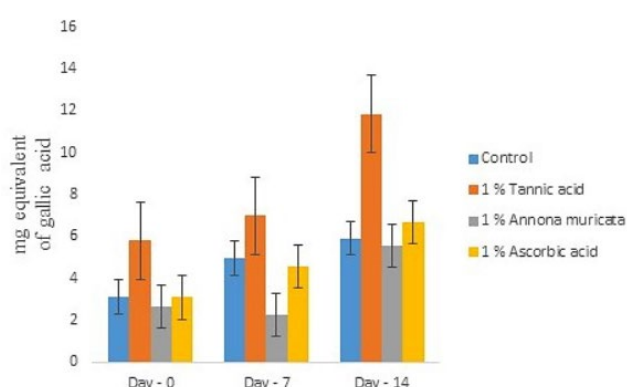


Fig. 2. Total polyphenolic content (TPC) of apple (*Malus domestica*) after treatment and storage at 4 °C for 0, 7 and 14 days. Data are presented as mean \pm standard error of mean (SEM), n = 3

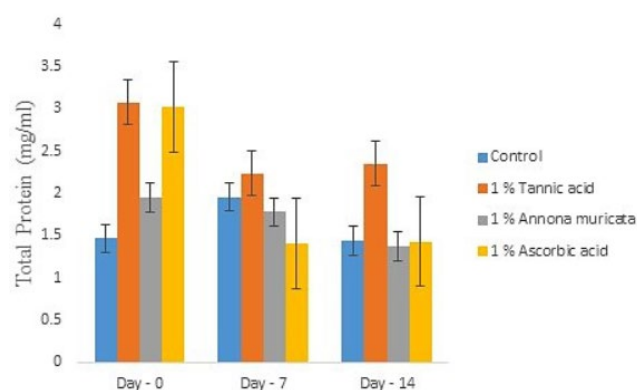


Fig. 3. Total protein concentrations of apple (*Malus domestica*) after treatment and storage at 4 °C for 0, 7 and 14 days. Data are presented as mean \pm standard error of mean (SEM), n = 3

temperature using a UV spectrophotometer (Jenway, Staffordshire, UK). POD activity was expressed as Units of enzyme activity (U) and defined as that amount of enzyme which catalyzes the production of one milligram of purpurogallin in 20 seconds at a pH of 6.0.

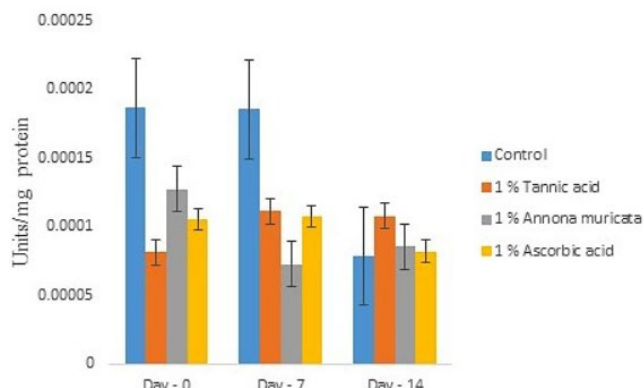


Fig. 4. Polyphenol oxidase activity of apple (*Malus domestica*) after treatment and storage at 4 °C for 0, 7 and 14 days. Data are presented as mean \pm standard error of mean (SEM), n = 3

Determination of Total Polyphenol Content

The total polyphenol content (TPC) was determined according to the method described by Adeyemi and Owoseni (2015). Briefly, 0.1 μ L of apple supernatants was added to 0.4 μ L Na_2CO_3 in a test tube. Equal volume of Folin-ciocalteu reagent was added to the test tube and incubated at room temperature for 60 minutes. Absorbance was measured at 725 nm against the air. The concentration of polyphenols in the supernatant of the apple was derived from the standard curve of gallic acid. The TPC was expressed as gallic acid equivalents (GAE) in mg/100 g material.

Statistical Analysis

Data were analyzed using the one-way ANOVA (GraphPad Software Inc., San Diego, CA) and presented as the mean \pm standard error of mean (SEM). Differences among the group means were determined by the Tukey's test. Mean values at $p < 0.05$ were considered to be significant.

RESULTS

All the treatments reduced the browning index of apple slices stored at 4 °C for 7 and 14 days (**Fig. 1**) when compared to the control. On the contrary, only gallotannin treatment preserved the polyphenolic content of the apple slices following storage at 4 °C for 7 and 14 days (**Fig. 2**). In the same manner, gallotannin treatment preserved the total protein content of the apple slices following storage at 4 °C for 7 and 14 days (**Fig. 3**). Furthermore, treatment of apple slices with 1% - gallotannin, *A. muricata* and ascorbic acid reduced the PPO activity only for storage days 0 and 7 (**Fig. 4**). However, for all storage days, the POD activity was not affected by these treatments (**Fig. 5**).

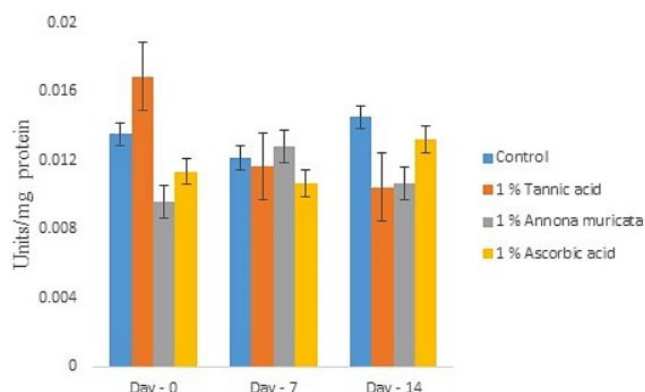


Fig. 5. Peroxidase activity of apple (*Malus domestica*) after treatment and storage at 4 °C for 0, 7 and 14 days. Data are presented as mean \pm standard error of mean (SEM), n = 3

DISCUSSION

In this study, we determined the anti-browning and inhibitory efficacy of naturally derived compounds. We showed that *A. muricata* and gallotannin not only inhibited PPO activity but reduced browning in apple. While several studies have reported anti-browning compounds with PPO-inhibiting activities (Landi et al. 2013, Samanta et al. 2010), the present finding lends credence to the prospects of natural products as alternative anti-browning agents. The reduction in the browning index exhibited by the treatments may be linked to their potential to reduce the activity of PPO. High PPO activity has been shown to correlate well with increased browning index (Jeong et al. 2008, Landi et al. 2013, Taranto et al. 2017). Meanwhile, gallotannin has been implicated for inhibitory potential against several target enzymes (Goel et al. 2005). Additionally, ascorbic acid used as internal control in the present study reduced PPO activity as well as browning index. This is consistent with existing findings (Landi et al. 2013, Samanta et al. 2010). In contrast, all treatments showed no effect on the activity of POD. This is not unexpected as it has been demonstrated that PPO usually has higher activity than POD and thus could largely be responsible for the overall browning index (Ali et al. 2015a, Furumo and Furutani 2008, Queiroz et al. 2008).

This may yet explain why the relative inhibition of the activity of PPO and not that of POD led to the reduction in the browning index. Taken together, the finding may indicate a link between the PPO-inhibitory potential and anti-browning action of gallotannin and *A. muricata* extract. Furthermore, while the treatments reduced the PPO activity as well as the browning index relative to the control, only gallotannin treatment preserved the polyphenolic content and total protein concentration of the apple slices during storage period. The capacity of gallotannin treatment to spare the polyphenolic content of the apple slices from being oxidized by PPO may not be unconnected with their inhibitory potential against PPO activity. Further, correlation analyses of the data revealed negative association between browning index and PPO ($r = -0.926$), browning index and polyphenolic content ($r = -0.843$) as well as between PPO and polyphenolic content ($r = -0.578$). Overall, the present data support the anti-browning potential of gallotannin and *A. muricata*. Also, results implicate the ability of gallotannin and *A. muricata* to inhibit PPO activity. Taken together, data do not only glean new perspective into the biochemical and mechanistic action of these naturally derived materials but warrant further exploration of gallotannin and *A. muricata* as natural and/or biocompatible adjuvant treatment to prevent enzymatic browning in fruits and vegetables.

In conclusion, the prevention of browning in fruits remains a great concern in the food industry. Findings in this work do not only support the anti-browning potential of gallotannin and *A. muricata* extract but demonstrated their potential to inhibit PPO activity, thus providing insights into likely biochemical mechanisms of the anti-browning action. Therefore, the finding in this work suggests additional ways to control enzymatic browning in fruits so as to protect against spoilage.

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