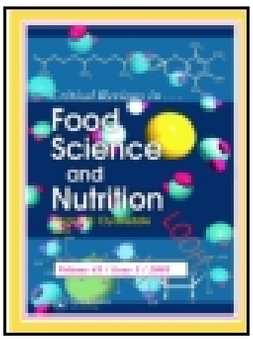

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REVIEW

Inhibitory effects of organic acids on polyphenol oxidase: From model systems to food systems

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ABSTRACT

Organic acids are widely utilized in the food industry for inhibiting the activity of polyphenol oxidase (PPO) and enzymatic browning. This review discusses the mechanisms of inhibition of PPO and enzymatic browning by various organic acids based on studies in model systems, critically evaluates the relevance of such studies to real food systems and assesses the implication of the synergistic inhibitory effects of organic acids with other physicochemical processing techniques on product quality and safety. Organic acids inhibit the activity of PPO and enzymatic browning via different mechanisms and therefore the suitability of a particular organic acid depends on the structure and the catalytic properties of PPO and the physicochemical properties of the food matrix. Studies in model systems provide an invaluable insight into the inhibitory mechanisms of various organics acids. However, the difference in the effectiveness of PPO inhibitors between model systems and food systems and the lack of correlation between the degree of PPO inhibition based on in vitro assays and enzymatic browning imply that the effectiveness of organic acids can be accurately evaluated only via direct assessment of browning inhibition in a particular food system. Combination of organic acids with physical processing techniques is one of the most viable approaches for PPO inhibition since the observed synergistic effect helps to reduce the undesirable organoleptic quality changes from the use of excessive concentration of organic acids or intense physical processing.

KEYWORDS

Organic acids; polyphenol oxidase; inhibition mechanism; catalytic activity; enzymatic browning; model systems; food systems

Introduction

Polyphenol oxidase (PPO) refers to a group of copper-containing enzymes, widely distributed in animals, plants, fungi and bacteria (Mayer 1986). PPO has been intensively studied in food science because of its involvement in browning of plant based products and crustaceans such as shrimp. Enzymatic browning is the result of the PPO catalyzed hydroxylation of monophenols to *o*-diphenols and the oxidation of *o*-diphenols to *o*-quinones (Espín, Jolivet, and Wichers 1998; Yoruk and Marshall 2003a). Browning of fruits, vegetables and beverges is considered as one of the main causes of deterioration in food quality during postharvest handling and processing (Mathew and Parpia 1971). Changes in nutritional properties and appearance of fruits and vegetables by enzymatic browning reduces consumer's acceptance and value of the products. It is estimated that up to 50% loss of tropical fruits occurs due to enzymatic browning. Thus, inhibition of PPO activity in fruits and vegetables postharvest will create enormous economic and quality benefits (Queiroz et al. 2008; Whitaker and Lee 1995).

Thermal treatment is the most effective and economical method to inactivate PPO and control enzymatic browning in canned or frozen fruits and vegetables. However, it is not suitable for fresh products such as fresh-cut fruits and vegetables (McEvily, Iyengar, and Otwell 1992). The effects of alternative nonthermal preservation techniques such as high pressure (Chakraborty, Kaushik, et al. 2014; Terefe, Buckow, and Versteeg 2014), ultrasound (Islam, Zhang, and Adhikari 2014), high pressure carbon dioxide (Hu et al. 2013) and pulsed electric field processing (Terefe, Buckow, and Versteeg 2015b) on PPO from various sources have been widely studied. Many of these studies showed that PPOs from many plants are resistant to inactivation by these nonthermal techniques (García-Parra et al. 2016; Terefe et al. 2010; Van Loey, Verachtert, and Hendrickx 2001; Zhou, Liu, Stockmann, et al. 2018; Zhou, Liu, Xiong, Zou, Liu, et al. 2016). Therefore, organic acids are commonly used in combination with these non-thermal techniques to control enzymatic browning. Besides, the application of organic acid helps to reduce the intensity of heat required for enzyme and microbial inactivation and thus diminish the undesirable heat induced changes in products during thermal processing.

Organic acids that are commonly used as food ingredients and PPO inhibitors, are naturally present in plants. Many studies have investigated the effect of organic acids on

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the activity of PPO. Carboxylic acids are the most common organic acids employed in such application. Among carboxylic acids, aliphatic carboxylic acids such as citric acid, oxalic acid, tartaric acid and malic acid are the most widely used anti-browning agents (Ali et al. 2015; Deng et al. 2015; Huang et al. 2013; Zhou, Liu, Stockmann, et al. 2018). Aromatic carboxylic acids comprise two main series; benzoic acid series and cinnamic acid series. Benzoic acid, salicylic acid and gentisic acid are the most common benzoic acids and derivatives. The cinnamic acid series include cinnamic acid, ferulic acid, and coumaric acid. More and more aromatic carboxylic acids are being discovered in plants that are proven to be effective inhibitors of PPO (Robert, Rouch, and Cadet 1997; Shi et al. 2005; Siddiq and Dolan 2017; Zhou et al. 2015). In addition to carboxylic acids, other organic acids such as ascorbic acid, kojic acid and phytic acid are widely studied and used for the inhibition of browning in food products (Liu et al. 2015; Mishra, Gautam, and Sharma 2012; Zhu et al. 2009). Model systems offer a direct and easy way to investigate the effect of organic acid on PPO activity. Inhibitory effects of organic acids on PPO can be determined by dose-effect relationship and kinetic analysis in buffer system. Inhibition mechanisms are elucidated by analyzing the inhibition modes, lag times and structural changes of PPO. Several inhibition mechanisms of organic acids on PPO have been proposed. Different organic acids inhibit PPO in different modes and some organic acids have dual or multiple effects.

In food systems, organic acids as food additives have an advantage that they can move freely through cells because of their simple structure and small molecular weight (Theron and Lues 2007). Besides the anti-browning effect, organic acids also act as antimicrobial agents to prolong the shelf life of products. Therefore, many of them are widely utilized by adding directly or indirectly to the products (Bégin and Van Calsteren 1999; Theron and Lues 2007). There are several studies on the mechanistic aspects of PPO inhibition by various organic acids in model systems. However, the complexity of food systems limit their validity to real food systems. Food components such as sugars and salts and physicochemical properties such as pH significantly influence the activity and stability of enzymes (Riahi and Ramaswamy 2004; Terefe et al. 2014; Weemaes et al. 1998; Zhou, Liu, Stockmann, et al. 2018). Moreover, in food systems, PPO exists in two forms; soluble and membrane-bound (Liu et al. 2015; Zaini et al. 2013). Organic acids may have different inhibitory effects on soluble and membrane-bound PPO, which may limit the applicability of data in model systems to food systems since membrane-bound PPO may not be completely extracted. The effects of organic acid on PPO activity and enzymatic browning may also vary depending on the structure of the food matrix such as fresh-cut, juice and puree as that determines PPO-substrate interaction and potentially the accessibility of PPO to the inhibitor (Rocha and De Morais 2005; Yi and Ding 2014).

In recent years, more and more organic acids are proven to effectively inhibit PPO and some recent reviews are focused on updating the literature with these recently discovered inhibitors (Chang 2009; Kim and Uyama 2005; Loizzo, Tundis, and Menichini 2012). There are also a number of reviews on the application of organic acids for preventing browning in minimally processed fruits and vegetables (Dhall 2013; Oms-Oliu et al. 2010; Rojas-Graü, Soliva-Fortuny, and Martín-Belloso 2009). However, very little attention has been paid to the mechanisms of inhibition of PPO by the various organic acids. Thus, the objectives of this review are to discuss the mechanisms of inhibition of PPO and enzymatic browning by various organic acids based on studies in model systems, critically evaluate the relevance of such studies to real food systems and assess the combined effects of organic acids and physical processing techniques on the inactivation of PPO in model and real food systems. The overall objective is to derive insights from the literature which enables the rational selection of organic acids for prevention of enzymatic browning in food systems based on the structure and composition of the food matrix, the intended storage time and condition and the preservation technique employed.

Inhibitory effect and mechanism of inhibition of PPO by organic acids in model systems

Acidulants

PPO is sensitive to pH and the native enzyme unfolds with structural changes at acidic pH condition (Ionita et al. 2014). At pH 3.0, PPO from mushroom was strongly inactivated and conformational changes were observed (Liu et al. 2013). The optimum pH of PPO varies with the enzyme source, variety and growing condition in case of plant PPOs. According to previous studies, most PPOs exhibit an optimum pH range of 6.0–7.0 (McEvily et al. 1992; Palma-Orozco et al. 2011; Queiroz et al. 2011; Siddiq and Dolan 2017). An optimum pH of weak acidic pH ranging from 4.0 to 6.0 was observed in relatively fewer PPOs such as PPO from gooseberry, pear, loquat and mango (Bravo and Osorio 2016; Liu et al. 2009; Palma-Orozco et al. 2014; Zhang and Shao 2016). At pH lower than 3.0, PPOs from most sources are strongly inhibited at times with no PPO activity detected (Altunkaya and Gökmen 2012; Batista et al. 2014; Bravo and Osorio 2016; Cheema and Sommerhalter 2015). Lowing the pH by organic acids may induce the denaturation of the enzyme and keep the media pH lower than the optimal pH.

Citric acid, tartaric acid and malic acid are the common organic acids which inhibit PPO activity as acidulants. Citric acid is the most widely used one in the food industry. In a study by Liu et al (2013), the pH values of 10, 30 and 60 mM citric acid in phosphate buffer (pH 6.8, 50 mM) were 5.54, 3.54 and 3.01, respectively whereas the pH values of 10, 30 and 60 mM citric acid in double-distilled water were 2.76, 2.36 and 2.20, respectively (Liu et al. 2013). These results indicate that citric acid is an effective acidulant especially in non-buffered systems. As shown in Table 1, in model systems, citric acid has been successfully used to inhibit PPO from various sources such as potato (Mosneaguta, Alvarez, and Barringer 2012), apple (Du, Dou, and Wu 2012; Liu et al. 2015), chestnut (Jiang, Pen, and Li

Table 1. Summary of representative studies on PPO inhibitory effects of organic acids in model systems.

Source of PPO	Organic acid	Concentration	Inhibition degree and Inhibition mode	Processing media	References
Mushroom	Citric acid	10 and 30 mM	18.0% and 75.8%	50 mM phosphate buffer (pH 6.8)	Liu et al. (2013)
Mushroom	Malic acid	10 and 20 mM	24.5% and 89.7%	50 mM phosphate buffer (pH 6.8)	Zhou, Liu, Xiong, Zou, Chen, et al. (2016)
	Cinnamic acid	3 and 5 mM	75.1% and 84.3%, mixed-type		
Mushroom	2-chlorcinnamic acid	0.765 mM	50%, uncompetitive	50 mM phosphate buffer (pH 6.8)	Hu et al. (2014)
	2,4-dichlorcinnamic acid	0.295 mM	50%, uncompetitive		
Mushroom	Cinnamic acid	2.10 mM	50%, noncompetitive	50 mM phosphate buffer (pH 6.8)	Shi et al. (2005)
	4-Hydroxycinnamic acid	0.50 mM	50%, competitive		
	4-Methoxycinnamic acid	0.42 mM	50%, competitive		
Mushroom	Salicylic acid	4.30 mM	50%, competitive	50 mM phosphate buffer (pH 6.8)	Zhang et al. (2006)
	4-Methoxysalicylic acid	2.28 mM	50%, noncompetitive		
	4-Methysalicylic acid	1.65 mM	50%, mixed-type		
Mushroom	Acetylsalicylic acid	28.35 mM	50%, mixed-type	Buffer (pH 6.25)	Z. Wang et al. (2013)
Mushroom	Citric acid	0.1 M (pH 3.5)	96.6% (substrate isolated from mushroom) and 50% (L-tyrosine)	100 mM citric acid (pH 3.5)	McCord and Kilara (1983)
Mushroom	Ascorbic acid	0.5 and 10 mM (after 2 h incubation)	~50% and ~80%	50 mM sodium phosphate buffer (pH 7.0)	Arias et al. (2007)
Mushroom	Pyruvic acid	0.1–0.75 mM	Competitive	10 mM phosphate buffer (pH 6.8)	Gheibi et al. (2009)
	Acrylic acid	0.2–1.2 mM	Competitive		
	Propanoic acid	0.5–1.5 mM	Uncompetitive		
	2-Oxo-butanoic acid	0.4–2.5 mM	Competitive		
	2-Oxo-octanoic acid	0.5–2.0 mM	Competitive		
Mushroom	Gentisic acid	0.09–0.36 mM	Mixed-type	50 mM sodium phosphate buffer (pH 6.8)	Zhou, Xiong, et al. (2017)
Mushroom	Oxalic acid	0.12 mM	50%, uncompetitive	5 mM phosphate buffer (pH 7.0)	Dedeoglu and Guler (2009)
	L-cysteine	0.15 mM	50%, uncompetitive		
Mushroom	*p*-hydroxybenzoic acid	0.25–1.00 mM	Competitive	50 mM phosphate buffer (pH 6.8)	Chen et al. (2005)
	p-methoxybenzoic acid	0.1–0.4 mM	Noncompetitive		
	p-propoxybenzoic acid	0.25–1.00 mM	Uncompetitive		
	p-ethoxybenzoic acid	0.1–0.4 mM	Mixed-type		
Mushroom	Ascorbic acid	0.02 mM	50%	McIlvaine buffer (pH 7.0)	Öz et al. (2013)
	Benzoic acid	5.02 mM	50%		
Mushroom	Ascorbic acid	0.1–0.8 mM	Reductant and irreversible inactivation	140 mM phosphate buffer (pH 6.5)	Golan-Goldhirsh and Whitaker (1984)
Apple	Benzoic acid	1–3 mM	Competitive	50 mM phosphate buffer (pH 6.5)	Janovitz-Klapp et al. (1990)
	Cinnamic acid	0.1 mM	Competitive		
Apple	Oxalic acid	8 mM	93.1%	100 mM acatate buffer (pH 5.0)	Yoruk and Marshall (2003b)
Cashew apple	Ascorbic acid	0.01 mM	100%	10 mM phosphate buffer (pH 6.5)	Queiroz et al. (2011)
	Citric acid	5 mM	50%		
Apple	Ferulic acid	39.74 μg/mL	15.19%	100 mM phosphate buffer (pH 7.0)	Sukhonthara et al. (2016)
Apple	Phytic acid	0.1 mM	99.2%	10 mM sodium phosphate buffer (pH 6.0)	Du et al. (2012)
	Ascorbic acid	0.1 mM	~85%		
	Citric acid	0.1 mM	~10%		
Apple	Ascorbic acid	2.81 and 1.82 mM	50% (soluble PPO) and 50% (membrane-bound PPO)	50 mM sodium phosphate buffer (pH 6.8)	Han et al. (2019)
	L-cysteine	2.12 and 1.80 mM	50% (soluble PPO) and 50% (membrane-bound PPO)		
	Citric acid	44.28 and 47.55 mM	50% (soluble PPO) and 50% (membrane-bound PPO)		
Apple	Ascorbic acid	0.05 mM	50%	50 mM phosphate buffer (pH 6.5)	Can et al. (2014)
	Benzoic acid	9.8 mM	50%		
Apple	L-cysteine	0.03–0.19 mM	Fit no type of reversible inhibition	100 mM citrate buffer (pH 5)	de la Rosa et al. (2011)
Mamey	Kojic acid	0.1, 1 and 10 mM	0, 57% and 100%	200 mM phosphate buffer (pH 7.0)	Palma-Orozco et al. (2011)
	Ascorbic acid	0.1, 1 and 10 mM	100%, 100% and 100%		
	Succinic acid	0.1, 1 and 10 mM	58%, 100% and 100%		
Potato	Citric acid	0.3–1.6 mL 3% solution	Depending on pH	Crude extract	Mosneaguta et al. (2012)
	Malic acid	0.3–1.6 mL 3% solution	Depending on pH		
Potato	*p*-alkylbenzoic acids	0.1–0.4 mM	Noncompetitive	50 mM phosphate buffer (pH 6.8)	Lin et al. (2010)
Potato	Ferulic acid	39.74 μg/mL	15.21%	100 mM phosphate buffer (pH 7.0)	Sukhonthara et al. (2016)

(*continued*)

Table 1. Continued.

Source of PPO	Organic acid	Concentration	Inhibition degree and Inhibition mode	Processing media	References
Potato	Cysteine	1.11×10^{-6}–1.11×10^{-2} mM	Activity decreased with increasing concentration of cysteine	100 mM sodium phosphate buffer (pH 6.5)	Ali et al. (2016)
Potato	3-hydroxybenzoic acid	0.36–2.12 mM	Competitive inhibition		Bayrak et al. (2019)
Sweet potato	Ascorbic acid	5.08 mM	50%	100 mM potassium phosphate buffer (pH 6.8)	Lim et al. (2019)
Lettuce	*Ascorbic acid*	0.1 and 1.0 mM	32% and 90%	Crude enzyme extract	Landi et al. (2013)
Lettuce	Cysteine	0.03–0.7 mM	Competitive	100 mM sodium phosphate buffer (pH 6.5)	Ali et al. (2015)
	Citric acid	0.03–0.7 mM	Noncompetitive		
	Ascorbic acid	0.03–0.7 mM	Competitive		
Lettuce	Ascorbic acid	0.01–0.02 mM	Competitive	Distilled water	Altunkaya and Gokmen (2008)
	Oxalic	0.1–0.14 mM	Noncompetitive		
	Citric acid	0.1–0.14 mM	Noncompetitive		
Lettuce	Ascorbic acid	2.69 mM	50%	50 mM phosphate buffer (pH 7.0)	Zlotek and Gawlik-Dziki (2015)
	L-cysteine	0.84 mM	50%		
	Citric acid	0.99 mM	50%		
	p-Hydroxybenzoic acid	9.08 mM	50%		
Lettuce	Citric acid	10 mM	44%	100 mM sodium phosphate buffer (pH 7.4)	Lee, Shin, and Kim (2014)
	Ascorbic acid	10 mM	45%		
Lettuce	Ascorbic acid	0.133–0.367 mM; 0.10–0.20 mM	Mix-type (catechol) competitive (methylcatechol)	50 mM phosphate buffer (pH 7.0)	Doğan and Salman (2007)
Butter lettuce	Ascorbic acid	4.61 mM	50%	50 mM phosphate buffer (pH 6.8)	Gawlik-Dziki et al. (2008)
	L-cysteine	10.39 mM	50%		
	Citric acid	62.91 mM	50%		
	p-Hydroxybenzoic acid	4.54 mM	50%		
Chestnut	Phytic acid	0.02% and 0.05%	Competitive	100 mM citrate phosphate buffer (pH 5.6)	Li et al. (2017)
Chestnut	Salicylic acid	0.3, 0.4 and 0.5 g/L	27.0%, 50.0% and 56.4%, competitive	50 mM citrate phosphate buffer (pH 5.6)	Zhou et al. (2015)
Chestnut	Citric acid	100 mM	~90%	50 mM phosphate buffer (pH 6.8)	Jiang, Pen, and Li (2004)
Artichoke	Ascorbic acid	0.09 and 0.08 mM	50% (head and leaves)	100 mM phosphate buffer (pH 7.0)	Tuncay and Yagar (2011)
	L-cysteine	0.097 and 0.079 mM	50% (head and leaves)		
	Citric acid	41.3 and 25.5 mM	50% (head and leaves)		
	Benzoic acid	3.9 and 4.5 mM	50% (head and leaves)		
Artichoke	Ascorbic acid	2 mM	89%, 32% and 92% (Violetto di Sicilia, Violetto di Provenza and Tema 2000)	100 mM phosphate buffer (pH 6.5)	Todaro et al. (2010)
	Tartaric acid	100 mM	72%, 66% and 5% (three cultivars as above)		
	Citric acid	100 mM	56%, 37% and 6% (three cultivars as above)		
Soursop	Ascorbic acid	0.1, 1.0, 10.0 mM	100%	50 mM phosphate buffer (pH 7.0)	Palma-Orozco et al. (2019)
Blueberry	Benzoic acid	0.2, 1.0, 2.0 mM	7.5%, 9.8% and 21.8%	100 mM citrate phosphate buffer (pH 6.1)	Siddiq and Dolan (2017)
	t-Cinnamic acid	0.2, 1.0, 2.0 mM	17.8%, 19.8% and 24.7%		
	Ascorbic acid	0.2, 1.0, 2.0 mM	86.7%, 99.7% and 99.7%		
	L-cysteine	0.2, 1.0, 2.0 mM	98.0%, 98.6% and 99.4%		
Banana	Ascorbic acid	1.0, 2.0 and 4.0 mM	About 30%, 90% and 100%	50 mM phosphate buffer (pH 7.0)	Chaisakdanugull and Theerakulkait (2009)
Tadela	Ascorbic acid	0.1, 10 and 2.0 mM	About 40%, 90% and 100%	50 mM phosphate buffer (pH 7.0)	Benaceur et al. (2019); Ünal (2007)
Anamur banana	Ascorbic acid	0.2 and 0.8 mM	99.8% and 100%	100 mM phosphate buffer (pH 6.8)	
	Citric acid	10 and 20 mM	61.6% and 69.7%		
Boletus erythropus	Benzoic acid	10.2 mM	50%	50 mM acetate buffer (pH 5.0)	Özel et al. (2010)
	Ascorbic acid	0.08 mM	50%		
Coffee	Cinnamic acid	1–5 mM	About 50% to 90%	50 mM phosphate buffer (pH 6.0)	Mazzafera and Robinson (2000)
Cape gooseberry	Ascorbic acid	0.1, 1, 10 mM	89.3%, 93.6% and 96.4%	200 mM sodium phosphate buffer (pH 6.0)	Bravo and Osorio (2016)
	L-cysteine	0.1, 1, 10 mM	83.6%, 91.0% and 98.0%		

(*continued*)

Table 1. Continued.

Source of PPO	Organic acid	Concentration	Inhibition degree and Inhibition mode	Processing media	References
	Tannic acid	0.1, 1, 10 mM	17.2%, 52.7% and 63.5%		
	Tartaric acid	0.1, 1, 10 mM	20.2%, 32.6% and 42.0%		
Cotton	Citric acid	1.0 and 10 mM	~5% and ~15%	100 mM phosphate buffer (pH 6.5)	Kouakou et al. (2009)
	Ascorbic acid	1.0 and 10 mM	~85% and ~100%		
Durum wheat	Ascorbic acid	10–20 mM	K_i = 0.4, uncompetitive	50 mM phosphate buffer (pH 6.5)	Altunkaya and Gökmen (2012)
	Cysteine	15–25 mM	K_i = 0.9, competitive		
	Citric acid	30–60 mM	K_i = 1.7, Noncompetitive		
	Oxalic acid	20–50 mM	K_i = 1.8, Noncompetitive		
Eggplant	Ascorbic acid	10–30 μM	Competitive	50 mM phosphate buffer (pH 6.5)	Mishra et al. (2012)
	Kojic acid	0.05–0.2 mM	Competitive		
	Citric acid	20–40 mM	Mixed-type		
Eggplant	Tartaric acid	0.42 mM	About 50%	100 mM phosphate buffer (pH 7.0)	Todaro et al. (2011)
	Citric acid	0.42 mM	About 95%		
	Acetic acid	0.42 mM	About 70%		
Grape	Ascorbic acid	0.5 and 5 mM	25.0% and 74.7%	McIlvaine buffer (pH 6.0)	Zheng et al. (2012)
	L-cysteine	0.5 and 5 mM	69.8% and 100%		
	Citric acid	0.5 and 5 mM	11.4% and 14.6%		
Grape	Ascorbic acid	0.16 and 0.4 mM	15.1% and 100%	100 mM phosphate buffer (pH 6.8)	Ünal (2007)
	Citric acid	40 and 100 mM	18.6%, 30.2% and 46.6%		
Jackfruit	Kojic acid	0.23	50%, noncompetitive	50 mM phosphate buffer (pH 7.0)	Tao et al. (2013)
	Ascorbic acid	0.30	50%, mixed-type		
	L-cysteine	0.19	50%, noncompetitive		
Lotus seeds	Citric acid	50 mM	42.7%	200 mM phosphate buffer (pH 7.0)	Cai et al. (2015)
	Ascorbic acid	50 mM	100%		
Loquat	Oxalic acid	0.005 and 0.025 mM	5.37% and 99.64%	100 mM sodium phosphate buffer (pH 6.5)	Zhang and Shao (2016)
	Citric acid	0.005 and 0.025 mM	6% and 37.89%		
	L-cysteine	0.01 and 0.05 mM	53.7% and 98.99%		
	Ascorbic acid	0.01 and 0.05 mM	11.75% and 75.9%		
Lonicera confusa	Kojic acid	1, 5 and 10 mM	55%, 82% and 86%	20 mM tris-HCl buffer (pH 7.5)	Feng et al. (2014)
	Ascorbic acid	1, 5 and 10 mM	78%, 80% and 88%		
	Citric acid	1, 5 and 10 mM	12%, 57% and 60%		
Longan	Ascorbic acid	1 mM	100% and 100% (substrate-epicatechin and catechol)	10 mM phosphate buffer (pH 6.8)	J. Sun et al. (2010)
	L-cysteine	1 mM	100% and 97.6% (substrate-epicatechin and catechol)		
Lychee	Ascorbic acid	100 mM	97.9% and 98.1% (substrate-epicatechin and catechol)	10 mM phosphate buffer (pH 6.8)	Sun et al. (2008)
	L-cysteine	100 mM	100% and 90.5% (substrate-epicatechin and catechol)		
	L-cysteine	1.0 and 2.0 mM	About 65% and 100%		
Litchi	L-cysteine	0.1 and 0.2 mM	45% and 63%, 3 and 5 min lag period	50 mM phosphate buffer (pH 7.5)	Liu et al. (2007)
Leaves of *Cleome gynandra* L.	Ascorbic acid	1.0 and 10 mM	93% and 100%	20 mM tris-HCl buffer (pH 7.5)	Gao, Liu, and Xiao (2011)
	Citric acid	1.0 and 10 mM	15% and 26%		
	L-cysteine	1.0 and 10 mM	100% and 100%		
Medlar	Ascorbic acid	0.11 mM	50%	100 mM phosphate buffer (pH 7.0)	Yolmeh and Sadeghi Mahoonak (2016)
	Benzoic acid	0.95 mM	50%		
Mango	Kojic acid	0.02, 0.2, 2 and 20 mM	4%, 60%, 96% and 100%	60 mM sodium phosphate buffer (pH 5.8)	Cheema and Sommerhalter (2015)
	Citric acid	0.02, 0.2, 2 and 20 mM	−1%, 12%, 47% and 100%		
	EDTA	0.02, 0.2, 2 and 20 mM	0, 31%, 73% and 77%		
	Benzoic acid	0.02, 0.2, 2 and 20 mM	3%, −3%, −1% and 56%		
Mango	Kojic acid	0.1, 1 and 10 mM	48%, 76% and 100%	50 mM sodium phosphate buffer (pH 7.0)	Palma-Orozco et al. (2014)
	Ascorbic acid	0.1, 1 and 10 mM	100%, 100% and 100%		
	Benzoic acid	0.1, 1 and 10 mM	31%, 36% and 52%		
	Citric acid	0.1, 1 and 10 mM	30%, 29% and 33%		
	Succinic acid	0.1, 1 and 10 mM	18%, 24% and 31%		
Plantain	Ascorbic acid	0.5 mM	62.8% and 70.7% (peel and pulp)	100 mM phosphate buffer (pH 6.8)	Chong, Cheng, and Aziz (2011)
	L-cysteine	0.25 mM	37.5% and 56.0% (peel and pulp)		
	Citric acid	5 mM	18.1% and 23.6% (peel and pulp)		

(*continued*)

Table 1. Continued.

Source of PPO	Organic acid	Concentration	Inhibition degree and Inhibition mode	Processing media	References
Parsley	L-cysteine	1 and 3 mM	Noncompetitive	100 mM phosphate buffer (pH 6.8)	Lin, Ng, and Wong (2016)
	Citric acid	1 and 3 mM	Competitive		
Peach	L-cysteine	0.02, 0.04, 0.08, 0.15 and 0.2 mM	16.81%, 40.32%, 67.35%, 89.74% and 94.83%	50 mM phosphate buffer (pH 6.8)	Liu et al. (2015)
Peach	Ascorbic acid	0.29 mM	50%, reducing agent	50 mM phosphate buffer (pH 6.5)	Garro and Gasull (2010)
Red Swiss chard leaves	Citric acid	1 and 10 mM	25% and 35%	20 mM tris-HCl buffer (pH 7.5)	Gao et al. (2009)
	L-cysteine	1 and 10 mM	93% and 100%		
	Ascorbic acid	1 and 10 mM	88% and 100%		
Rosmarinus officinalis L	Ascorbic acid	0.167 and 0.333 mM	Mixed-type	100 mM phosphate buffer (pH 6.5)	Doğan et al. (2011)
	L-cysteine	0.167 and 0.333 mM	Competitive		
Rosmarinus Officinalis L	Ascorbic acid	0.0396 mM	50%, competitive	50 mM phosphate buffer (pH 7.0)	Aydemir (2010)
	L-cysteine	0.058 mM	50%, competitive		
	Citric acid	16.7 mM	50%, noncompetitive		
Plum	Citric acid	0.5, 5, 50 mM	24.12%, 32.18% and 46.44%	McIlvaine buffer (pH 6.0)	Ioniţă et al. (2017)
	Oxalic acid	0.5, 5, 50 mM	16.24%, 31.31% and 36.44%		
	Ascorbic acid	0.5, 5, 50 mM	30.01%, 100 % and 100%		
	L-cysteine	0.5, 5, 50 mM	12.74%, 37.74 % and 99.32%		
	Glycine	0.5, 5, 50 mM	9.11%, 11.59 % and 17.20%		
Pineapple	Ascorbic acid	0.5–1.5 mM	15%–97%, 0.8–2.5 min lag phase	100 mM phosphate buffer (pH 7.0)	Das et al. (1997)
	L-cysteine	0.5–1.5 mM	20%–87%, 0.6–2.0 min lag phase		
	Cinnamic acid	1 and 3 mM	60% and 85%, 0 min lag phase		
Solanum lycocarpum fruits	Ascorbic acid	30 mM	70.3%, uncompetitive	50 mM sodium phosphate buffer (pH 6.5)	Batista et al. (2014)
	Citric acid	10 mM	24.3%, uncompetitive		
	L-cysteine	10 mM	74.5%, competitive		
Sugarcane	Ascorbic acid	1 and 10 mM	81% and 100%	20 mM phosphate buffer (pH 6.8)	Zhao et al. (2011)
	L-cysteine	1 and 10 mM	30% and 96%		
	Citric acid	1 and 10 mM	20% and 70%		
Vanilla bean	Citric acid	1 mM	20.97%	100 mM sodium phosphate buffer (pH 6.5)	Waliszewski et al. (2009)
	L-cysteine	1 mM	77.46%		
	Benzoic acid	1 mM	78.55%		
	Ascorbic acid	1 mM	99.77%		
Wheat	Ascorbic acid	11.18 mM	50%, noncompetitive	50 mM phosphate buffer (pH 6.5)	Erat et al. (2010)
	Oxalic acid	77.33 mM	50%, competitive		
	L-cysteine	183 mM	50%, noncompetitive		
Yam	Ascorbic acid	1 and 10 mM	100% and 100%	0.1 M citrate/0.2 M sodium phosphate buffer (pH 7)	Fujita et al. (2006)
	L-cysteine	1 and 10 mM	100% and 100%		
	Citric acid	1 and 10 mM	11.6% and 37%		
	Acetic acid	1 and 10 mM	14.5% and 33.3%		
Yam	Ascorbic acid	0.5 mM	~90%	100 mM phosphate buffer (pH 6.0)	Li et al. (2015)
	L-cysteine	0.5 mM	~85%		
	Oxalic acid	Oxalic acid	~10%		
	Phytic acid	0.5 mM	~15%		
Chinese pear	Ascorbic acid	3 mM	68.8%	McIlvaine buffer (pH 4.5)	Liao et al. (2020)
	Citric acid	60 mM	70.7%		
	Ferulic acid	5 mM	57.2%		
	Salicylic acid	5 mM	62.7%		
Fragrant pear	Ascorbic acid	3 mM	82.3%	McIlvaine buffer (pH 4.5)	Liao et al. (2020)
	Citric acid	60 mM	77.5%		
	Ferulic acid	5 mM	59.1%		
	Salicylic acid	5 mM	67.9%		
Lentil	Ascorbic acid	2 mM	43.86%	5 mM tris-pH (pH 6.5)	Sikora et al. (2019)
	L-cysteine	2 mM	21.35%		
	Citric acid	2 mM	37.88%		

2004), mushroom (Zhou, Liu, Xiong, Zou, Chen, et al. 2016), yam (Fujita et al., 2006; R Li et al. 2015), lotus seeds (Cai et al. 2015), lettuce (Altunkaya and Gokmen 2008; Gawlik-Dziki, Złotek, and Świeca 2008; Zlotek and Gawlik-Dziki 2015), mango (Cheema and Sommerhalter 2015; Palma-Orozco et al. 2014), eggplant (Mishra et al. 2012; Todaro et al. 2011), grape (Ümit Ünal, Şener, and Şen 2007; Zheng, Shi, and Pan 2012), artichoke (Tuncay and Yagar 2011) and banana (Ünal 2007). The concentrations of citric acid used in these studies vary but most of the effective concentrations are higher than 10 mM (Table 1). Citric acid concentrations higher than 30 mM are usually required to strongly inhibit PPO

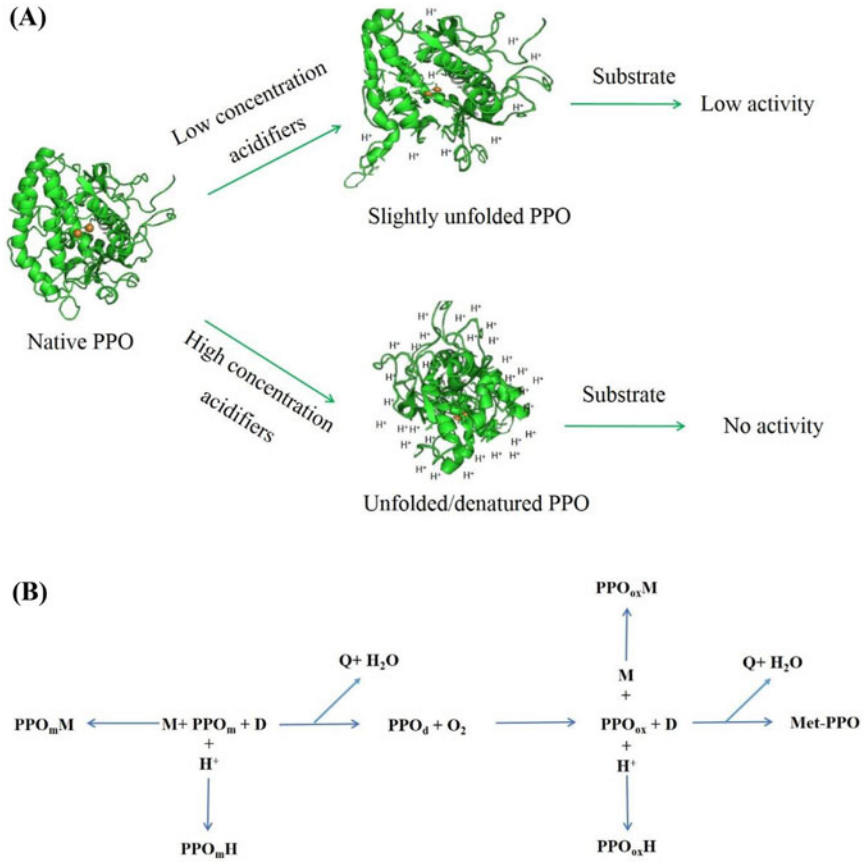

Figure 1. Inhibitory mechanism of organic acid as acidulants.

since the media pH dramatically decreases at these conditions and the extent of decrease depends on the concentration and pH of the buffer system (Ioniță et al. 2017; Liu et al. 2013, 2015; Todaro et al. 2010). The pH of 30 mM citric acid which is dissolved in phosphate buffer (pH 6.8, 50 mM) is 3.54. At this condition, citric acid inhibited the activity of mushroom PPO and the secondary and tertiary structure of PPO was significantly altered (Liu et al. 2013). The secondary structure of mushroom PPO consists of α-helix, β-sheet, β-turn and random coil structures, and the α-helix component is very important for the activity of PPO. Tertiary structure refers to the three-dimensional structure of a polypeptide chain, which results from the folding and arrangement of the secondary structural elements such as α-helix and β-sheet. A decrease in activity might be related to the unfolding of the structure of PPO (Liu et al. 2013). Ionita et al. (2014) reported mushroom PPO was unfolded with the exposure of the hydrophobic residues at this pH. Moreover, our previous study showed that the inhibitory effect of citric acid on mushroom PPO was mainly attributed to the decrease of pH (Zhou, Liu, Xiong, Zou, Chen, et al. 2016). Mosneaguta et al. (2012) made a similar observation that the effectiveness of citric acid on potato PPO was mainly due to a pH effect.

Other acidulants such as malic acid, oxalic acid, acetic acid and tartaric acid are also widely studied for the inhibition of PPO (Table 1). Relatively high concentrations (> 10 mM) of these acidulants were used in these studies to inhibit PPOs from mushroom (Zhou, Liu, Xiong, Zou, Chen, et al. 2016), plum (Ioniță et al. 2017), potato (Mosneaguta et al. 2012), wheat (Erat et al. 2010) and artichoke (Todaro et al. 2010). In the viewpoint of Zhou, Liu, Xiong, Zou, Chen, et al. (2016) and Mosneaguta et al. (2012), mailc acid inhibits PPO in the same mode as citric acid which was attributed to a decrease in pH. The inhibitory mechanism of acidulants on PPO is shown in Figure 1. Different concentrations of acidulants and buffer system induce different pH decrease in media. Moderately low pH (4.0 to 6.0) induce limited unfolding of most PPOs, which can influence enzyme-substrate interaction and thus decrease the catalytic efficiency of PPO. On the other hand, acidifiers with high concentration cause very low pH environments (pH < 4.0). This leads to unfolding and denaturation with substantial conformational changes, resulting in a very low or no activity (Figure 1a). Furthermore, protons produced by acid pH cause the dissociation of the substrate and change the protonation state of the enzyme. Protons can interact with the enzyme in competitive-type inhibition and the protonation of the *met*-PPO (hydroxo-bridged dicopper (II), *met*-form) and *oxy*-PPO (μ-η^2: η^2 peroxo dicopper (II), *oxy*-form) might hinder the access of the substrate to the active site, resulting in the inhibition of PPO catalyzed oxidation (Maria-Solano et al. 2016) (Figure 1b).

PPO inhibitors

Several studies and reviews on special PPO inhibitors have been published (Chang 2009; Oms-Oliu et al. 2010). The inhibition of PPO can be divided into two major classes,

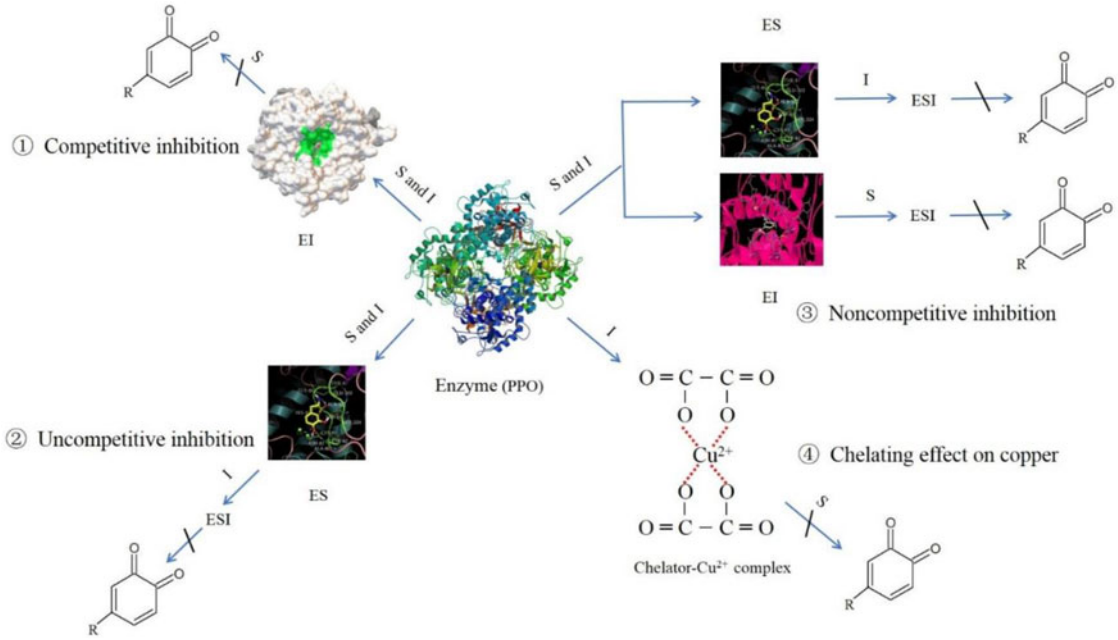

Figure 2. Inhibitory mechanism of organic acid as PPO inhibitors (L. Zhang et al. 2017; Zhou, Liu, Xiong, Zou, Chen, et al. 2016; Zhou, Xiong, et al. 2017). E, S and I represent enzyme, substrate and inhibitor, respectively.

reversible and irreversible inhibition. Most of the studies indicate that the inhibition of PPO by organic acids is mainly reversible inhibition (Aydemir 2010; Doğan and Salman 2007; Lin, Ng, and Wong 2016; Zhou, Liu, Xiong, Zou, Chen, et al. 2016). Reversible inhibition can be further subdivided into competitive, uncompetitive, noncompetitive and mixed types. Among reversible inhibitors, organic acids play an important role. Most of the studies in this respect are focused on aromatic carboxylic acids. Aromatic carboxylic acids are classified into two main series; benzoic acid series and cinnamic acid series. The other organic acids of interest in this category are organic acids which act as chelating agents that can bind to the copper in active site of PPO. These include oxalic acid, citric acid, EDTA and malic acid. This section discusses organic acids as PPO inhibitors and their mechanism of action.

Aromatic carboxylic acids in the benzoic acid series are frequently reported as PPO inhibitors in model systems (Table 1). Benzoic acid effectively inhibited PPO from various plants such as palmito (Robert et al. 1997), artichoke (Tuncay and Yagar 2011), vanilla bean (Waliszewski, Márquez, and Pardio 2009), apple (Can et al., 2014; Janovitz-Klapp et al. 1990) and medlar (Yolmeh and Sadeghi Mahoonak 2016). Concentration of benzoic acid in most of these studies were below 10 mM with some even below 1 mM. Compared to acidulants such as citric acid, benzoic acid at the same concentration showed much higher inhibitory effect on PPOs from vanilla bean (Waliszewski et al. 2009) and artichoke (Tuncay and Yagar 2011). Benzoic acid derivatives such as *p*-hydroxybenzoic acids (Gawlik-Dziki et al. 2008), *p*-alkoxybenzoic acids (Chen et al. 2005), *p*-alkylbenzoic acids (Lin et al. 2010), salicylic acid family compounds (Z. Wang et al. 2013; Zhang et al. 2006; Zhou et al. 2015) and vanillic acid (Robert et al. 1997) at a relatively low concentration range from 0.1 to 5.0 mM also showed effective inhibition of PPO activity (Table 1). Cinnamic acid series are another family of PPO inhibitors which have been shown to be effective in model systems. Cinnamic acid has low toxicity and a broad spectrum of biological activity and widely occurs in a number of plants (Sova 2012; Zhou, Liu, Xiong, Zou, Chen, et al. 2016). PPOs from various sources such as mushroom (Shi et al. 2005; Zhou, Liu, Xiong, Zou, Chen, et al. 2016), apple (Janovitz-Klapp et al. 1990), blueberry (Siddiq and Dolan 2017), palmito (Robert et al. 1997) and pineapple (Das, Bhat, and Gowda 1997) were effectively inhibited by cinnamic acid (Table 1). Concentrations of cinnamic acid used in all these studies were below 5 mM, indicating cinnamic acid is a strong PPO inhibitor (Table 1). A number of studies have also shown that cinnamic acid derivatives are effective PPO inhibitors (Table 1). Coumaric acid, ferulic acid, 2-chlorcinnamic acid and other derivatives with concentration lower than 1 mM exhibited effective inhibition on PPOs from mushroom (Hu et al. 2014; Shi et al. 2005), apple (Sukhonthara, Kaewka, and Theerakulkait 2016), potato (Sukhonthara et al. 2016) and palmito (Robert et al. 1997).

The mechanism of PPO inhibition by benzoic acid and cinnamic acid series compounds is well investigated and different types of inhibitory mechanisms are observed (Figure 2). Among the four inhibition types, competitive inhibition is the most commonly observed inhibitory mechanism. Competitive inhibitors bind to the active site of PPO and form enzyme-inhibitor (EI) complex, hindering the entrance of the substrate (Figure 2). Generally, competitive inhibitors have a similar structure with the substrate and the occupation of the catalytic center by these compounds induce the inhibition of PPO. Inhibition kinetics and computational simulations are widely used to analyze the competitive inhibition mechanism (Y. Wang et al. 2014; Zhou et al. 2015). With regard to uncompetitive inhibition, the inhibitor

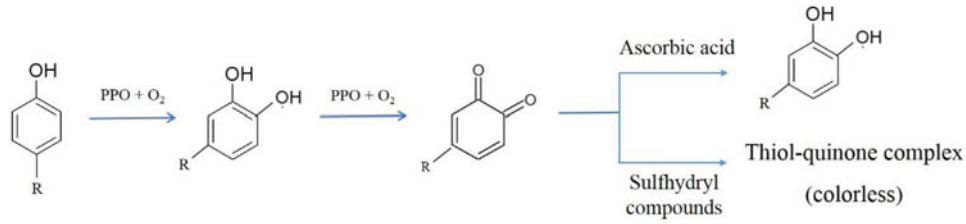

Figure 3. Inhibitory mechanism of organic acids as reducing agents.

can only interact with enzyme-substrate (ES) complex (Figure 2). As a result, the ternary complex of enzyme-substrate-inhibitor (ESI) is formed and terminate the oxidation of the substrate. In noncompetitive inhibition, inhibitors generally bind with the enzyme at somewhere other than the active site (Figure 2). This changes the conformation of the enzyme so that even if the substrate binds to the active site of the enzyme, the catalytic efficiency is reduced inhibiting the formation of oxidation products. Mixed inhibition is another form of noncompetitive inhibition where the inhibitors influence the binding of the substrate to the enzyme. Mixed type inhibition is frequently reported as one of the mechanisms through which organic acid inhibit the activity of PPO (Chen et al. 2005; Zhang et al. 2006; Zhou, Liu, Xiong, Zou, Chen, et al. 2016).

PPO contains a coupled binuclear copper site which plays an important role in the transportation of oxygen and the oxidation of the substrate (Wilcox et al. 1985). Organic acids such as oxalic acid, citric acid, kojic acid, EDTA and malic acid are the commonly used chelating agents in the food industry with the ability to bind to copper in active site of PPO (Figure 2). Yoruk and Marshall (2003b) reported that inhibition of PPO by oxalic acid was due to the chelation of the active site copper and oxalic acid was a more effective inhibitor compared with other acids with similar structures. Oxalic acid showed a strong affinity to form chelates with copper ion and the inactivated PPO partially recovered its activity after the addition of cupric ion (Son, Moon, and Lee 2000). Moreover, the activity of PPO incubated with oxalic acid was not restored via dialysis, indicating that the inhibition of PPO by oxalic acid was irreversible (Son et al. 2000). Similar results were observed in the study of Roux et al. (2003), where the activity of apple PPO inhibited by the chelation of copper was slightly recovered via exhaustive dialysis. These results suggest that irreversible inhibition might be the major type of PPO inhibition by metal chelators. Citric acid can also chelate copper and that was considered as one of the main mechanism through which it inhibited the activity of PPO from red Swiss chard (Gao, Han, and Xiao 2009; Wu and Tsai 2007). Kojic acid is known to be a strong metal chelator and was shown to bind strongly to dinuclear copper on the active site of PPO in two steps, preventing the entrance of the catechol substrate (Battaini et al. 2000). Among the various chelators, kojic acid is the most effective. Kojic acid at concentration lower than 1 mM induced strong inhibition on PPOs from various sources in many studies (Cheema and Sommerhalter 2015; Mishra et al. 2012; Palma-Orozco et al. 2014; Tao et al. 2013). Compared with kojic acid, oxalic acid and citric acid with low concentration (<10 mM) exhibited limited inhibitory effects on PPO (Fujita et al. 2006; Ioniță et al. 2017). In general, relatively high concentration (>20 mM) of oxalic acid and citric acid are required to show effective inhibition on PPO (Erat et al. 2010; Jiang, Pen, and Li 2004; Ünal 2007). High concentrations of oxalic acid and citric acid induce obvious decrease in pH even in buffer systems with high ionic strength. According to Warner and Weber (1953), the chelation of cupric ion by citric acid occurs at pH 6 to 7. It seems that chelation plays a major role in the inhibition of PPO by kojic acid whereas acidification might be a more important factor in the case of citric acid, oxalic acid and malic acid than chelation.

Reducing agents

The first step in the PPO catalyzed oxidation of polyphenols and enzymatic browning is the formation of the colorless intermediate *o*-quinone, which subsequently polymerizes to form brown pigments. Reducing agents can chemically reduce the *o*-quinones back to polyphenols or react with *o*-quinones to form the colorless thiol-quinone complexes (Figure 3) (Bottino et al. 2009; McEvily et al. 1992; Munoz et al. 2007), inhibiting enzymatic browning. However, reducing agents are gradually consumed by the oxidation products during the enzymatic reaction. Therefore, the inhibitory effect on enzymatic browning might be temporary which depends on the amount of reducing agents and oxidation products. In model systems, a lag period is frequently observed during the activity assay of PPO treated with reducing agents.

Ascorbic acid is an important component of anti-browning formulations on the market. In model systems, ascorbic acid is also the most widely studied organic acid on the inhibition of browning. Strong inhibition by ascorbic acid of PPOs from various sources such as lettuce (Altunkaya and Gokmen 2008; Landi et al. 2013), apple (Du et al. 2012; Han et al. 2019; Landi et al. 2013; Palma-Orozco et al. 2011), blueberry (Siddiq and Dolan 2017), plum (Ioniță et al. 2017), yam (Fujita et al. 2006; R Li et al. 2015), mango (Palma-Orozco et al. 2014), sweet potato (Lim et al. 2019) and Tadela (Benaceur et al. 2019) has been reported (Table 1). Most of the ascorbic acid concentrations in these studies are below 1 mM and complete inhibition of browning was observed in most cases (Table 1), indicating that ascorbic acid is very effective at inhibiting PPO activity in model systems. Das et al. (1997) observed that ascorbic acid was the most effective inhibitor of pineapple PPO and the lag period increased as the ascorbic acid concentration increased. The

amount of ascorbic acid consumed during PPO catalyzed oxidation depends on the reaction time with more ascorbic acid consumed by the more *o*-quinones produced during longer enzymatic oxidation time. Therefore, the reported inhibition degree of reducing agents on PPO depends on the measuring time during the activity assay by spectrophotometry. Besides, the lag period is inversely proportional to the PPO concentration since more *o*-quinones are formed per unit time at higher PPO concentration to react with ascorbic acid (Golan-Goldhirsh and Whitaker 1984). Although ascorbic acid results an acidic environment in solution, the reduction in pH is usually limited due to the buffering capacity of the matrix and low concentration of ascorbic acid. In a study by Golan-Goldhirsh and Whitaker (1984), ascorbic acid had little effect on O_2 uptake during the mushroom PPO activity assay by polarography (Golan-Goldhirsh and Whitaker 1984), which indicated that ascorbic acid acted more as a reducing agent than as a PPO inhibitor or an acidulant.

L-cysteine is a semi-essential amino acid with sulfhydryl moiety. Many studies investigated the inhibitory effect of L-cysteine on the activity of PPOs from various sources such as blueberry (Siddiq and Dolan 2017), plum (Ioniță et al. 2017), yam (Fujita et al. 2006), Cape gooseberry (Bravo and Osorio 2016), apple (de la Rosa et al. 2011) and longan (J. Sun et al. 2010) (Table 1). As in the case of ascorbic acid, L-cysteine at concentrations lower than 1 mM induced 90% or higher inhibition on PPO in most studies (Table 1). Moreover, effective inhibition on PPO from peach (Liang Liu et al. 2015), potato (Ali et al. 2016), loquat (Zhang and Shao 2016) and artichoke (Tuncay and Yagar 2011) was observed with L-cysteine concentrations lower than 0.1 mM (Table 1). Unlike ascorbic acid, the lag phase observed in L-cysteine treated PPO activity assay was due to formation of cysteine-quinone adduct and not quinone reduction (Dudley and Hotchkiss 1989). Analysis of oxidized products by loquat PPO showed similar results that L-cysteine inhibited browning by reacting with *o*-quinone to form a thiol-conjugated product (Ding et al. 2002) (Figure 3). Nevertheless, L-cysteine might inhibit PPO in dual mechanisms. A direct interaction of L-cysteine with PPO to inhibit the catalytic activity was also reported in some studies based on polarography study (O_2 uptake) (Dudley and Hotchkiss 1989; Golan-Goldhirsh and Whitaker 1984). Ali et al. (2015) observed that L-cysteine at high concentration (>1.0%) reacted with *o*-quinone to form colorless products while it acted as a competitive inhibitor at low concentration. These results indicate that L-cysteine has a multiple role in inhibiting browning in model systems. To investigate the effect of reductants on the activity of PPO, the combination of spectrophotometric assays with polarographic method is suggested to obtain more detailed information on the mechanism of inhibition.

Inhibitory effects of organic acids on PPO activity in food systems

Organic acids are widely used in foods to inhibit the enzymatic browning of plant based products. Compared with model systems, food systems are more complex and the food components influence the activity and stability of PPO. Moreover, the effect of organic acids on PPO activity may be dependent on the structure of the food matrix with different degree of inhibition in intact fruit or vegetable, fresh-cut, puree and juice. Products susceptible to browning such as banana, mushroom, litchi, longan and mango brown rapidly during storage and transportation. Some processing operations such as peeling, cutting, pressing and pureeing damage the integrity of plant tissues which enable PPO to have easy access to its natural substrates and O_2. Faster and serious browning occurs and results in shorter shelf-life of processed foods like apple juice, potato slices, strawberry and pear puree. The most frequently used organic acids to inhibit browning in food systems are ascorbic acid, citric acid, oxalic acid and phenolic acids such as salicylic acid (Table 2). Dipping, adding and spraying are the main methods of applying organic acid treatment in foods (Table 2).

Ascorbic acid

Ascorbic acid is the most widely used anti-browning agent in foods. Ascorbic acid with different concentrations has exhibited inhibition on PPO activity in real food systems such as apple juice (Yi and Ding 2014), peach slices (Zhu et al. 2009), sweet potato slices (Ojeda, Sgroppo, and Zaritzky 2014), apple cubes (Pizzocaro, Torreggiani, and Gilardi 1993; Rocha and De Morais 2005), lettuce slices (Ali et al. 2015), plum (Shao et al. 2011), litchi (D. Sun et al. 2010), carambola slices (Teixeira et al. 2008), sugarcane juice (Hithamani et al. 2018; Mao, Xu, and Que 2007), mung bean sprouts (Sikora and Świeca 2018) and mango puree (Guerrero-Beltrán, Swanson, and Barbosa-Cánovas 2005) (Table 2). Dipping is the most frequently used method in the application of ascorbic acid and the concentration commonly ranges from about 0.2% to 2% (Table 2). Zhu et al. (2009) reported that dipping with 0.2% ascorbic acid inhibited PPO activity and browning of peach slices by acting as an oxygen scavenger or reduction of enzymatically formed *o*-quinones to diphenols. In most studies, the effective dipping concentration of ascorbic acid to inhibit PPO activity is around 57 mM (1%) (Abbasi et al. 2013; Ali et al. 2015; D. Sun et al. 2010; Teixeira et al., 2008) (Table 2). However, dipping with relatively high concentration of ascorbic acid (500 mM) was used in sweet potato slices (Ojeda et al. 2014). Besides concentration, effect of ascorbic acid on the inhibition of PPO activity and enzymatic browning varies depending on dipping time and the food matrix. A longer dipping time might allow more ascorbic acid to transfer to the food system. The size and components of food also influence the absorption of ascorbic acid by the food matrix. Compared to dipping with 0.5% ascorbic acid, vacuum infiltration with ascorbic acid at the same concentration resulted in a higher internal ascorbic acid level, lower PPO activity and browning rate in the intact plum fruit during storage (Shao et al. 2011). Similarly, vacuum infiltration of ascorbic acid into the apple and potato improved the inhibitory efficiency (Sapers, Garzarella, and Pilizota 1990). These results

Table 2. Inhibition of organic acid on PPO in food system.

Source of PPO	Organic acid	Concentration and treatment	Results	Processing media	References
Peach	Ascorbic acid	0.2%, dipping	PPO and browning decreased	Peach slices	Zhu et al. (2009)
Sweet potato	Ascorbic acid	0.5 M, dipping	Effective inhibition in PPO and browning	Sweet potato slices	Ojeda et al. (2014)
Golden delicious apple	Ascorbic acid + citric acid	1 g/L +1 g/L, 10 g/L + 2 g/L dipping	46.7% and 87.1% inhibition in PPO	Apple cubes	Pizzocaro et al. (1993)
Lettuce	Ascorbic acid	1%, dipping	Inhibited the PPO activity and browning	Lettuce slices	Ali et al. (2015)
	Citric acid	1%, dipping	Inhibited the PPO activity and browning	Lettuce slices	
	Cysteine	1%, dipping	Inhibited the PPO activity and browning	Lettuce slices	
Apple (cv. Fuji)	L-cysteine	0.1–1.0 g/L, adding	Inhibited PPO activity and suppressed browning	Apple juice	Yi and Ding (2014)
	Ascorbic acid	0.1–1.0 g/L, adding	Inhibited PPO activity and suppressed browning	Apple juice	
Loquat fruit	Citric acid	250–750 mg/L, immersing for 2 min	Inhibited PPO activity and reduced browning	Intact loquat fruit	Abbasi et al. (2013)
	Ascorbic acid	250–750 mg/L, immersing for 2 min	Inhibited PPO activity and reduced browning	Intact loquat fruit	
Sugarcane	Citric acid	Below pH 3.9	Strongly inhibited PPO activity	Sugarcane juice	Hithamani et al. (2018)
	Ascorbic acid	Below pH 4.1	Completely inhibited PPO activity		
Grape	Ascorbic acid	3 and 6 mM	Partly inhibited PPO activity	Grape cluster	Lo'ay and El-Boray (2018)
	Salicylic acid	2 and 4 mM	Strongly inhibited PPO activity		
Plum	Ascorbic acid	Vacuum infiltration of 0.5% ascorbic acid	Inhibited browning rate and PPO activity	Intact plum	Shao et al. (2011)
Litchi fruit	Ascorbic acid	Dipping in 40 mM ascorbic acid for 15 min	Retarded the increase of PPO activity and browning during storage	Intact litchi fruit	D. Sun et al. (2010)
Carambola	Ascorbic acid	0.5% or 1% ascorbic acid immersing for 5 min	Reduced the PPO activity	Carambola slices	Teixeira et al. (2008)
Sugarcane	Ascorbic acid	0.1% ascorbic acid, adding	Prevented browning with reduced PPO activity	Sugarcane juice	Mao et al. (2007)
Mung bean sprouts	Ascorbic acid	2 and 20 mM	Reduced PPO activity and enzymatic browning during storage	Mung bean sprouts	Sikora and Świeca (2018)
Mango	Ascorbic acid	250–1000 mg/kg, adding	Inhibited PPO activity and reduced browning	Mango puree	Guerrerobeltran et al. (2005)
	Cysteine	100–300 mg/kg, adding	Inhibited PPO activity and reduced browning	Mango puree	
Avocado	Ascorbic acid	200 ppm, adding	Increased the kinetic rate of PPO activity inhibition	Avocado puree	Soliva-Fortuny et al. (2002)
Chinese pear	Ascorbic acid	3 mM, adding	Slightly inhibited PPO activity, greatly inhibited browning	Pear puree	Liao et al. (2020)
	Citric acid	120 mM, adding	Inhibited PPO activity and browning		
	Ferulic acid	10 mM, adding	Increased PPO activity, inhibited browning		
	Salicylic acid	10 mM, adding	No effect on PPO activity and inhibited browning		
Fragrant pear	Ascorbic acid	3 mM, adding	Increased PPO activity and greatly inhibited browning	Pear puree	Liao et al. (2020)
	Citric acid	120 mM, adding	Inhibited PPO activity and browning		
	Ferulic acid	10 mM, adding	No effect on PPO activity, inhibited browning		
	Salicylic acid	10 mM, adding	Effectively inhibited PPO activity, slightly inhibited browning		
Apple	Ascorbic acid	42.6 mM, dipping	Reduced PPO activity and color changes	Apple cubes	Rocha and De Morais (2005)
Litchi	L-cysteine	0.25% dipping	Significantly reduced brown index and PPO activity	Intact litchi	Ali et al. (2016)
Potato	Citric acid	1% and 2%, dipping	Effectively inhibited PPO activity and browning	Potato piece	Tsouvaltzis and Brecht (2017)
Banana	Malic acid	80 mM, dipping for 5 min	Inhibited PPO activity and alleviated chilly injury	Intact banana	Huang et al. (2016)
Chinese yam	Citric acid	20 mg/L, dipping for 25 min	Inhibited the PPO activity	Yam slices	Jia et al. (2015)
Yacon	Citric acid	1%, adding	97% inhibition of PPO activity	Yacon juice	Lago and Noreña (2014)
Strawberry	Citric acid	0.5, 1.0 and 2.5 g/100 g, adding	Inhibition of PPO activity and decrease in color difference were observed at 1.0 and 2.5 g/100 g	Strawberry puree	Holzwarth et al. (2013)

(*continued*)

Table 2. Continued.

Source of PPO	Organic acid	Concentration and treatment	Results	Processing media	References
Peach	Oxalic acid	5 mM oxalic acid dipping for 10 min	Increased PPO activity	Intact peach	Zheng et al. (2007)
Muskmelon	Oxalic acid	50 mM, dipping	Increased PPO activity and controlled postharvest disease	Intact muskmelon	Deng et al. (2015)
Banana	Oxalic acid	8 and 20 mM, dipping	PPO inhibition on the 8th day of storage, Browning delay	Intact banana	Huang et al. (2013)
Persimmon	Oxalic acid	5 mM, dipping	Suppressed PPO activity and alleviated chilling injury	Intact persimmon	Li et al. (2018)
Banana	Salicylic acid	1.0 mM, dipping	Effectively inhibited PPO	Intact banana	Khademi et al. (2019)
Sponge gourd	Salicylic acid	1.5 mM, dipping	Effectively inhibited PPO activity and alleviated chilly injury	Intact sponge gourd	Cong et al. (2017)
Anthurium flowers	Salicylic acid	2 mM, dipping	Result in low PPO activity, chilling injury and browning index	Anthurium cut flowers	Aghdam et al. (2016)
Mushroom	Salicylic acid	250 μM, dipping	Result in low PPO activity and browning index	Intact mushroom	Dokhanieh and Aghdam (2016)
Mango	Salicylic acid	2 mM, spraying	Increased PPO activity	Intact mango	Damodaram et al. (2015)
Pomegranate	Salicylic acid	250 μM, dipping	Reduced browning and PPO activity	Pomegranate aril	Dokhanieh, Aghdam, et al. (2016)
Pineapple	Salicylic acid	5.0 mM, immersion	Inhibited PPO activity and reduced browning index	Intact pineapple	Lu et al. (2011)
Peach	Salicylic acid	0.5–2.0 mM, dipping	PPO activity decreased at salicylic acid concentration higher than 0.5 mM	Intact peach	Tareen et al. (2012)
Bamboo shoot	Salicylic acid	1.0 mM, dipping	Reduced PPO activity and controlled chilling injury	Intact bamboo shoot	Luo et al. (2012)
"Qingnai" plum	Salicylic acid	1.5 mM, dipping for 10 min	Suppressed the increase of PPO activity and controlled chilling injury	Intact plum	Luo et al. (2011)
Laminaria japonica sporophyte	Salicylic acid	0.5 mM, dipping for 4 h	PPO was inhibited	Intact *L*aminaria japonica sporophyte	Zhou, Tang, and Wang (2010)
Mango	Salicylic acid	200, 400 and 600 ppm	Effectively inhibited PPO activity	Intact mango	Prasad and Sharma (2018)
Mushroom	4-methoxy cinnamic acid	100 μM, dipping for 60 s	Inhibited the PPO activity to control the browning	Intact mushroom	Hu et al. (2015)
Longan	α-aminoisobutyric acid	100 mM, vacuum-infiltration for 3 min	Activity of PPO was down-regulated with lower pericarp browning index	Intact longan fruit	Wang et al. (2015)
	β-aminoisobutyric acid	1 mM, vacuum-infiltration for 3 min	Activity of PPO was down-regulated with lower pericarp browning index	Intact longan fruit	
Anthurium flowers	γ-aminobutyric acid	5 mM, dipping for 15 min	Result in low PPO activity, chilling injury and browning index	Anthurium cut flowers	Aghdam et al. (2015)
"Golden Delicious" apple	chlorogenic acid	0.5 mM, spraying	Inhibited PPO activity and russet formation	Intact apple	L. Wang et al. (2014)
Iceberg lettuce	Gibberellic acid	0.1 mg/mL, spraying	Inhibited the PPO activity and browning	Intact iceberg lettuce	Tian et al. (2014)
Apple	Phytic acid	0.2% v/v, dipping	Increased PPO activity and controlled postharvest disease	Intact apple	Mahunu et al. (2016)

indicate that vacuum infiltration facilitate the transfer of exogenous organic acids to food tissue.

On the other hand, adding ascorbic acid is a more effective method and the concentration used is much lower than dipping method (Table 2). Mao et al. (2007) and Guerrero-Beltrán et al. (2005) reported that addition of 0.1% ascorbic acid suppressed the browning of sugarcane juice and mango puree with lower PPO activity. Addition of ascorbic acid at lower than 1 g/L also inhibited PPO activity in apple juice and avocado puree (Soliva-Fortuny et al. 2002; Yi and Ding 2014). Nevertheless, direct addition of ascorbic acid is only suitable for fruit juice and puree, but not for fresh-cut slices and intact fruit. Compared to model systems, the concentration of ascorbic acid needed to inhibit PPO in real food systems is much higher and this is the case for both dipping and direct addition. As stated above, ascorbic acid acts mainly as a reducing agent in inhibition of PPO, therefore, the inhibitory effect of ascorbic acid in food systems depends on the rate of *o*-quinones formation and ascorbic acid consumption. In model systems, the inhibitory effect is measured via PPO activity assay using spectrophotometry once enzyme and substrate are mixed. Thus, data from such

studies are based on short term observation. In contrast to model systems, exogenous ascorbic acid in food systems is gradually consumed as a consequence of enzymatic oxidation of the natural PPO substrates during storage, hence the need for higher concentration of ascorbic acid. Once the ascorbic acid is completely consumed, *o*-quinones are no longer reduced and browning may occur (Nicolas et al. 1994; Oms-Oliu et al. 2010). Consequently, ascorbic acid is suggested to be used in plant based foods intended for a relatively short storage period.

Citric, oxalic and malic acid

Citric and oxalic acid are the other two widely used anti-browning agents which are naturally present in some plants. In food systems, the inhibitory effects of citric acid on PPO and enzymatic browning has been studied in products such as potato pieces (Tsouvaltzis and Brecht 2017), lettuce slices (Ali et al. 2015), yam slices (Jia et al. 2015), yacon juice (Lago and Noreña 2014), strawberry puree (Holzwarth et al. 2013) and intact loquat fruit (Abbasi et al. 2013). The commonly used citric acid concentration for dipping ranges from about 50 to 100 mM (Ali et al. 2015; Tsouvaltzis and Brecht 2017), while some studies found that dipping with citric acid at concentrations lower than 5 mM also showed inhibition on PPO and browning in yam slices and loquat fruit (Abbasi et al. 2013; Jia et al. 2015) (Table 2). For direct addition of citric acid, concentration ranged from 0.5% to 2.5% are used in fruit juices and purees (Abbasi et al. 2013; Jia et al. 2015). Our recent study found that the pH of pear puree decreased from 4.4 to 3.7 after dipping in 1.5% citric acid for 30 min (Zhou, Liu, Stockmann, et al. 2018). Moreover, pear puree was acidified to pH 2.5 by addition of citric acid (2% w/w), resulting in lower PPO activity and reduced browning in pear puree (Zhou, Liu, Stockmann, et al. 2018).

Oxalic acid at concentrations ranging from 5 to 50 mM have been used in the dipping treatment of intact banana fruit (Huang et al. 2013), muskmelon (Deng et al. 2015), persimmon (Li et al. 2018) and peach (Zheng et al. 2007) (Table 2). PPO in banana and muskmelon were inhibited by oxalic acid while a slight increase in PPO activity was observed in peach (Deng et al. 2015; Huang et al. 2013; Zheng et al. 2007) (Table 2). The increase in the activity of peach PPO might be due to the low oxalic acid concentration used in the dipping process. Slight decrease in pH by dipping with 1 or 5 mM oxalic acid was not enough to inactivate PPO and the dipping treatment may have facilitated the extractability of the enzyme resulting in a higher PPO activity compared to the untreated samples. The suitability of malic acid for browning inhibition has also been investigated in a number of studies (Table 2). Huang et al. (2016) reported that the activity of PPO and skin browning in banana were suppressed by dipping in 80 mM malic acid. In addition to browning inhibition, these exogenous organic acids are found to affect many other enzymes and physiological responses such as the activities of peroxidase (POD) and superoxide dismutase, stress tolerance, accumulation of H_2O_2 and expression of defense proteins in fresh cut products (Huang et al. 2016; Zheng et al. 2007).

In many studies, the effective concentrations of citric and oxalic acids that are used in food systems are similar to model systems with different buffer solutions (Tables 1 and 2). The pH values decrease in both systems. Dipping or addition of citric, oxalic and malic acids can acidify the food and decrease PPO activity. The acidification degree depends on many factors such as the processing conditions, level of tissue disruption and the buffering capacity of the food system (Terefe et al. 2016). The inhibition of PPO and browning is dependent on the characteristics of the enzyme, especially the acid-pH stability. Many plants PPOs show high activity and stability in the pH range from 6.0 to 7.0, while the activity of PPO decreases drastically at pH lower than 4.0 (Altunkaya and Gökmen 2012; Cai et al. 2015; Cheema and Sommerhalter 2015; Dedeoglu and Guler 2009). On the other hand, several plants PPOs can still show relatively high activity at pH around 4 (Batista et al. 2014; Bravo and Osorio 2016; Liu et al. 2009). To avoid the excessive acidification of food, acidifiers such as citric, malic and oxalic acid are suggested to be used in food systems with PPO of low acid-pH stability. In such cases, partial or complete inactivation of PPO by decreasing pH can prolong the storage time of foods with sustained anti-browning effects.

Aromatic carboxylic acids

Salicylic acid is a natural and ubiquitous phenolic acid in plants that can be used during postharvest processing to extend storage life of fruit and vegetables (Dokhanieh and Aghdam 2016; Lu et al. 2011) (Table 2). In addition, salicylic acid can alleviate chilling injury of fruits and vegetables, prevent cardiovascular disease and exhibit other benefits for human health such as reducing risk of developing atherosclerosis and colorectal cancer (Deng et al. 2001; Janssen et al. 1997; Peng and Jiang 2006). Therefore, fruits and vegetables with high salicylic acid are recommended in a healthy diet (Scheier 2001). The inhibitory effects and mechanisms of many aromatic carboxylic acids on PPO including competitive inhibition have been well investigated in model systems, while salicylic acid is the only one which has been widely studied for the inhibition of PPO in food systems. Dipping with salicylic acid has been evaluated in foods such as mushroom (Dokhanieh and Aghdam 2016), banana (Khademi, Ashtari, and Razavi 2019), sponge gourd (Cong, Wang, and Dong 2017), Chinese water chestnut slices (Peng and Jiang 2006), Pomegranate aril (Dokhanieh, Aghdam, and Sarcheshmeh 2016), peach (Tareen, Abbasi, and Hafiz 2012), bamboo shoot (Luo et al. 2012), Mango (Prasad and Sharma 2018) and plum (Luo, Chen, and Xie 2011) (Table 2). PPO activity and browning rate were inhibited in these food systems with a relatively low salicylic acid concentration ranging from 0.25 to 5 mM (Table 2). These salicylic acid concentrations are similar to those used in studies in model systems. Salicylic acid acts as a competitive PPO inhibitor, which can reversibly and sustainably inhibit PPO

in model systems (Zhang et al. 2006; Zhou et al. 2015). In most of food systems, salicylic acid acts as a PPO inhibitor while activity increase from 7.5 to 15.0 U/mg was reported with the use of salicylic acid on mango PPO (Damodaram et al. 2015). For Chinese water chestnut, salicylic acid at concentration higher than 2 mM significantly increased PPO activity in a model system. Nevertheless, increasing salicylic acid concentrations enhanced the inhibition of PPO in Chinese water chestnut (Peng and Jiang 2006). Limited information is available on the impact of other aromatic carboxylic acids on PPO activity in food systems. Chlorogenic acid and 4-methoxycinnamic acid have been found to effectively inhibit PPO in intact apple (L. Wang et al. 2014) and mushroom (Hu et al. 2015), respectively. Hu et al. (2015) reported that PPO activity and browning in intact mushroom were significantly inhibited by dipping in 100 μM 4-methoxycinnamic acid for 60 s. Spraying exogenous chlorogenic acid at concentration of 0.5 mM could inhibit PPO activity to ~50% in intact apple (L. Wang et al. 2014) (Table 2).

The effect of aromatic carboxylic acids on fresh cuts and intact fruits and vegetables is complex since the treatment influences other physiological and metabolic activities of the tissue. Several studies have found that treatment by aromatic carboxylic acids affects the activities of other enzymes such as phenylalanine ammonia-lyase (PAL), POD, superoxide dismutase, glutathione reductase, catalase and ascorbate POD (Aghdam et al. 2016; Peng and Jiang 2006), which are all related to response of plants to applied stress. The activity of PAL is responsible for the accumulation of polyphenols, the substrates in enzymatic browning reactions, in the phenylpropanoide pathway as a response to stress during peeling, slicing etc. in fresh cut production. Moreover, POD and PPO might have synergistic effect on the browning of fruit and vegetables (Tomás-Barberán and Espín 2001).

The evaluation of the effectiveness of treatments by aromatic acids on PPO activity in real food systems involves the extraction of the enzyme from the tissue after the acid treatment. In the extraction process, the concentration of the inhibitor decreases after addition of the extraction solution and the inhibitory effect might weaken because of the reversible mode of inhibition. Thus, the measured activity reduction may not truly reflect the actual effect in situ. Indeed, the activity of PPO in aromatic carboxylic acids treated food can't be accurately determined by in vitro analysis involving extraction and spectrophotometry. According to Gomes et al. (2014), inferences on food browning based on the activity of PPO can be misleading and measurement of food color is suggested as a reliable method to assess the effectiveness of anti-browning additives in food systems. Because of safety concerns, their poor water solubility and perhaps cost, far less aromatic carboxylic acids have been studied in food systems and used in the food industry compared to model systems. Therefore, toxicological tests are required for applying these new PPO inhibitors. In order to improve the application of natural aromatic carboxylic acids, delivery systems such as emulsion, liposome and edible coatings are suggested for controlled release of the inhibitors into food matrices.

L-cysteine

L-cysteine is one of the most effective anti-browning agents and its effect have been widely studied in fruits and vegetables (Ali et al. 2015; Ding et al. 2002; Gorny et al. 2002; Guerrerobeltran, Swanson, and Barbosacanovas 2005) (Table 2). Among these food systems, the effect of L-cysteine on the activity of PPOs from litchi, lettuce, apple and mango have been investigated (Ali et al. 2015; Ali, Khan, and Malik 2016; Guerrerobeltran et al. 2005; Yi and Ding 2014) (Table 2). As with other PPO inhibitors, dipping and direct addition of L-cysteine are the main methods with the concentration used in direct incorporation much lower than that used in dipping (Table 2). For dipping treatment, 0.25% to 1% L-cysteine is effective in inhibiting PPO and browning of litchi and lettuce (Ali et al. 2015; Ali et al. 2016). Unlike dipping, addition of L-cysteine at relatively low concentration (0.01%–0.03%) exhibited strong inhibition on PPO and browning in mango puree (Guerrerobeltran et al. 2005). Similar result was observed in apple juice by adding L-cysteine at 1.0 g/L (Yi and Ding 2014) (Table 2). As stated above, L-cysteine inhibits PPO following dual mechanisms. At high concentration, reaction with *o*-quinone to from colorless products is the main mode of inhibition by L-cysteine. Ding et al. (2002) reported that the required concentration of L-cysteine to effectively inhibit browning of loquat juices was dependent on the concentration of endogenous phenolic substrates inherent in different cultivars. Some studies reported that application of L-cysteine can substantially inhibit the browning of fruits and vegetables, indicating the direct inhibition of L-cysteine on the PPO (Ali et al. 2016; Pace et al. 2015). In general, the inhibitory effect of L-cysteine on browning of food systems is a complex phenomenon, which is not only influenced by processing conditions, but also the properties of the food matrix such as PPO activity and concentration of endogenous substrates.

Model systems versus real food systems

Comparison of studies in model systems and real food systems indicate that there is a difference in the effectiveness of organic acids for inhibition of browning and PPO activity in model and real food systems. In our recent study, different inhibitory effects of ascorbic, citric, ferulic and salicylic acid on pear PPO were observed in model systems and pear puree (Liao et al. 2020). Results showed that all the four organic acids could effectively inhibit pear PPO in model system while their inhibitory effect was significantly weakened in pear puree. Besides, no significant correlation was observed between the browning of pear puree and the measured PPO activity (Liao et al. 2020). The difference in the effectiveness of organic acids for inhibition of PPO activity in model and food systems can be attributed to several factors; (1) Food components such as sugars, salts and pectin may have protective effects on enzymes including PPO and

improve their stability in food systems. (2) Differences in PPO source (cultivar, growing conditions etc.) and concentrations as well as differences in substrate compounds and their concentration may result in different inhibitory effect because of potential differences in enzyme structure between different isoforms of PPO as well as the substrate specificity of enzymatic reactions. (3) The use of organic acids acting as acidulants may result in different acidification degree because of the difference in buffering capacity between model systems and food systems. (4) PPO activity assay in vitro can not exactly determine PPO activity in vivo because of the changes in the concentration of the organic acid during enzyme extraction and the use of an artificial substrate (which may not actually be present in the food matrix in such assays) during the in vitro assaying of the enzyme with the difference more obvious especially in the case of organic acids acting as reducing agents.

Combining organic acids with other preservation technologies for inhibition of PPO and enzymatic browning

Organic acids are frequently used in food processing as adjuvants for enhancing the inhibition of pathogenic and spoilage organisms as well as undesirable quality changes such as enzymatic browning. The use of such adjuvants enable reduction in process intensity resulting in better quality retention. The use of organic acids is especially important for the commercial applications of emerging non-thermal processing technologies such as high pressure as these processes have limited effects on enzymes under commercially feasible conditions (Terefe et al. 2013). This section discusses the application of various organic acids for inhibition of PPO and enzymatic browning in combination with preservation technologies such as high pressure processing, thermal processing and edible coating.

High pressure processing

High pressure processing (HPP) is considered as a better alternative to thermal processing for preservation of food products since it minimizes the degradation of nutritional compounds and quality attributes (Terefe et al. 2013). Several studies have reported that HPP under commercially relevant condition (600 MPa, 3–5 min hold times) has limited inactivation effect on enzymes such as PPO, POD, pectin methylesterase and polygalacturonase both in model and food systems (Terefe et al. 2014). Most PPOs from plants show very high resistance to HPP induced inactivation at room temperature (Terefe et al. 2014; Zhou, Liu, Stockmann, et al. 2018). Therefore, a combination of HPP with other hurdles such as temperature and organic acids have been investigated for the inhibition of PPO and browning in fruits and vegetables. Citric acid, ascorbic acid, benzoic acid and L-cysteine are the most widely used organic acids in combination with HPP. Combination of HPP and citric acid could effectively inactivate PPO in tomato puree (Plaza et al. 2003), chopped garlic (Hong and Kim 2001), pear puree (Zhou, Liu, Stockmann, et al. 2018), mango pulp (Kaushik, Nadella, and Rao 2015) and pineapple puree (Chakraborty, Rao, and Mishra 2014) (Table 3). The pH values of pear puree, mango pulp and pineapple puree in these studies significantly decreased after citric acid treatment and the lower pH resulted in higher degree of PPO inactivation at the same HPP condition (Chakraborty, Rao, and Mishra 2014; Kaushik et al. 2015; Zhou, Liu, Stockmann, et al. 2018). As discussed in "*Acidulants*," citric acid is the most widely used acidulant in the food industry. Some studies have observed that lowering the pH by acids could increase the susceptibility of PPO to HPP inactivation (Chakraborty, Rao, and Mishra 2014; Weemaes et al. 1998). Mushroom PPO at pH 4 is more sensitive to pressure inactivation than PPO at pH 5, which is in turn considerably more sensitive than PPO at pH 6.5 (Weemaes et al. 1999). Besides, reducing pH from 8.0 to pH 4 resulted in a decrease in minimum inactivation pressure from 850 MPa to 450 MPa (Weemaes et al. 1998). As shown in Table 3, many fruit based products such as mango pulp, pineapple and pear puree were acidified by dipping with citric acid to pH 4.0, pH 3.0 or even lower, which enhanced the inactivation of PPO by HPP (Chakraborty, Rao, and Mishra 2014; Kaushik et al. 2015; Zhou, Liu, Stockmann et al., 2018).

Ascorbic acid is another widely used organic acid in combination with HPP. PPO in chopped garlic (Hong and Kim 2001), apple juice (Valdramidis et al. 2009), mango puree (Guerrerobeltran et al. 2005), and peach puree (Guerrerobeltran, Barbosacanovas, and Swanson 2004) were effectively inhibited by a combination of ascorbic acid and HPP (Table 3). However, HPP at 400 or 600 MPa combined with 0.04% ascorbic acid could not inhibit PPO activity in Nectarine puree perhaps due to the low concentration (García-Parra et al. 2014). Synergistic effects were observed in inhibiting PPO in mango puree when HPP at 586 MPa and 20 min was combined with 500 ppm ascorbic acid (Guerrerobeltran et al. 2005). The concentrations used in most of these studies ranged from 300 ppm to 1000 ppm (Table 3). Compared with citric acid, the concentration of ascorbic acid required for effective inactivation of PPO is much lower. This might be due to the different inhibitory mechanisms of citric acid and ascorbic acid on PPO. Combined HPP treatment (379 to 586 MP, 5 to 25 min) with L-cysteine at relatively low concentrations of 200 and 300 ppm were used in the processing of mango puree (Guerrerobeltran et al. 2005) and peach puree (Guerrerobeltran et al. 2004), and results showed that the inhibition of PPO was improved after the combined treatment. Benzoic acid at 5 mM did not stabilize or sensitize PPO in model system toward high pressure (Weemaes et al. 1999) while 50 mM benzoic acid caused sensitization toward pressure inactivation (Weemaes et al. 1997). This sensitizing effect induced by benzoic acid was not be due to decrease in pH since only 0.02 units pH drop was observed by the addition of 50 mM benzoic acid (Weemaes et al. 1997). Overall, in combination with HPP, organic acids act as acidulants, inhibitors and reducing agents depending on the type of organic acid to exhibit synergic effect on PPO inactivation.

Table 3. Combining organic acids with other processing techniques for the inhibition of PPO and enzymatic browning.

Source of PPO	Combination method	Combination condition	Results	Processing media	References
Garlic	HPP	Addition with citric acid (10 g/kg), ascorbic acid (10 g/kg) or a mixture of two (5 g/kg) and HPP (600 MPa - 1 min) treated	PPO activity was reduced to nearly zero	Chopped garlic	Hong and Kim (2001)
Pear	HPP	Dipping in 1.5% citric acid and HPP (600 MPa, 1–5 min) at 55 and 90 °C	Combined treatment was more effective in reducing PPO activity and browning of puree	Pear puree	Zhou, Liu, Stockmann, et al. (2018)
Pineapple	HPP	Adjust to pH 3.0 with 1% citric acid and HPP (100–600 MPa, 20–70 °C, 0–30 min)	Lower pH induced by citric acid increased inactivation rate of PPO	Pineapple puree	Chakraborty, Rao, and Mishra (2014)
Mango	HPP	Adjust to pH 3.5 or pH 4.0 with 1% citric acid and HPP (400–600 MPa, 40–70 °C, 6–20 min)	Lower pH resulted in higher PPO inactivation	Mango pulp	Kaushik et al. (2015)
Nectarine	HPP	HPP (400 and 600 MPa) combined with 0.04% ascorbic acid	Ascorbic acid could not decrease PPO activity especially when HPP was used	Nectarine puree	García-Parra et al. (2014)
Tomato	HPP	HPP (50–600 MPa) combined with citric acid (0–2%, w/w)	Significantly improve PPO inactivation	Tomato puree	Plaza et al. (2003)
Apple	HPP	HPP (750 MPa, 10–50 °C, 0–90 min) with 300 ppm added ascorbic acid	Highest reduction of PPO with 51.47% was achieved at 50 °C for 25 min	Apple juice	Valdramidis et al. (2009)
Mango	HPP	Addition of 500 ppm ascorbic acid or 200 ppm L-cysteine combined with HPP (379–568 MPa for 0–20 min)	Inhibited PPO activity and improved the color stability	Mango puree	Guerrerobeltran et al. (2005)
Peach	HPP	Addition of 1000 ppm ascorbic acid or 300 ppm L-cysteine combined with HPP (207–512 MPa for 0–25 min)	Inhibited PPO activity and discoloration	Peach puree	Guerrerobeltran et al. (2004)
Mushroom	HPP	Addition of 5 mM benzoic acid combined with HPP (750–900 MPa)	The benzoic acid did not stabilize or sensitize the PPO	100 mM phosphate buffer (pH 6.5)	Weemaes et al. (1999)
Peach	HPP and vacuum packaging	1% (w/v) ascorbic acid and 1% (w/v) citric acid dipping, HPP (500 MPa/5 min) and vacuum-packed	Reduced browning and PPO activity	Peach cubes	Denoya, Vaudagna, and Polenta (2015)
Mushroom	Thermal treatment/ pressure treatment	Addition of 50 mM benzoic acid combined with HPP or thermal treatment	Benzoic acid at 50 mM protected the PPO toward temperature but caused sensitization toward pressure	100 mM phosphate buffer (pH 6.5)	Weemaes et al. (1997)
Pomegranate	Thermal processing	Dipping in 250 μM salicylic acid at 45 °C for 30 s	Reduced browning and PPO activity	Pomegranate aril	Dokhanieh, Aghdam, et al. (2016)
Mango	Thermal processing	Homogenizing mango with 0.2% citric acid (w/v 1:1) and blanching at 90 °C for 4 min	Complete inactivation of PPO	Mango puree	Guiamba and Svanberg (2016)
Radish	Thermal processing	Immersion in water for 1.5 min at 50 °C followed by immersion in 2% ascorbic acid solution for 5 min	Prevented browning and inhibited PPO activity	Radish slices	Goyeneche et al. (2015)
Lotus root	Heat treatment and modified-atmosphere packing	Immersion at hot water (55 °C) for 45 s and dipped with 1% ascorbic acid + 0.5% chamomile, and packed with 100% CO_2	Effectively inhibited PPO and extended the shelf-life to 21 day at 5 °C	Lotus root slices	Son et al. (2015)
Purple sweet potato	Thermal processing	Immersing in a water bath at 90 °C for 10 min combined with addition of 1% w/v sodium borate, 1% w/v oxalic acid and 1% w/v citric acid	Completely inactivation of PPO and increasing polyphenolic yields	Purple sweet potato cubes	de Aguiar Cipriano et al. (2015)
Yacon	Thermal treatment	Acidified with of 1% citric acid prior to blanching (0–10 min, 80–100 °C)	Effectively inhibited PPO activity	Yacon juice	Lago and Noreña (2014)
Apple	Thermal processing	Heating at 45 or 60 °C in the presence of 1 mM ascorbic acid	The combined treatment was more effective in reducing PPO activity	20 mM malate buffer (pH 3.8)	Chow et al. (2011)

(*continued*)

Table 3. Continued.

Source of PPO	Combination method	Combination condition	Results	Processing media	References
Apple	Thermal treatment	Heating at 50 or 70 °C in the presence of 1 mM ascorbic acid	Ascorbic acid inhibited the thermal inactivation of PPO	20 mM malate buffer (pH 4.5)	Aka et al. (2013)
Sugarcane	Thermal treatment	Blanching in boiling water for 5 min and adding 0.1% ascorbic acid	Prevented browning with completely inactivation of PPO activity	Blanching: peeled stems Adding acid: sugarcane juice	Mao et al. (2007)
Mushroom	Ultrasound	20 kHz ultrasound with 10, 20 and 30 mM malic acid	The combination method effectively inactivated PPO	50 mM sodium phosphate buffer (pH 6.8)	Zhou, Liu, Xiong, Zou, Liu, et al. (2016)
Banaa	Ultrasound	40 kHz ultrasound with 1 mM salicylic acid	Combined treatment effectively inactivated PPO	Intact banana	Khademi et al. (2019)
Mushroom	Ultrasound and modified atmosphere package	Ultrasound (400 W/20 kHz/10 min) in citric acid (10 g/L) with modified atmosphere package	Inhibited PPO activity and extended shelf life to 12 days at 4 °C	Intact mushroom	Lagnika et al. (2014)
Radish	Ultrasound	Ultrasound (180 W) for 5 min followed by acetic acid (0.05%, w/w) solution for 5 min	Retained the product color and inhibited PPO activity	Radish slices	Goyeneche et al. (2014)
Whole-wheat raw noodles	Ultrasound	50, 100 and 150 s ultrasound combined with 1 g/100 g ascorbic acid	∼30%, ∼55% and ∼90% inhibition of PPO, effectively prevented the browning	Suspensions of whole-wheat raw noodles	Niu et al. (2014)
Fuji apple	Ultrasound	40 kHz ultrasound with 1% ascorbic acid for 1 min	46% and 98% inhibition rate on browning and PPO activity	Apple cubes	Jang et al. (2009)
Yam	UV-C	1% (w/v) ascorbic acid and 0.1% (w/v) calcium chloride dipping and 6.84 kJ m^{-2} UV-C treating	Lower browning index and PPO activity	Yam slices	Teoh et al. (2016a)
Potato	UV-C	1% (w/v) ascorbic acid and 0.1% (w/v) calcium chloride dipping and 6.84 kJ m^{-2} UV-C treating	Lower PPO activity and higher total phenolic content	Potato slices	Teoh et al. (2016b)
Eggplant	Gamma irradiation	Combination of gamma irradiation (0.25–1 kGY) and ascorbic acid (2%)	Effectively inhibited PPO activity and prevented the surface browning	Eggplant pieces	Hussain et al. (2014)
Papaya	Coating	Chemical dipping (0.18 M calcium chloride, 0.058 M ascorbic acid and 0.008 M vanillin) for 3 min and *Aloe vera* gel (30 min)	Lower browning index and relatively low PPO activity during storage of 12 days	Papaya cubes	Kuwar et al. (2015)
Apple	Coating	Carboxymethyl cellulose (1% w/v) coating in combination with $CaCl_2$ (0.5%) and ascorbic acid (2%)	Suppressed browning and lower PPO activity	Apple slices	Saba and Sogvar (2016)
Plum	Coating	Dipping in a solution containing 40 mM ascorbic acid and 1% (w/v) chitosan	Significantly decreased PPO activity and contributed to the shelf-life extension and quality maintenance	Intact plum	Liu et al. (2014)
Persimmon	Coating	Apple pectin (10 g/kg) coating combined with 10 g/kg citric acid and 10 g/kg $CaCl_2$	Effectively inhibited browning and PPO activity	Persimmon pieces	Sanchís et al. (2016)
Banana	Coating	Chitosan nanoparticles with 1% citric acid or 1% acetic acid	Effectively inhibited PPO activity	Banana slices	Kim et al. (2013)
Grape	Coating	Xanthan gum (10% g/mL) enriched ascorbic acid (1% w/v) or citric acid (1% w/v) coating	Significantly suppressed PPO activity	Intact grape	Golly et al. (2019)
Mushroom	Coating	Coating with 0.1% (w/v) chitosan/tripolyphosphate nanoaggregates solution loaded with 0.7% (w/v) ascorbic acid	Significantly reduced PPO activity and delayed browning of mushrooms.	Mushroom slices	Ojeda et al. (2019)
Apple	Coating	15% aloe vera gel or 1% carboxy methylcellulose coating enriched with 1% ascorbic acid and 1% $CaCl_2$	PPO activity was low and browning was inhibited	Apple wedges	Kumar et al. (2018)
Grape	Coating	Chitosan/polyvinyl alcohol mixed with salicylic acid at 1 or 2 mM	Inhibited PPO activity and extended shelf life of grape	Grape cluster	Lo'ay and El-khateeb (2018)

(*continued*)

Table 3. Continued.

Source of PPO	Combination method	Combination condition	Results	Processing media	References
Apple	Coating	Montmorillonite (3 g/100 g) and citric acid (5, 10 g/100 g) dispersed in whey protein isolate film	Reduced PPO activity and enzymatic browning	Apple slices	Azevedo et al. (2018)
Persimmon	Coating and modified atmosphere packing	Apple pectin (10 g/kg) coating combined with 10 g/kg citric acid and 10 g/kg $CaCl_2$, packed in modified atmosphere packing (5 kPa O_2, balance N_2)	Effectively inhibited PPO activity during storage	Persimmon pieces	Sanchis et al. (2017)
Pomegranate	Coating	Dipping in 1% (w/v) chitosan and 1% (v/v) acetic acid for 1 min	Suppressed PPO activity and delayed the decrease of total phenolics	Aries	Ghasemnezhad et al. (2013)
Apple	Coating	1% Chitosan coating combined with 2% ascorbic acid and 0.5% $CaCl_2$	Retarded the increase of PPO activity during storage	Apple slices	Qi et al. (2011)
Litchi fruit	Coating	Dipping in mixed solution containing 40 mM ascorbic acid and 1% chitosan	Retarded the increase of PPO activity and browning during storage	Intact litchi fruit	D. Sun et al. (2010)
Peach	Coating	Chitosan coating with 2.0 and 4.0 mg/mL citric acid	PPO and browning decreased	Apple slices	Pilon et al. (2015)
Sweet potato	Coating	Cassava coating added with 0.5 M ascorbic acid	Effective inhibition in PPO and browning	Sweet potato slices	Ojeda et al. (2014)
Pear	Coating	Xanthan gum based edible coating (2.5 g/L) enriched with cinnamic acid (1 g/L)	Reduced browning and PPO activity	Pear slices	Sharma and Rao (2015)
Celery	Modified atmosphere packing	Packed in 20% atmospheric air + 80% N_2 after dipping with 1.5% citric acid for 5 min or 0.5% L-cysteine for 10 min	Effectively inhibited PPO activity during storage	Celery slices	Tamer et al. (2013)
Carambola	controlled atmosphere	1% ascorbic acid immersing for 5 min and under 0.4% O_2 at a flow rate of 50 mL/min	Inhibited PPO activity and prevented browning	Carambola slices	Teixeira et al. (2008)
Mushroom	Modified atmosphere packing	MAP in combination with chemical treatments (sorbitol 0.05 g/100 g, $CaCl_2$ 1.0 g/100 g and citric acid 3.0 g/100 g)	Effectively inhibited PPO activity	Intact mushroom	Xiao et al. (2011)
Potato	Sodium acid sulfate	1% sodium acid dip treatment including 1% citric acid and ascorbic acid	Lower PPO activity and browning	Potato slices	Calder et al. (2011)
Apple	Calcium chloride	Dipping in 1% calcium chloride with 1% citric acid or ascorbic acid	Inhibited the increase of PPO activity during storage	Apple slices	Chiabrando and Giacalone (2012)
Mango	Calcium nanoparticles	Immersing in calcium nanoparticles blended with ascorbic acid (6 or 9 mM)	Significantly reduced PPO activity and internal browning of mango	Intact mango	Lo'ay and Ameer (2019)
Apple	NaCl	Combination of NaCl (0.1 M) ascorbic acid (0.001 or 0.05 M) dipping	Effectively inhibited PPO and browning	Apple slices	Li et al. (2015)
Grape	Yeast	Combination of yeast *Hanseniaspora uvarum* suspension of 1×10^8 CFU/mL and 2 mM salicylic acid	Significantly reduced browning index, controlled gray mold and increased PPO activity	Grape berries	Qin et al. (2015)
Litchi	Glutathione	Dipping in water containing 10 mM glutathione and 100 mM citric acid for 5 min	Inhibited PPO activity and prevented browning	Intact litchi fruit	Jiang and Fu (1998)
Peach	NO	0.5 μM NO + 0.2% ascorbic acid, dipping	Effective inhibition in PPO and browning	Peach slices	Zhu et al. (2009)
Banana	N_2	Addition of 0.2% citric acid and 0.2% ascorbic acid and infusion of N_2	Lower browning index and PPO activity	Banana smoothies	S. Wang et al. (2013)
	CO_2	Addition of 0.2% citric acid and 0.2% ascorbic acid and infusion of CO_2	Lower browning index and PPO activity	Banana smoothies	
Lemon	Methyl jasmonate	Dipping in 10 μM methyl jasmonate plus 2 mM salicylic acid	Effectively enhanced chilling tolerance and inhibited PPO	Intact lemon	Siboza et al. (2014)
Apricot	Plant proteases	100 and 500 mg of ascorbic acid and 10, 50 and 100 mg of protease preparations per 100 g of fresh apricots	Effectively inhibited PPO and browning	Apricot puree	Derardja et al (2019)

Thermal processing

Thermal processing is recognized as the most effective, economical and widely used method to inhibit PPO and enzymatic browning (Awuah, Ramaswamy, and Economides 2007; Zhou, Liu, et al. 2017). Compared with PPO in model systems, PPO in food systems seems to be more resistant to thermal inactivation (Zhou, Liu, Stockmann, et al. 2018). However, thermal processing especially at high temperature might cause detrimental changes in product's sensory and nutritional attributes (Terefe et al. 2014). Accordingly, other methods are often used in combination with thermal processing to reduce the intensity of the thermal process and the consequent undesirable changes in fruits and vegetables. Organic acids that act as acidulants and PPO inhibitors are commonly used in foods during thermal processing. Mango puree, purple sweet potato cubes and yacon juice were acidified by citric acid prior to thermal processing (80–100 °C/0–10 min), resulting in effective or completely inactivation of PPO (de Aguiar Cipriano et al. 2015; Guiamba and Svanberg 2016; Lago and Noreña 2014) (Table 3). The yacon juice was acidified to pH 2.93 and the final pH of the mango puree were 3.9 (Guiamba and Svanberg 2016; Lago and Noreña 2014). Therefore, citric acid acted as an acidulant rather than a chelator since the complexes between cupric ion and citric acid are normally formed at pH 6 to 7 (Warner and Weber 1953). Liu et al. (2013) found that mushroom PPO treated with citric acid was more susceptible to thermal treatment (50–65 °C/0–60 min) and the thermostability of PPO decreased significantly. Similar results were observed in our previous studies that citric acid (2%, w/v) induced sensitizing effect on pear PPO to thermal inactivation both in buffer system and pear puree (Zhou, Liu, Stockmann, et al. 2018; Zhou, Liu, and Terefe 2018).

Ascorbic acid is commonly combined with mild heating processes to effect acidification and enhance the inactivation of PPO. Relatively high ascorbic acid concentrations of 1% and 2% were used to dip fresh-cut lotus root (Son et al. 2015) and sliced radish (Goyeneche et al. 2015), respectively (Table 3). Ascorbic acid immersion for 5 min and mild thermal shock at 50 °C for 1.5 min were successful hurdles to inactivate PPO and preserve the radish slices (Goyeneche et al. 2015). Similar result was observed in fresh-cut lotus root treated with thermal processing at 55 °C for 45 s (Son et al. 2015). With regard to addition of ascorbic acid, 1 mM ascorbic acid combined with heating at 45 °C was effective in reducing apple PPO activity in model system and the inactivation of PPO increased by 30% compared to heating alone at the same condition (Chow et al. 2011). Similarly, salicylic acid, oxalic acid and benzoic acid combined with heat treatment were evaluated for inhibiting PPO in pomegranate aril (Dokhanieh, Aghdam, et al. 2016), purple sweet potato cubes (de Aguiar Cipriano et al. 2015) and PPO extract from mushroom (Weemaes et al. 1997). The detailed processing condition and results are summarized in Table 3. Overall, organic acids such as citric acid act as acidulants reducing the thermostability of PPO, and increasing its sensitivity toward thermal inactivation. Reducing agents such as ascorbic acid combined with mild heat treatment are effective hurdles to inhibit PPO and browning especially in fresh cut fruits and vegetables. The use of organic acids helps to reduce undesirable changes in fruits and vegetables that can be caused by thermal processing.

Ultrasound

The application of ultrasound technology in the food industry has been studied for many years (Terefe, Buckow, and Versteeg 2015a). Low frequency (20–1000 Hz) high power ultrasound is mainly used in food processing whereas high frequency (1–10 kHz) low power ultrasound is commonly used for nondestructive measurement of food properties (Mason, Paniwnyk, and Lorimer 1996; Terefe, Buckow, and Versteeg 2015a). Ultrasound can be used to modulate the activity of enzyme although ultrasound treatment alone does not cause enzyme and microbial inactivation sufficient for food preservation (Terefe, Buckow, and Versteeg 2015a; Zhou, Liu, Xiong, Zou, Liu, et al. 2016). Therefore, a combination of ultrasound and other hurdles such as heat, ultraviolet, and organic acid treatments have been evaluated for inhibiting the activity of quality degrading enzymes such as PPO (Başlar and Ertugay 2013; Cheng, Zhang, and Adhikari 2013; Zhou, Liu, Xiong, Zou, Liu, et al. 2016). In this regard, organic acids such as ascorbic acid (Jang, Kim, and Moon 2009; Niu et al. 2014), malic acid (Zhou, Liu, Xiong, Zou, Liu, et al. 2016), citric acid (Lagnika et al. 2014), and acetic acid (Goyeneche, Roura, and Di Scala 2014) have been evaluated for such application (Table 3).

In a study by Jang et al. (2009), ascorbic acid or ultrasound alone could not effectively inhibit PPO and browning in apple cubes, while ultrasound treatment with ascorbic acid effectively inhibited enzymatic browning and PPO activity by 46% and 98%, respectively (Table 3). Lagnika et al. (2014) reported that the combined treatment of ultrasound (400 W/20 kHz/10 min) and citric acid (10 g/L) could extend the shelf-life of white mushroom from 4 to 12 days at 4 °C. A combination of organic acid and ultrasound was shown to be an effective treatment for inhibiting enzymatic browning in wheat raw noodles and radish slices (Goyeneche et al. 2014; Niu et al. 2014) (Table 3). Our previous study showed that a combination of ultrasound (20 kHz, 0–60 min) and malic acid (10, 20 and 30 mM) resulted in higher degree of inactivation of mushroom PPO in model system than the sum of malic acid alone and ultrasound alone, indicating synergistic effect. The inactivation rate constant of ultrasound treated PPO increased from $0.26 \times 10^{-2}\,\mathrm{min}^{-1}$ to $1.30 \times 10^{-2}\,\mathrm{min}^{-1}$ after combing with 10 mM malic acid (Table 3). The conformation of PPO was disrupted with a higher decrease of α-helix content induced by the combined method (Zhou, Liu, Xiong, Zou, Liu, et al. 2016). Organic acids may increase the sensitivity of PPO to ultrasound inactivation. On the other hand, ultrasound induced cell disruption can improve the exposure of PPO to organic acids in food systems, resulting in a higher degree of inhibition of PPO and browning in fruits and vegetables. Therefore, the application of ultrasound in combination

with organic acids is a promising approach for controlling PPO activity and browning in plant based products.

Edible coating

Edible coating is an environmentally friendly method that is applied in the food industry to modify the moisture transfer and oxidation reaction of food products (Dhall 2013). In the last two decades, the application of edible coating to extend the shelf life of fruits and vegetables has been given increasing attention (Dhall 2013). Polysaccharides, lipids and proteins are used as components in edible coating matrices with polysaccharides being the most widely used compounds. The commonly used polysaccharides are chitosan, cellulose and pectin. Chitosan, a nontoxic, abundant polymeric product obtained from the outer shell of crustaceans, has been widely accepted as an ideal compound in edible coating formulations because of its good film-forming and biochemical properties (Hirano 1999; Liu et al. 2014; Pilon et al. 2015). Organic acids as active ingredients incorporated into the edible coating matrix have been widely investigated for application in both intact fruits and vegetables and fresh cut products. For instance, chitosan nanoparticles with 2% citric acid as a solvent were successfully used for coating apple slices, resulting in lower PPO activity and browning index (Pilon et al. 2015). A combination of chitosan coating with ascorbic acid were found to be effective for controlling PPO activity and browning in intact plum (Liu et al. 2014), intact grape (Golly et al. 2019), mushroom slices (Ojeda et al. 2019), apple slices (Qi et al. 2011) and litchi fruit (D. Sun et al. 2010) (Table 3). Ghasemnezhad et al. (2013) reported that the application of chitosan with acetic acid suppressed PPO activity and prolonged the postharvest life of pomegranate arils. High methoxy pectin can form good films and apple pectin coating with citric acid effectively inhibited PPO and browning in persimmon pieces (Sanchís et al. 2016; Sanchis et al. 2017). Xanthan gum based edible coating enriched with 1 g/L cinnamic acid caused lower PPO activity and browning index compared to fresh cut pear coated with xanthan gum alone and control uncoated samples (Sharma and Rao 2015) (Table 3). Likewise, ascorbic acid as antibrowning agent has been incorporated into edible coatings such as cassava coating (Ojeda et al. 2014), carboxymethyl cellulose coating (Saba and Sogvar 2016) and *Aloe vera* gel coatings (Kumar et al. 2018; Kuwar, Sharma, and Tadapaneni 2015) (Table 3), these treatments resulted in lower browning and PPO activity. The high membrane solubility of organic acids helps them enter the cytoplasm by simple diffusion (Dhall 2013; Ricke 2003), rendering the effective inhibitors of enzymatic browning. However, effective browning inhibition is not sufficient and the preservation of other quality attributes such texture, nutritional quality and original taste during storage should be considered in the development of organic acid incorporated edible coatings.

Other preservation techniques

Other technologies where organic acids were investigated as adjuvants for enhancing the inhibition of enzymatic browning include gamma irradiation (Hussain et al. 2014), UV-C irradiation (Teoh et al. 2016a, 2016b), modified atmosphere packing (Tamer et al. 2013; Teixeira et al. 2008; Xiao et al. 2011) and treatment by chemicals such as sodium acid sulfate, calcium chloride, sodium chloride and glutathione (Calder et al. 2011; Chiabrando and Giacalone 2012; Li, Wills, and Golding 2015; Siboza, Bertling, and Odindo 2014; S. Wang et al. 2013; Zhu et al. 2009) (Table 3). Radiation processing at 1.0 kGy with 2.0% w/v ascorbic acid could significantly inhibit PPO activity, surface browning and maintain other quality attributes of fresh cut eggplant for up to 6 days (Hussain et al. 2014). Similarly, lower browning index and PPO activity of yam slices were observed after treating with UV-C at 6.84 kJ m^{-2} and 1.0% w/v ascorbic acid (Teoh et al. 2016a) (Table 3).

Modified atmosphere packaging alters the gaseous environment by adding and removing gases to manipulate the levels of CO_2, N_2 and O_2 (Tamer et al. 2013), which is one of the main reactants in the enzymatic reaction catalyzed by PPO. Tamer et al. (2013) reported that citric acid treatment with CO_2 and N_2 could effectively inhibit PPO activity of celery slices during storage (Table 3). Modified atmosphere packing with low O_2/CO_2 combined with chemical treatments including citric acid was found to be beneficial in maintaining quality and shelf-life of mushroom (Xiao et al. 2011). Interestingly, carambola slices treated with 1% ascorbic acid and low-oxygen atmospheres showed slower browning and loss in visual quality compared with 1% ascorbic acid alone, although this was not accompanied by a change in PPO activity (Teixeira et al. 2008). This might be due to the difference between PPO activity in situ under low-oxygen environment and the spectrophotometric PPO activity assay in vitro which is conducted under atmospheric condition. As mentioned before, PPO activity determined in vitro is not a good predictor of browning in plant based products packed under modified atmosphere condition.

Hurdle technology is widely used for the inhibition of PPO and browning in fruits and vegetables as well as other quality deteriorating processes. Chemical treatments by combining organic acids with chemical agents such as NO (Zhu et al. 2009), sodium acid sulfate (Calder et al. 2011), calcium chloride (Chiabrando and Giacalone 2012) and glutathione (Jiang and Fu 1998) is one of the approaches that is employed for quality preservation (Table 3). Organic acids act as acidulants are commonly combined with other browning inhibitors because of the limited PPO inhibitory effect of low pH in some food products with PPOs stable in acidic environment. Calder et al. (2011) reported that a combination of sodium acid sulfate, citric acid and ascorbic acid had an advantage for inhibiting PPO in potato slices because of the combined effects of acidification, chelating and reducing in one treatment. In order to avoid the excessive acidification of food or the negative effect induced by a high concentration of a PPO inhibitor, the combination of

different chemical agents and organic acid could be a potential method to safely inhibit PPO and browning.

Conclusion

Organic acids are traditionally used for inhibition of enzymatic browning in food products that are susceptible to browning. In addition to their effect on enzymatic browning, organic acids also help to inhibit undesirable microbial growth via pH reduction. Their use is indispensable for the manufacture of fruit and vegetable-based products and will continue into the future until alternative processing techniques or effective natural inhibitors are developed for effective inhibition of enzymatic browning while maintaining other quality attributes. Various organic acids inhibit the activity of PPO via different mechanisms and therefore the suitability of a particular organic acid for inhibition of enzymatic browning depends on the structure and the catalytic properties of PPO and the physicochemical properties of the food matrix. Studies in model systems provide an invaluable insight into the inhibitory effects of organics acids on PPO and their mechanisms of action. However, the gap in the effectiveness of PPO inhibitors between model systems and food systems implies that the effect of organic acids on PPO can be accurately evaluated only in real food systems. Moreover, PPO activity based on in vitro activity assay is not always a good predictor of browning in fruits and vegetables, since that involves dilution of activity as well as the added inhibitor during extraction and activity assay under a completely different condition.

The most common organic acids that are commonly used for inhibition of enzymatic browning in foods are the acidulant and metal chelator, citric acid, and the reducing agent ascorbic acid. Nevertheless, these organic acids are not as effective with some acid stable PPOs and in such cases, they are used in combination with other chemical inhibitors of PPO such as sulfates. Thus, several aromatic carboxylic acids, of both the benzoic acid and cinnamic acid series, are being explored for use as inhibitors of the activity of (PPO) and enzymatic browning. Many of these acids such as benzoic acid, cinnamic acid and their derivatives have been shown to be very effective for inhibiting PPO activity in model systems. However, limited information is available on their effectiveness in real food systems. So far, due to safety concerns, their poor water solubility and cost, far less aromatic carboxylic acids have been studied in food systems and used in the food industry compared to model systems. Salicylic acid is the most studied aromatic carboxylic acid in food systems with varying degree of effectiveness in different food systems. Future toxicological evaluations and development of delivery systems such as emulsions and edible coatings for improving solubility and controlled release of these inhibitors into food matrices may result in wider applications of these organic acids in food systems. The use of these phenolic acids at concentrations lower than their toxicity threshold in combination with other organic acids may also be an effective approach from the potential synergy between the different inhibitors.

Organic acids are frequently used as adjuvants during thermal and nonthermal processing of fruits and vegetables as adjuvants for enhancing the inhibition of pathogenic and spoilage organisms as well as enzymatic browning. The synergy between the applied process and the additives helps to reduce the undesirable organoleptic quality changes from the use of excessive concentration of organic acids or severe physical processing. The use of organic acids is especially crucial for the commercial application of non-thermal processing technologies such as high pressure since they have limited effect on the activity of PPO and other quality degrading enzymes under economically feasible processing conditions. Some of the aromatic carboxylic acids under exploration may find use in these applications.

Conflict of interests

The authors declare no conflict of interest.

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