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Effect of Green Tea "*Camellia sinensis*" Extract on Antioxidant Activity of Fresh-cut Apple during Cold Storage

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Abstract

An evaluation of the influence of green tea extract on phenolic contents, flavonoids compounds, antioxidants activity, and lipid oxidation of *Golden delicious* apple cubes during storage at 4°C was the main aim of the paper. The phenolic compounds, flavonoids contents, ascorbic acid, antioxidant activity, and lipid oxidation of apple spices were measured after 0; 15 and 30 days of refrigerated storage. The results revealed that the treatment of apple cubes with 1% (w/v) green tea extract maintained relatively higher levels of total phenolic compounds and flavonoids in comparison with apples treated with water after 15 days of storage. The application of tea extract induced an increase in antioxidant activity estimated at 65% compared to apple control. There was no effect in the levels of MDA in apples treated with tea extract compared to apple cubes treated with ascorbic acid. These results suggest that the green tea extract may be will used as a potential natural antioxidant in the cold storage of apple cubes for a short time.

Keywords: Apple, Green tea extract, Total phenolic contents, Antioxidative activity, Lipid peroxidation

Introduction

In recent years, more attention has been attracted to the benefits of regular consumption of fruits and vegetables on human health. This nutritional value is due to their bioactive molecules, such as fibers, carotenoids, and vitamins and phenolic compounds (Jessica & Mcaleese, 2007). Among the most desired fruits, apple contains the highest amount of antioxidants compounds, especially polyphenols (Boyer & Liu, 2004). This fruit is an extremely perishable foodstuff; a major part of its nutritional value is lost during the agro-food journey, as it undergoes deterioration, rotting, drying out and injuries, due to mechanization during harvesting, transport, and storage (Eissa *et al*., 2014). All of these phenomena can induce an oxidative alteration of its polyphenols (McEvily *et al*., 1992). What causes the appearance of unpleasant flavors and color change. Indeed, the enzymatic oxidation of fruit polyphenols by polyphenoloxidase (PODs) takes place during the loss of cell integrity (for example during cutting) and in the presence of oxygen (Holderbaum *et al*., 2010). Another enzyme, peroxidase, may also be involved in enzymatic browning. PODs oxidize a hydrogen donor to transform it into specific hydrogen peroxide (Robards *et al*., 1999). These reactions are largely responsible for the deterioration of the fruit and encourage the food industry to research and develop preservation processes to ensure high taste, organoleptic and nutritional quality (Prokopov & Tanchev, 2007). Among the processes which provide fruit protection against oxidation is the use of natural antioxidants, these are used to substitute synthetic antioxidants such as ascorbic acid and thus avoid all risks and consequences harmful to consumer health (Hussain *et al*., 2012). Recently, the role of polyphenols in extending shelf life and delaying oxidation has been the subject of numerous studies (Pires *et al*., 2017); this role is often associated with their intrinsic antioxidant properties. Green tea is one of the plants that attract attention due to its richness in polyphenols, mainly catechins (Yang *et al.*, 2018). Indeed, tea polyphenols are widely described by their higher free radical scavenging and metal-chelating capability (Tang *et al*., 2002). However, few studies have investigated the effect of tea polyphenols on the storage of fresh-cut fruit (Soysal, 2009; Wessels *et al*., 2014). Analysing the effects of the phenolic compounds of green tea extract on the antioxidant properties, phenolic compounds and lipid oxidation of fresh cut-apple during cold storage was the main objective.

Materials and Methods

Chemicals

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Folin-Ciocalteu, Gallic acid, ascorbic acid, Quercetin, thiobarbituric acid, and trichloroacetic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). analytical grade solvents and chemicals were employed.

Plant Material

Golden delicious apples (*Malus domestica*) were purchased from the local market; this cultivar was selected because of its wide popularity as a food.

Preparation of Green Tea Extract

Green tea leaves (*Camellia sinensis*) were obtained from a local market. The dried tea leaves were ground to obtain a powder which was used to carry out the extraction by maceration technique. The method of extraction used in this work is described by Anesini *et al.* (2008). Briefly, 10 g of powder was treated with 100 mL of ethanol for 24 h at room temperature and in the dark. The extracts were filtered through Wattman n°1 filter paper. The filtrate obtained was evaporated using a rotary evaporator to obtain a dry extract.

Samples Treatment and Storage Conditions

The selected apples were carefully cleaned with cold water, dried, cut into unpeeled pieces, and prepared as per Chen *et al*.'s (2014) methodology with some enhancements. In this study, the apple pieces were dipped separately in three different solutions for 2h, i.e.: 1% (w/v) green tea extract, 1% ascorbic acid (considered as the positive control), or distilled water (negative control). After treatment, apple cubes were drained on absorbent paper then placed in separated jars and stored at 4°C for 30 days. At each time interval (0, 15, and 30days) during cold storage at 4°C, samples were taken for phytochemical and antioxidative analyses.

Phytochemical Analysis

Determination of Phenolic Compounds

5g of the apple pieces obtained from the different solutions (ascorbic acid, the green tea extract, or distilled water) were homogenized with 50mL of a mixture of pure methanol (49.5 mL) and 37% HCl (1N)). The mixture was ground and then stirred for 30 min in the dark and at room temperature (Chen *et al*., 2014). After the filtration, the total phenolic compounds were estimated after 0, 15, and 30 days of storage as per the Folin-Ciocalteu reaction method (Singleton *et al.,* 1999). Briefly, 500µL of the apple extract was added to 500µL of Folin-Ciocalteu reagent (0.2 N), the mixture was incubated at room temperature for 10 min before 500 µL of sodium carbonate (7.5%, w/v) were added. The reaction mixture was then incubated for 30 min at room temperature and in dark. The absorbance was recorded at 760 nm on a blank without extract using a spectrophotometer (Shimadzu Corporation, Japan). The content of phenolic compounds is shown as mg Gallic acid Equivalent per 100g of dry weight (mg GA/100g d.w).

Flavonoids Contents

The content of the total flavonoids apple pieces was determined by the method of Bahorun *et al*. (1996). A 1mL of the apple extract solution was added to 1mL of Aluminum chloride (2% w/v), after 30 min of incubation, the absorbance was read at 430 nm using a UV-visible spectrophotometer. Total flavonoid contents are presented as mg Quercetine equivalent per 100 g dry weight (mg Q /100g d.w.).

Antioxidants Activities Analysis

Ferric Reducing Antioxidant Power (FRAP) Assay

Iron's (Fe^{3+}) power of reduction in the apple extract was determined according to Yen and Duh, (1993). Methodology 500 µL of the extract at different concentrations were mixed with 500 µL of phosphate buffer solution (0.2 M, pH 6.6) and 500 µL of a potassium ferricyanide solution (1%, w/v). The mixtures were incubated at 50°C for 20 min, after incubation, 500 µL of trichloroacetic acid (10%, w/v) was added to stop the reaction. Finally, 1 ml of the upper layer was mixed with 1mL of distilled water and 500 µL of ferric chloride (0.1%, w/v), the absorbance was measured at 700 nm against a blank. A heightened reaction absorbance corresponds to an increase in the reducing power of the tested extract. The reducing potential of the extract and standards is expressed by the effective concentration values at 50% (EC50).

Ascorbic Acid Determination

The content of ascorbic acid was established by Jacota and Dana (1982), 500 µL of apple extract was added to 200 µL of Folin Ciocalteu's reagent, after incubation in the water bath for 10 min at37°C. The absorbance measurement, of the developed blue color, was performed at a wavelength of 769nm. The intensity of the coloration obtained is proportional to the ascorbic acid content present in the sample. The concentration is expressed in mg/mL and was determined using a calibration curve obtained with 100mg/mL stock solution of ascorbic acid.

Determination of Lipid Peroxidation (TBARS test)

MDA levels were assessed using the method described by Chen *et al*. (2014).10g of apple pieces from different conditions and storage times were homogenized with 25 mL of 5% trichloroacetic acid and then centrifuged for 10 min at 4000xg and 4°C. The collected supernatant (0.5mL) was mixed with 3mM of 0.5% thiobarbituric acid previously dissolved in 1N HCl. The solution of this reaction mixture was heat-treated for 20 min at 95°C in a water bath, then cooled rapidly in cold water for 10 min, and centrifuged for 10 min at 4000xg to clarify the supernatant. Absorbance was measured at 532 nm.

Statistical Analysis

The results obtained were expressed as means ± standard deviation (SD). the STATISTICA software (version 6.1, Stat soft,

Tulsa. OK, USA) was used to analyze the data. The comparison of the means was carried out via ANOVA with a factor, followed by the LSD test. A value of p <0.05 was used as the significance level.

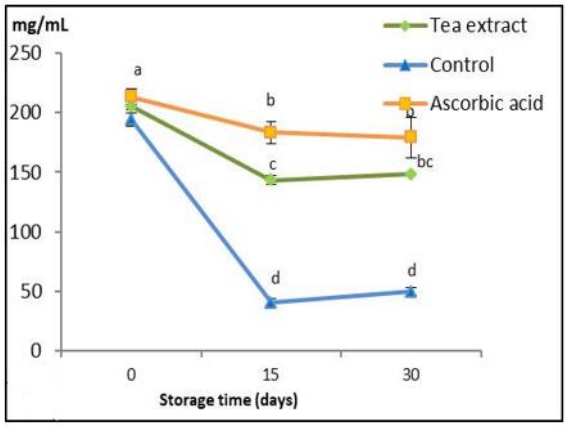
Results and Discussion

Effect of Green Tea Extract on the Total Phenolic Compounds and Flavonoids

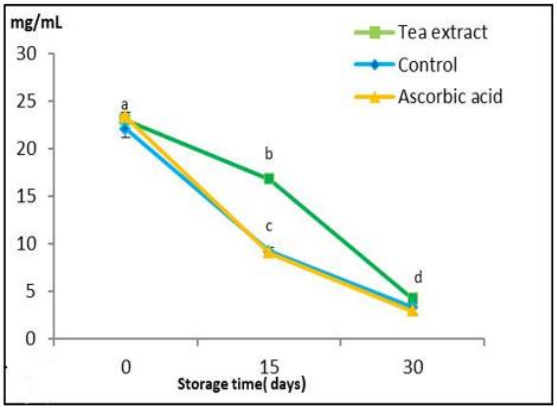
The effect of green tea extract on the contents of total phenols in apple pieces during storage at 4C°is shown in **(Figure 1)**. The results showed that the contents of polyphenols of apple cubes dipped in different solutions decreased as the cold storage increased. However, high concentrations of phenolic compounds were observed between 15 and 30 days of storage, in comparison with apples treated with water; that was reflected by the stability of polyphenols contents at this period. After 30 days of storage, the polyphenols contents of green tea extract-treated apples were 196% significantly higher than control samples.

The flavonoid contents decreased rapidly along with increasing storage time **(Figure 1)**, while the fruits treated with distilled water and ascorbic acid revealed a greater reduction in flavonoids contents in comparison with apple pieces treated with green tea extract. At 15 days of storage, the contents flavonoids of apple cubes treated with green tea extract were 83% and 86% significantly higher than the apples treated with distilled water and ascorbic acid respectively.

The treatment of apple cubes with green tea extract maintained relatively higher levels of phenolic contents throughout refrigerated storage. This finding can be attributed to the phenolic compounds in tea extract added to apples. Indeed, tea is a very important source of antioxidants, in particular catechins and flavonols whose role is the neutralization of free radicals (Cabrera *et al*., 2013). This allows for better storage by inhibiting the oxidation process. These results agree with Chen *et al*.'s (2014) reports, which confirmed that application of tea polyphenols (1%) kept high contents of total phenolic of litchi fruit after 30 days of cold storage.



a)



b)

Figure 1. Effect of green tea extract on the content of total polyphenols (a) and flavonoids (mg/mL) (b) in apple cubes during cold storage.
Different letters (a,b,c,d) indicate significant differences (p<0.05).

Influence of Green Tea Extract on Ascorbic Acid Contents

The contents of ascorbic acid of apple pieces treated with ascorbic acid or green tea extract were reduced during cold storage **(Table 1)**. However, it is interesting to note that the apple pieces treated with green tea extract showed good storage, this is reflected by the ascorbic acid contents stability between 15 and 30 days of storage. On the other hand, our results indicated that the ascorbic acid contents in apples treated with tea extract after 30 days were significantly higher (65%) than that obtained in apples treated with distilled water. The results of this research support previous studies that the application of ascorbic acid or citric acid effectively inhibited the oxidation process of phenolic compounds *Golden Delicious* apples (Pizzocaro *et al.*, 1993) and fresh-cut cantaloupe melon (Lamikanra & Watson, 2001).

Table 1. Influence of tea extract on ascorbic acid contents (mg/mL) in apple cubes during cold storage

Storage time (days)	Treatment		
	Control	Tea extract	Ascorbic acid
0	72.78±1.73^{Aa}	82.83±18.77^{Aa}	323.21±15.81^{Ba}
15	23.72±2.05^{Ab}	40.19±0.44^{Bb}	315.28±31.97^{Ca}
30	22.95±0.11^{Abc}	37.92±8.34^{Bab}	57.86±2.76 ^{Cb}

The values represented are the mean ±SD. Different letters (a,b,c) indicate significant differences (p<0.05).
Different capital and small letters in the same column and row indicate statistically significant differences.

Ferric Reducing Antioxidant Power (FRAP)

The results of the extracts' ferric reducing activity, obtained from apple cubes treated with different solutions are shown in **(Figure 2)** (They are expressed as EC50).

These results revealed that the treatment of apple spices with green tea extract increased significantly ($p<0.05$) the ferric reducing power at 15 days of storage, in comparison with the control samples. However, the apples preserved in green tea extract exhibited a significantly ($p<0.001$) higher antioxidant activity than the fruit treated with water after 30 days storage, which is estimated at 65% in tea extract-treated apples compared with control fruit. It seems that the antioxidant activity can be related to the contents of phenolic compounds, which are protected in the apples treated with green tea extract or ascorbic acid.

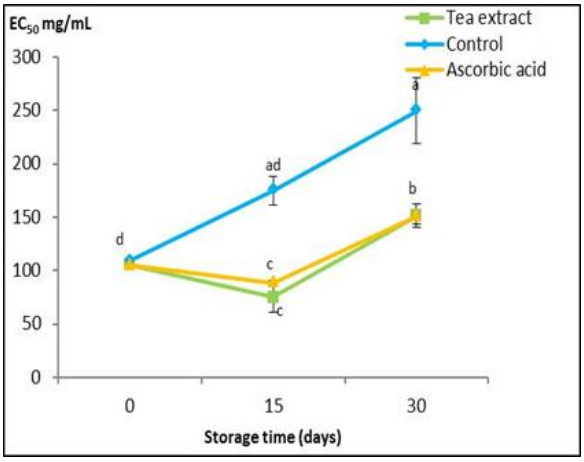


Figure 2. Effect of green tea extract on total antioxidant activity FRAP (EC50) in fresh-cut apple during cold storage. Different letters (a,b,c,d) indicate significant differences ($p<0.05$)

These results are consistent with those obtained by Chen *et al*. (2014), who suggested that the antioxidant activity was assessed by the radical scavenging activity (DPPH°), of litchi fruit is influenced by the content of total phenolic compounds. The authors explained that the antioxidant properties and the total phenolic compounds are much correlated. In addition, they confirmed that the tea polyphenols improved the antioxidant activity by decreasing the reactive oxygen species content in the fruit. Several studies have reported that the catechins in green tea have a more powerful antioxidant activity than that of vitamin C and E (Mukai *et al*., 2005; Yang *et al*., 2018).

Lipid Peroxidation

The results showed that the malondialdehyde (MDA) levels of apple pieces treated with green tea extract were increased speedily during the storage period **(Figure 3)**. Whereas, ascorbic acid-treated samples showed a slight decrease in MDA levels after 30 days of storage. This decrease is estimated at 19% compared to the MDA levels of apples treated with water and tea extract. Indeed, ascorbic acid is a non-enzymatic antioxidant defense system, a reducing agent capable of influencing lipid peroxidation (Ming Lu *et al*., 2010).

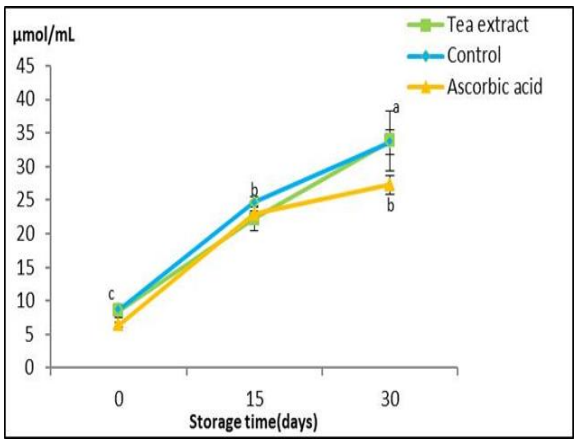


Figure 3. Effect of green tea extract on malondialdehyde content (µmol/mL) in fresh-cut apple during cold storage. Different letters (a,b,c) indicate significant differences ($p<0.05$).

Conclusion

The study has shown that the treatment of apple pieces with green tea extract effectively maintained the phenolic compounds, the flavonoids contents, and antioxidant potential throughout the cold storage period. This natural extract can be used as an alternative substance to a chemical agent for improving food quality during storage.

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Conflict of interest: None

Financial support: None

Ethics statement: None

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