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MiniReview

Potential Application of Ascorbic Acid, Citric Acid and Oxalic Acid for Browning Inhibition in Fresh-Cut Fruits and Vegetables

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Abstract

The market for fresh-cut fruits and vegetables has grown rapidly in recent decades as a result of their freshness, convenience, and human health benefits. However, fresh fruits and vegetables deteriorate very rapidly after processing, especially cut-surface browning resulting from wound-induced physiological and biochemical changes. The application of antibrowning agents is one of the most effective methods for controlling the enzymatic browning reaction in fresh-cut fruits and vegetables. This article reviews the use of nature identical antibrowning agents, which are generally recognized as safe (GRAS) including ascorbic acid, citric acid and oxalic acid for preventing browning in fresh-cut fruits and vegetables. Factors affecting inhibitory efficiency of the antibrowning agents and synergistic effects of the mixtures in various fresh-cut fruits and vegetables are presented.

Keywords: Ascorbic acid, citric acid, oxalic acid, enzymatic browning, fresh-cut fruits and vegetables, polyphenol oxidase

Introduction

Food consumption patterns are rapidly changing all over the world. Consumer food choices have been attributed, in part, to the rise in health problems. Therefore, the positive health impact of nutrients in fruits and vegetables has become one of the consumer's chief concerns. Consumption of fresh fruits and vegetables has been revealed to be beneficial for the alleviation of many degenerative diseases including cancer, heart disease, arthritis, inflammation, immune system decline, brain dysfunction and cataracts [1-5]. In addition to health considerations, the lifestyles of consumers play an important role in consumption patterns and purchase decisions. Currently, consumers increasingly demand convenience foods which are ready to eat or cook. Consequently, fresh-cut fruits and vegetables are emerging as an answer to the consumer's desire for greater convenience in their daily lives. The International Fresh-cut Produce Association defines fresh-cut products as fruits or vegetables that have been trimmed and/or peeled and/or cut into a 100 % usable product that is bagged or pre-packed to offer the consumer high nutrition, convenience and flavor while still maintaining its freshness [6]. However, the practices of fresh-cut processing promote a faster physiological deterioration, biochemical changes, enzyme activity and microbial degradation of the product. These changes in response to wounding have been shown to be the main factor in the loss of aesthetic and nutritional qualities and market value of fresh-cut fruits and vegetables [7]. Cut-surface browning of fresh-cut fruits and vegetables is one of the most fundamental factors affecting acceptability of the products by consumers. Most of them are caused by the action of polyphenol oxidase (PPO) on phenolic compounds released during the process of cutting (*called enzymatic browning*) [8]. Enzymatic browning may be controlled by various methods. Application of antibrowning agents is a popular approach for retarding enzymatic browning in fresh-cut fruits and vegetables. Surface treatments by dipping fresh-cut products in the appropriate antibrowning agents can effectively help to delay discoloration. Nature identical antibrowning agents are a favorite group

because they are generally recognized as safe (GRAS) status and are non-toxic. Several nature identical antibrowning agents extensively used to control excessive browning include ascorbic acid (AA), citric acid (CA) and oxalic acid (OA) that are weak organic acids found in fresh fruits and vegetables [9-11]. This review is focused on the application of AA, CA and OA for inhibiting the enzymatic browning in fresh-cut fruits and vegetables, by describing the factors affecting the antibrowning activity of the compounds and presenting the synergistic effects of the mixtures in various fresh-cut fruits and vegetables.

Quality Parameters of Fresh-Cut Fruits and Vegetables

The quality of fresh-cut fruits and vegetables determines the value to the consumer and is a combination of parameters including appearance (size, shape, color, gloss and defects), texture (firmness, crispness and juiciness), flavor (sweetness, sourness, astringency and bitterness) and nutritional value (vitamins, minerals and dietary fiber). The relative importance of each quality depends on the product. Color is one of the most important attributes affecting the consumer's decision to purchase. However, subsequent purchases depend on the consumer's gratification in terms of texture, flavor and nutritional value of the products [12,13].

Enzymatic Browning of Fresh-Cut Fruits and Vegetables

Enzymatic browning of fresh-cut fruits and vegetables is a crucial limiting factor determining the shelf-life of the products. Enzymatic browning is the discoloration that results from the action of a group of enzymes called PPO. PPO is a copper-containing enzyme predominantly located in the chloroplast thylakoid membranes [14]. PPO is activated as a result of disruption of cell integrity and when the content of the plastid and vacuole are mixed. The PPO structure is composed of two atoms of copper (Cu^{2+}) held closely together by a polypeptide chain. Each atom of Cu^{2+} is tightly ligated to three histidine residues. The state of PPO in plant tissues is distributed as ~ 85 % MET-PPO and ~ 10 - 15 % OXY-PPO forms and is often isolated in the MET-PPO form [15]. The mechanism for enzymatic browning involves the interaction of phenolic compounds with PPO in the presence of oxygen [16]. PPO catalyzes two reactions including hydroxylation and oxidation. Hydroxylation (**Figure 1**) is catalyzed by monophenol monooxygenase (EC 1.14.18.1) to change monophenols to diphenols. The reaction sequence is summarized below:

oxy-enzyme [2 Cu(II)·O_2] + monophenol + $2H^+$ → met-enzyme [2 Cu(II)] + diphenol + H_2O

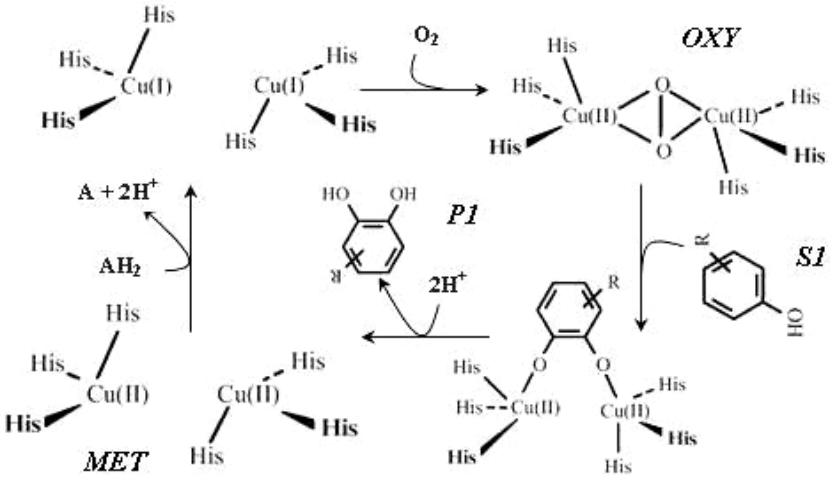

Figure 1 Hydroxylation reaction catalyzed by monophenol monooxygenase. S1 stands for monophenol. P1 stands for diphenol.
Source: Modified from [15,17]

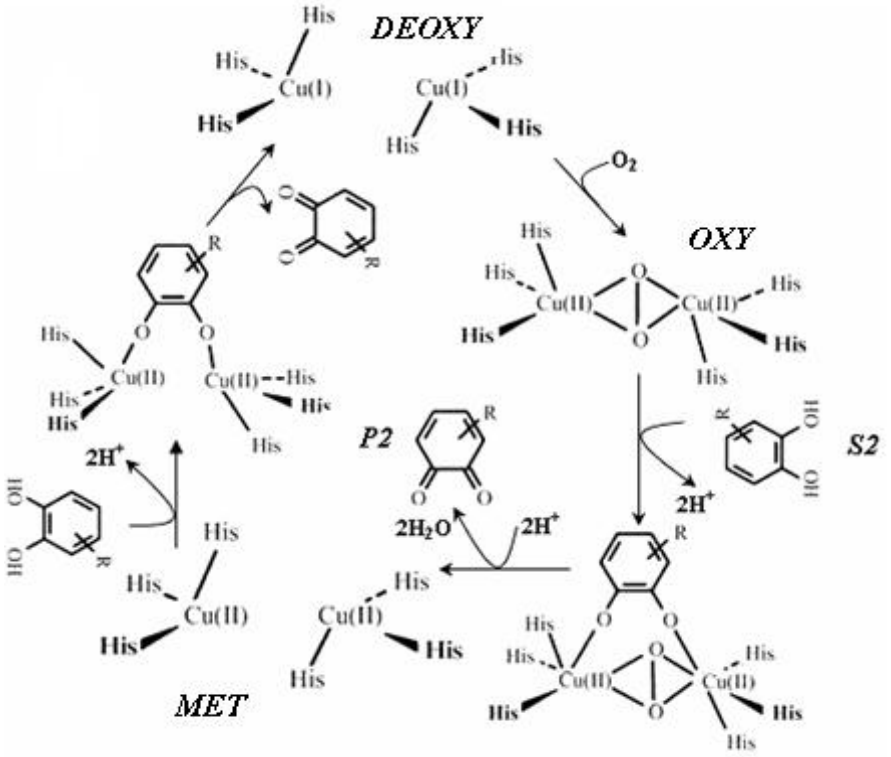

Figure 2 Oxidation reaction catalyzed by oxygen oxidoreductase. S2 stands for diphenol. P2 stands for quinone.
Source: Modified from [15,17]

Oxidation (**Figure 2**) is catalyzed by oxygen oxidoreductase (EC 1.10.3.1) to convert the diphenols to quinones. The reaction sequence is summarized below:

met-enzyme [2 Cu(II)] + diphenol →
deoxy-enzyme [2 Cu(I)] + quinone + $2H^+$

deoxy-enzyme [2 Cu(I)] + O_2 →
oxy-enzyme [2 Cu(II)·O_2]

oxy-enzyme [2 Cu(II)·O_2] + diphenol + $2H^+$ →
met-enzyme [2 Cu(II)] + quinone + $2H_2O$

The hydroxylation reaction is relatively slow and results in colorless products, while the oxidation reaction is relatively rapid and the resultant quinones are colored [18]. Subsequent reactions of the quinones with other molecules such as quinones, phenolic compounds, amino acids and proteins lead to melanin accumulation [19], resulting in the brown or black pigment associated with browning in the cut-surface of the fruits and vegetables. The degree of browning depends on the content and the types of phenolic compounds and PPO activity involved in the reaction. Therefore, the inhibition of enzymatic browning can be achieved by controlling enzymes (PPO), substrates (phenolic compounds) and/or products (quinones).

Antibrowning Agents

Antibrowning agents are compounds that act to prevent the browning reactions. Chemical treatment with the use of antibrowning agents is an effective and frequently employed method for controlling the enzymatic browning in several fresh-cut fruits and vegetables. Antibrowning agents can be divided into six groups including acidulants, reducing agents, chelating agents, complexing agents, enzyme inhibitors and enzyme treatments, based on inhibitory mechanisms [9,14,20,21]. Acidulants, such as citric acid, oxalic acid, tartaric acid, malic acid, phosphoric acid and

hydrochloric acid, retard browning by lowering the pH of the product to minimize the activity of PPO. At pH values below 4, PPO has little activity due to the loss of copper at the active site. Successful browning inhibition by reducing agents, such as ascorbic acid, erythorbic acid, ascorbyl-2-phosphate, ascorbyl-triphosphate and cysteine is attributed to the reduction of quinones back to diphenols or the reduction of Cu^{2+} to mononuclear copper (Cu^{+}) at the PPO active site. Efficiency of chelating agents, such as citric acid, oxalic acid, ethylenediamine tetraacetic acid, sodium pyrophosphate, sodium hexametaphosphate, sporix, chloride and honey, for delaying discoloration is associated with copper chelation at the PPO active site. Complexing agents, such as cyclodextrin and cysteine, prevent browning by entrapping or complexing PPO substrates or products of the reaction. The ability of enzyme inhibitors, such as 4-hexyl resorcinol, kojic acid and cinnamic acid, in browning inhibition is due to having a structure similar to PPO substrates and high affinity with the PPO active site. The effectiveness of enzyme treatments, such as ficin, bromelain and papain, in controlling browning is accomplished by attacking the PPO active site or inhibiting PPO by proteolysis.

Antibrowning Efficiency of AA, CA and OA

AA, CA and OA are weak organic acids widely found in plant tissues [13,19]. AA is a natural component in fresh fruits and vegetables and greatly accepted as an important nutrient for human health [22,23]. CA is the most abundant acid in plants, especially citrus fruits [23,24]. OA is a common constituent of many plants such as asparagus, broccoli, carrots, garlic and spinach [25,26]. These organic acids have been reported often for their antibrowning activity in fresh-cut fruits and vegetables [27-37] and have generally recognized as safe (GRAS) status [38]. However, according to the Thailand Food and Drug Administration, intake of OA should be limited to 378 mg per 1 kg body weight per day, to prevent the reduced absorption of dietary minerals such as calcium, magnesium and potassium. On the other hand, there has been no report on the limit of AA and CA consumption. Antibrowning efficiency of AA, CA and OA in fresh-cut fruits and vegetables relates to two main factors including the types and cultivars of produce, and concentrations of antibrowning agents.

1) Types and Cultivars of Produce

The effectiveness of AA, CA and OA for controlling browning in fresh-cut apples has been reported to differ among apple cultivars. In Liberty apples, 1 % OA showed the highest inhibitory activity on browning followed by 1 % CA and 1 % AA, respectively [9], while 1 % AA possessed better antibrowning efficiency than 1 % CA in Fuji apples [10]. Surprisingly, 1 % CA exhibited higher inhibitory activity on Golden Delicious apples PPO than 1 % AA, which did not inhibit, but activated PPO activity [27]. In fresh-cut mangosteens, dipping in 0.5 % OA was the most effective treatment for retarding browning and inhibiting PPO activity, followed by 0.5 % CA and 0.5 % AA, respectively (**Figure 3-4**). In contrast, no difference in antibrowning efficiency among 1 % AA, 1 % CA and 1 % OA was found in banana slices [33]. Likewise, comparable whiteness indexes ($WI = 100 - ((100 - L^*)^2 + a^{*2} + b^{*2})^{1/2}$) for potato slices treated with 1 % AA and 1 % CA were detected [34]. Considering lettuce PPO, inhibition constants (K_i) of 0.1 mM CA and 0.1 mM OA were not different, indicating an equivalent capability in browning inhibition between CA and OA [21].

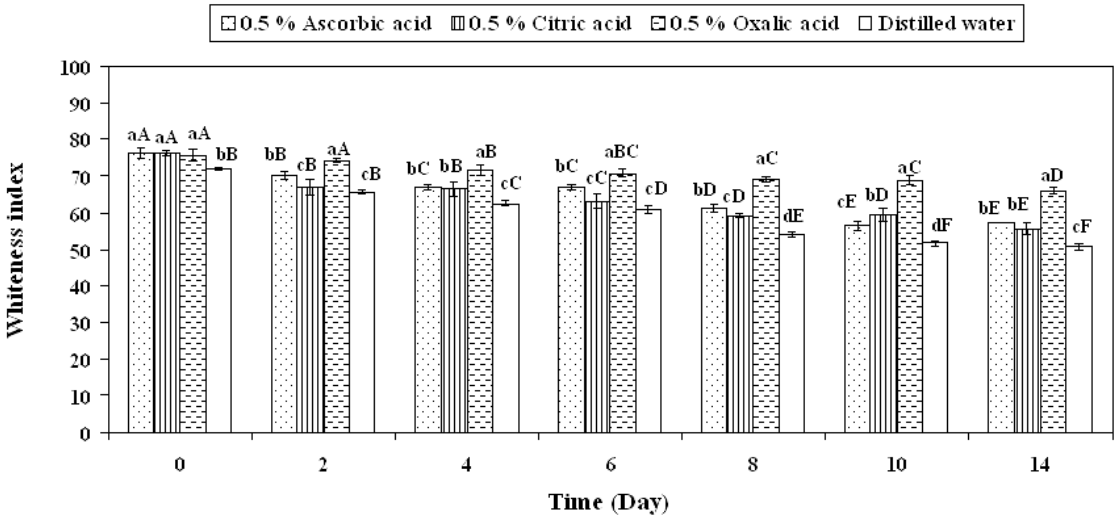

Figure 3 Whiteness indexes of fresh-cut mangosteens dipped in 0.5 % of ascorbic acid, citric acid, oxalic acid and distilled water during storage at 10 °C. Means (n = 3) followed by the same lower-case letter within the same time period or capital letter within the same solution are not significantly different ($p > 0.05$).

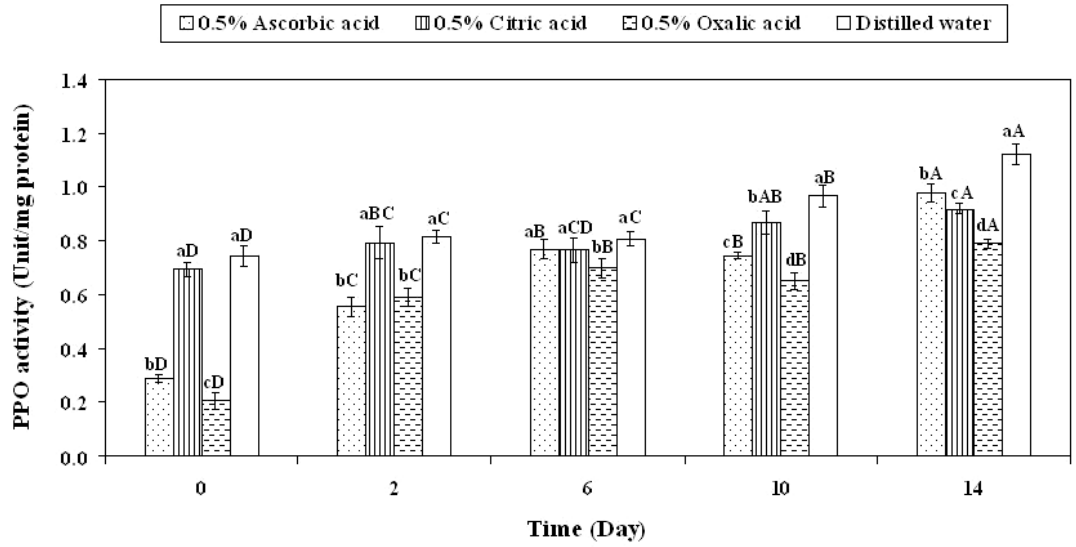

Figure 4 Polyphenol oxidase (PPO) activity of fresh-cut mangosteens dipped in 0.5 % of ascorbic acid, citric acid, oxalic acid and distilled water during storage at 10 °C. Means (n = 3) followed by the same lower-case letter within the same time period or capital letter within the same solution are not significantly different ($p > 0.05$).

The variation in inhibitive effects of AA, CA and OA on browning in various fresh-cut fruits and vegetables is probably the result of different PPO isozymes and/or phenolic substrates among product types and cultivars, affecting the sensitivity of PPO to the antibrowning agents. The affinity of plant PPO for specific phenolic substrates has been reported in longans [39], apples [40], medlar fruits [41], marula fruits [42], broccoli florets [43] and lettuce [21] (**Table 1**). Furthermore, the diverse pattern of inhibitory mechanisms of AA, CA and OA on PPO in various vegetables has been revealed (**Table 2**).

2) Concentrations of AA, CA and OA

Antibrowning efficiency of AA, CA and OA in fresh-cut fruits and vegetables has been reported to evidently correlate with the employed concentrations. In pineapple slices, treatment with 0.1 M AA resulted in a smaller extent of browning compared with 0.05, 0.025 and 0.01 M AA treatments [11]. Dipping fresh-cut Chinese water chestnut in 0.05 M CA stimulated PPO activity, whereas concentrations of CA at 0.1 M or higher conspicuously inhibited the PPO activity [47]. Correspondingly, concentrations of CA between 0.02 - 0.1 % activated PPO activity, while concentrations at 0.2 % or higher exhibited an inhibitive effect on the PPO activity in apples [27]. Regarding the *in vitro* effect of OA on the catechol-mushroom PPO reaction and oxygen uptake during the reaction, the increase in inhibition of catechol oxidation and oxygen consumption is associated with increases in concentrations of OA (**Figure 5**). Moreover, lower concentrations of OA were needed to inhibit PPO activity by 50 % (I_{50}), compared with CA, indicating the superior antibrowning efficiency of OA endowed with higher stability when compared with CA [46]. These results reveal that higher concentrations are required to effectively prevent browning when each individual antibrowning agent is employed.

Table 1 Phenolic substrate specificity of PPO for various fruits and vegetables.

Specific phenolic substrate	Source of PPO	Reference
Pyrogallo	Longan	[39]
p-cresol	Jonagored apple	[40]
4-methylcatechol	Medlar fruit	[41]
4-methylcatechol	Marula fruit	[42]
Catechol	Broccoli floret	[43]
Chlorogenic acid	Lettuce	[21]

Table 2 Inhibitory patterns of AA, CA and OA on PPO activity in various vegetables.

Inhibitor	Substrate	Vegetable	Inhibitory mechanism	Reference
AA	Chlorogenic acid	Lettuce	Competitive	[21]
	Catechol	Peppermint	Competitive	[16]
CA	Chlorogenic acid	Lettuce	Non-competitive	[21]
OA	Chlorogenic acid	Lettuce	Non-competitive	[21]
	Catechol	Artichoke	Non-competitive	[44]
	Catechol	Celery root	Competitive	[45]
	Catechol	Mushroom	Competitive	[46]

Synergistic Effects of AA, CA and OA

Synergistic effects of AA, CA and OA were found on apple slices by mixing the antibrowning agents. The mixed solution of 1 % CA and 0.02 % OA and the mixed solution of 1 % AA and 0.02 % OA clearly showed higher antibrowning efficiency than individual solutions of 1 % AA, 1 % CA and 0.02 % OA (**Figure 6**). Similarly, a mixed solution of 1 % AA and 0.1 % CA exhibited higher PPO inhibition in apple slices, compared with the solutions of 1 % AA and 1 % CA. Moreover, when 0.2 % CA instead of 0.1 % CA was added to 1 % AA, the degree of PPO inhibition increased from 36.3 % to 87.1 % (**Figure 7**). The use of mixtures of various antibrowning agents conducive to increased antibrowning efficiency is presumably due to the collaborative inhibitory mechanism of the constituents.

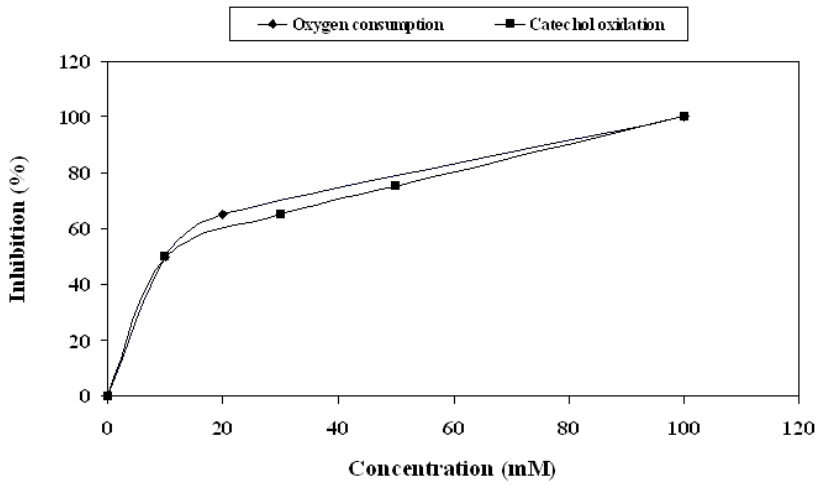

Figure 5 Inhibitory effect of oxalic acid at various concentrations on oxygen consumption and catechol oxidation.
Source: Modified from [46]

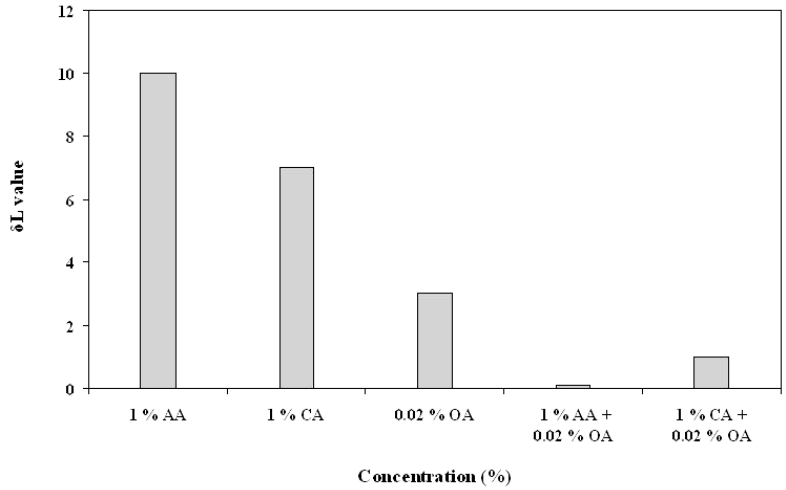

Figure 6 Degree of browning expressed by δL value (initial L value - L value at a given time) of apple slices treated with ascorbic acid (AA), citric acid (CA) and oxalic acid (OA) and mixtures of OA and AA or CA.
Source: Modified from [9]

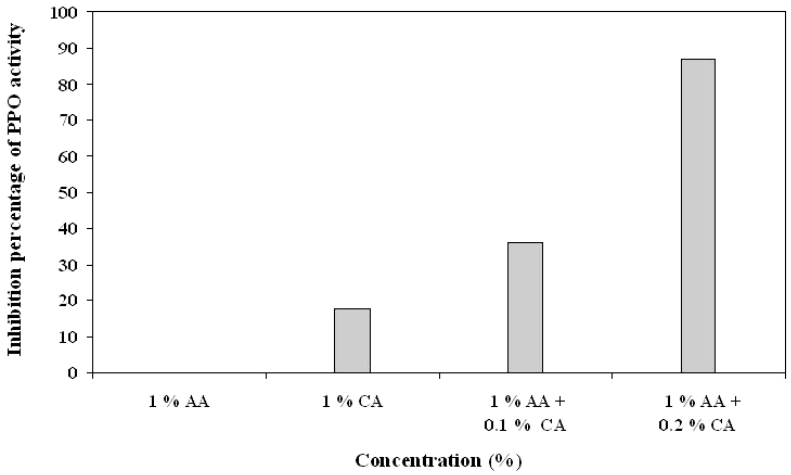

Figure 7 Degree of inhibition of polyphenol oxidase in apple slices treated with ascorbic acid (AA), citric acid (CA) and mixtures of AA and CA.
Source: Modified from [27]

Conclusions

Ascorbic acid (AA), citric acid (CA) and oxalic acid (OA) are natural identical antibrowning agents and generally recognized as safe (GRAS). The effectiveness on browning prevention is mainly dependent on the produce types and cultivars, and concentrations of antibrowning agents. The occurrence of different PPO isozymes and phenolic substrates in various produce types and cultivars may result in the diverse responses of PPO to the antibrowning agents. On the other hand, efficiency of respective AA, CA and OA on delaying browning can be enhanced by addition of an antibrowning agent possessing a different inhibitory mechanism and/or superior stability.

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