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REVIEW

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Heated plant extracts as natural inhibitors of enzymatic browning: A case of the Maillard reaction

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Abstract

Enzymatic browning is the second largest cause of quality loss in fruits, vegetables, and seafood. Methods to prevent browning are the subject of great research interest in the field of Food Science and Technology. Numerous strategies for inhibiting enzymatic browning have been proposed in literature. Recent research is focused on finding alternative anti-browning agents to synthetics such as sulfites. Amongst natural antioxidants, Maillard reaction products (MRPs) have proven to be effective. Although reviews have been published on the antioxidant and anti-browning activity of MRPs, none of these focused solely on enzymatic browning inhibition mechanism of MRPs generated via heated plant extracts. Therefore, this review explores the common factors associated with the Maillard reaction (temperature, time, and concentration) and enzymatic browning inhibition (enzyme, substrate and reaction time) in order to confirm the activity and presence of MRPs in heated plant extracts.

Practical applications

Chemical food additives applied in prevention of enzymatic browning are subjected to scrutiny. Therefore, alternative natural compounds are sought after. Plant extracts have been applied, however, they tend to impart their characteristic natural flavor into the product. Heating of these plant extracts have been proven to reduce the "planty, herby" flavors, whilst producing Maillard reaction. Maillard reaction products are known to exhibit anti-browning activity, and they are a cheap alternative to these chemical inhibitors. Therefore, these can be applied as potential anti-browning agents in food products.

KEYWORDS
enzymatic browning, heated plant extracts, inhibition, Maillard reaction, Maillard reaction products

1 | INTRODUCTION

Appearance is one of the key sensory attributes that is considered by consumers when selecting a food product. Amongst them color is a first critical determinant for the appeal and quality of food products. Non-enzymatic (NEB) and enzymatic browning (EB) reactions that occur during handling, processing and storage, affect the acceptability, and thus commercial value of products. Of the two browning reactions EB is one of the well-known chemical reactions (Ghoul, 2013). It is responsible for deterioration or enhancement of quality during handling, processing, and storage of food. Enzymatic browning reactions are usually adverse in cases where they lead to discoloration, off-flavor formation, and loss of nutritional value of fresh fruits, vegetables, nuts, tubers, and crustaceans (Lee, Seo, et al., 2016; Nooshkam et al., 2019). In conjunction with NEB, it is beneficial in the development of characteristic

color and flavor associated with food products such as coffee, cacao beans, black olives, dried fruits wine, and tea during processing (Cisneros-Yupanqui & Lante, 2020; Jati et al., 2002; Moon et al., 2020) Whether EB reactions are desirable, or not, some level of control is required.

The browning reaction mechanism is well characterised and involves the oxidation of phenolic compounds by a class of metallo-enzymes belonging to the group oxidoreductases (Redondo et al., 2016). Polyphenol oxidase and peroxidase are the chief enzymes responsible for enzymatic browning in food. In the plant or animal cell the phenolic substrates are located in the vacuole and the enzyme in the cytoplasm; due to this distinct compartmentalization browning does not occur. However, once the cell is ruptured by loss of firmness, shock, cutting, bruising, or pulping, the enzymes and substrate come in contact.

Polyphenol oxidase catalyses two main reactions in the presence of oxygen, first the hydroxylation of tyrosine to 3,4-dihydroxy phenylalanine (monophenolase activity), which is relatively slow and results in colorless products. The second reaction is rapid and gives colored oxidation products which converts 3,4-dihydroxy phenylalanine into quinones (diphenolase activity). The resultant quinones are either polymerized spontaneously or react with amino acids or proteins to form brown pigments (melanins) (Queiroz et al., 2008). The reaction of quinone with these primary metabolites causes bitterness, nutritional (reduction in or of protein digestibility), and loss of quality attributes such as color and flavor in food. The rate of enzymatic browning depends on the concentration and source of enzyme and phenolic substrates, oxygen availability, pH, and temperature (Lante et al., 2016).

To combat this reaction, various interventions were introduced. Many papers have reported several physical and chemical methods for controlling or inhibiting enzymatic browning. These methods are applied based on inactivating the enzyme (Zhou et al., 2016), removing essential components on the enzyme, or complexing with intermediate end-products. Physical methods aimed at exclusion of oxygen include immersion in solution, vacuum packing, use of controlled and modified atmospheric packaging (Tsouvaltzis & Brecht, 2017). Physical methods aimed at the enzyme include removing, lowering, arresting, and inactivation of enzyme activity by ultrafiltration, refrigeration, freezing, and blanching, respectively. Thermal methods are very efficient in combatting EB, however, it leads to modification of some product attributes such as texture, taste, and loss of nutrient (Rawson et al., 2011). At times processes like blanching would not be compatible with commodities such as banana, lettuce, apple, etc. As a result, researchers delved into some non-thermal alternative processing methods, these include high pressure processing (Sulaiman et al., 2015), pulsed electric field (Cao et al., 2018), gamma irradiation, ohmic heating (Makroo et al., 2020; Saxena et al., 2017) ultra violet light (Fan et al., 2017), and sonication.

Moreover, chemical inhibitors of enzymatic browning are divided into six categories based on their different modes of action, namely; reducing, chelating, and complexing agents (Guiamba & Svanberg, 2016), acidulants, enzyme inhibitors, and enzyme treatments (Bai et al., 2017). Compounds that inhibit EB target key reactants such as enzyme (PPO), substrate (phenol), co-substrate (oxygen), and intermediate reaction products such as o-quinones. The inhibition targeted toward enzymes exhibit at least four different mechanisms, namely: introducing compounds with a similar structure as the phenolic substrate which competitively binds on the active site forming an enzyme-inhibitor complex; by chelating the copper ion from the active site; binding of sulfhydryl, amino, or hydroxyl groups on the active site, bringing about conformational change on the enzyme structure or lowering the pH below the required optimum range (Ali et al., 2016; Chériot et al., 2007). Inhibition targeted toward the substrate involves complexation to prevent oxidation or enzyme catalyzed modification/alteration of the substrate by addition of a functional group. Co-substrate targeted inhibition involves the scavenging of molecular oxygen. Inhibition targeted toward the intermediate product o-quinone involves reducing it back to precursor diphenol or reacting to form stable colorless adducts (Lim & Wong, 2018). It is well recorded that EB inhibition mechanisms is somewhat linked to the antioxidant activity of a compound.

Chemical inhibitors are the most preferred choice due to low cost and high performance. However, commonly used inhibitors such as sulfites have increased regulatory scrutiny due to association with initiating reactions in asthmatic individuals (Vally et al., 2009) and, consequently, their application on fresh-cut fruits and vegetable food products has been banned by the U.S. Food and Drug Administration (Moon et al., 2020). Sulfites also reduce the uptake of vitamin thiamine, and thus consequently causing its deficiency in food. For this reason, the food industry has been focusing on other anti-browning agents such as ascorbic and citric acids that are, however, less effective than sulfiting agents, since ascorbic acid is irreversibly oxidized to dehydroascorbic acid, thus allowing browning to occur (Zocca et al., 2011). Moreover, Consumer awareness has resulted in demands for clean labeling and this has driven the food industry into looking for natural alternative anti-browning agents to synthetic food additives (Redondo et al., 2016; Zocca et al., 2011). Moreover, this shift has to be done without compromising efficiency, food safety, sensory quality, and cost. Most natural food additives are limited in terms of application due to the strong odor and color of compounds that make them up (Lee, Seo, et al., 2016). Thus, food scientists have a difficult task since synthetic food additives have proven superior.

Numerous studies have been devoted to the search for natural inhibitors. Some of those proposed to have had the inhibitory effects are namely; honey, cysteine (Redondo et al., 2016), glutathione (Phonpala et al., 2009), natural aliphatic alcohols, kojic acid, and Maillard reaction products (Nooshkam et al., 2019). This paper will not focus on discussing the effect of different browning inhibitors, but on the mechanisms of inhibition exerted by Maillard reaction products formed in heated plant extracts, and how they perform in comparison to known inhibitors.

Many papers have been published with reference to the use of plant extracts to inhibit EB (Wessels et al., 2014; Yapi et al., 2015) This paper will focus on MRPs derived from heated plant extracts.

TABLE 1 Summary of browning inhibition by heated plant extracts

Plant extract	Heating parameters	PPO source	Substrate/enzyme	In vivo product and treatment	Mode of action of inhibition	Method	References
Onion extract	100°C 10 min	Potato	0.2 M Catechol	5 mm potato slice Immerse in extract for 2 min and incubated at 30°C for 30 min	Non-competitive	Spectrophotometric 420 nm at 25°C/60 s	Lee et al. (2002)
Grape leaf	100°C 15 min	Lettuce	1 mM Catechol	Lettuce slurry 0, 1, 3, 6 & 24 hr at 25°C	Non-competitive	Spectrophotometric 420 nm Colour spectrophotometer	Altunkaya (2014)
Onion extract	100°C 10 min	Pear	0.2 M Catechol	Pear juice	Non-competitive	Spectrophotometric 420 nm at 25°C/60 s Spectrophotometric 400 nm	Kim et al. (2005)
Onion extract	100°C 10 min	Banana	0.2 M Catechol	–	Non-competitive	Spectrophotometric 420 nm at 25°C/60 s	Lee (2007)
Onion extract	100°C 10 min	Taro	10 mM Catechol 10 mM 4-methylcatechol 10 mM pyrogallol 10 mM resorcinol 10 mM hydroquinine 10 mM Catechin 10 mM L-DOPA	5 mm Taro slices immersed for 20 min at room temperature	–	Spectrophotometric 420, 410, 334, 400 & 475 nm at 25°C/60 s	Lee et al. (2007)
Onion by-products (juice, paste, bagasse)	Pasteurisation 100°C 17–31 min, Sterilisation 115°C 11–17 min	Avocado	0.07 M Catechol	–	–	Spectrophotometric 420 nm at 25°C/30 s	Roldán et al. (2008)
Onion by-products (juice, paste bagasse)	Pasteurisation 100°C 15 min,	Eggplant	Catechol	Eggplant homogenate	–	Spectrophotometric 505 nm/ (browning index) 420 nm at 25°C	Barbagallo et al. (2012)
Onion	100°C 10 min	Cassava leaf	10–100 mM Catechol	–	Non-competitive & irreversible	Spectrophotometric 410 nm at 20°C/360 s	Wong and Angel Lee (2014)
Rice bran protein extract	40, 60 and 80°C	Potato	0.2 M catechol	Potato puree	Mixed-type	Spectrophotometric 420 nm at 25°C	Kubglomsong and Theerakulkait (2014)
Thinned nectarine extracts	Microwave 500, 1,000, 1,500 W 90 s	Mushroom	1.5 mM DL-DOPA	Peaches slices Immersed for 15 min	Irreversible Mixed-type inhibitor	Spectrophotometric 475 nm at 30°C	Redondo et al. (2016)
Onion extracts	95°C 15 min	Ginger	0.1 M 4-methylcatechol	–	Mixed-type	Spectrophotometric at 410 nm/5 min room temperature	Lim and Wong (2018)

(Continues)

TABLE 1 (Continued)

Plant extract	Heating parameters	PPO source	Substrate/enzyme	In vivo product and treatment	Mode of action of inhibition	Method	References
Chilli pepper extracts	95°C 15 min	Ginger	0.1 M 4-methylcatechol	–	Un-competitive	Spectrophotometric at 410 nm/5 min room temperature	Lim and Wong (2018)
Pineapple juice	95°C 15 min	Ginger	0.1 M 4-methylcatechol	–	Non-competitive	Spectrophotometric at 410 nm/5 min room temperature	Lim and Wong (2018)
Onion	95°C 15 min	Ginger	0.1 M pyrocatechol	–	Mixed	Spectrophotometric at 410 nm/5 min room temperature	Lim and Wong (2018)
Chilli pepper extracts	95°C 15 min	Ginger	0.1 M pyrocatechol	–	Mixed	Spectrophotometric at 410 nm/5 min room temperature	Lim and Wong (2018)
Pineapple juice	95°C 15 min	Ginger	0.1 M pyrocatechol	–	Non-competitive	Spectrophotometric at 410 nm/5 min room temperature	Lim and Wong (2018)
Onion	95°C 15 min	Sweet potato	0.1 M 4-methylcatechol	Sweet potato	Un-competitive	Spectrophotometric at 410 nm/5 min room temperature	Lim et al. (2019)
Chilli pepper extracts	95°C 15 min	Sweet potato	0.1 M 4-methylcatechol	Sweet potato	Mixed	Spectrophotometric at 410 nm/5 min room temperature	Lim et al. (2019)
Pineapple juice	95°C 15 min	Sweet potato	0.1 M 4-methylcatechol	Sweet potato	Mixed	Spectrophotometric at 410 nm/5 min room temperature	Lim et al. (2019)
Onion	95°C 15 min	Sweet potato	0.1 M pyrocatechol	Sweet potato	Mixed	Spectrophotometric at 410 nm/5 min room temperature	Lim et al. (2019)
Chilli pepper extracts	95°C 15 min	Sweet potato	0.1 M pyrocatechol	Sweet potato	Un-competitive	Spectrophotometric at 410 nm/5 min room temperature	Lim et al. (2019)
Pineapple juice	95°C 15 min	Sweet potato	0.1 M pyrocatechol	Sweet potato	Mixed	Spectrophotometric at 410 nm/5 min room temperature	Lim et al. (2019)

Abbreviations: L-DOPA, L-dihydroxy phenylalanine; PPO, polyphenoloxidase.

2 | THE MAILLARD REACTION

The Maillard reaction or non-enzymatic browning occurs when a carbonyl-containing compound, namely reducing sugars, aldehydes, or ketones condenses with a free amino group of amino acids, peptides or proteins during processing and prolonged storage of foodstuffs. The MR plays a major role in thermally processed foods, and is of vital importance to the food industry (Nooshkam et al., 2019). The MR is sub-divided into early, intermediate and advanced stages with each step resulting in a myriad of reaction products. These are termed Maillard reaction products (MRPs), and they have an influence on certain desirable and undesirable effects on food quality (Laroque et al., 2008). Processes such as roasting, baking, or frying results in the formation of color and flavor which are amongst the favorable effects of the MR, while during drying, pasteurization, sterilization and storage the occurrence of the MR is unfavorable due to discoloration, off-flavor formation as well as reduction in nutritional value (Laroque et al., 2008). Owing to this, it is essential to better understand the MR progression in order to control the formation of desirable attributes, while ensuring and preventing or minimizing undesirable effects (Jaeger et al., 2010). Furthermore, the reaction is influenced by various factors such as the reaction time, temperature, pH, concentration (Matmaroh et al., 2006; Wang et al., 2013) water activity, type of reactants as well as the medium used. These factors influence the stages of the MR differently and as a result, the reaction products also vary.

2.1 | Sources of Maillard reaction products

The MR can be generated from any biological system that contains a carbonyl compound and a free amino group. Since its inception in 1912, the Maillard reaction has been produced and studied in numerous types of food, matrixes and model systems. Some of the models systems studied include oxidized fat-protein, sugar-amino (Xu et al., 2017), sugar-protein (Yu et al., 2018), sugar-protein hydrolysates (Nie et al., 2017), polysaccharide-protein, and food systems include; dairy (Nasrollahzadeh et al., 2017; Perusko et al., 2015), infant formula, fruit juices, and pulps (Selen Burdurlu & Karadeniz, 2003), cereal products (Rufián-Henares et al., 2009), baked goods (Fiore et al., 2012), potato-based products (Morales et al., 2014) meat and meat products (Li et al., 2013), and coffee (Rufián-Henares et al., 2009). The abovementioned systems have been investigated for their antioxidant, toxicity, allergenicity, antimicrobial (Habinshuti et al., 2019), anti-inflammatory, functional (Li et al., 2013), and anti-enzymatic browning properties (Xu et al., 2017). Regarding the latter property, numerous studies have focused on using sugar-amino acids than heated plant extracts. The present study focuses on reviewing Maillard reaction products formed during heat treating plant extracts and their subsequent effect in preventing enzymatic browning.

3 | PLANT EXTRACTS AS INHIBITORS OF ENZYMATIC BROWNI2019NG

As a means of finding alternative inhibitors to sulfites and other synthetic additives, studies have been conducted on the application of plant extracts in inhibiting EB. Several authors proved that compounds obtained from plant extracts, agro food by-products and waste had an inhibitory effect against polyphenoloxidase in various food matrices. Tinello and Lante (2017) reported the PPO inhibitory effect of bunch-thinned unripe grapes on fresh-cut potato and apple slices. Klimczak and Gliszczyńska-Świgło (2017) observed the anti-browning effectiveness of 0.001%–0.003% green tea leaf extracts added in apple juice for significantly decreasing PPO activity by 53%–96% after storage for 2 days at 4°C, they attributed the inhibition to the presence of catechins. Other authors investigated extracts obtained from garlic, sage, oregano (Wessels et al., 2014), onion (Yapi et al., 2015), cabbage on grape juice (Zocca et al., 2010), onion juice, paste, and baggase on avocado (Roldán et al., 2008), rice bran on apple and potato puree (Sukhonthara et al., 2016) and hydrosols from citrus peels (Lante & Tinello, 2015) and onion (Lante et al., 2020) against trosinase activity. These authors attributed the inhibition to the presence of polyphenolic compounds (flavonoids, phenolic acids), thiol (cysteine, glucosinolates) compounds, organic acids (citric, malic, and oxalic), terpenes, and peptides inherent in these plant extracts. Enzymatic browning inhibition by the afore-mentioned plant extracts were found to be competitive and non-competitive by acting as a metal chelators or reducing agents (Tinello & Lante, 2018).

4 | HEATED PLANT EXTRACTS AS INHIBITORS OF ENZYMATIC BROWNING

Upon witnessing the effect of plant extracts on EB, several authors decided to investigate the effect of heating as a solution to supress the inherent strong odor and flavor characteristics of these extracts (Lee, Seo, et al., 2016). Numerous studies have been devoted to investigating the effect of heating on the antioxidant activity of food commodities, especially those from fruit, vegetable and dairy origin, amongst these are mushrooms, apple juice, coffee, tomato, milk, onion, and garlic (Anese et al., 1999; Bae et al., 2014; Benkeblia, 2005; Javier Moreno et al., 2006; Kang et al., 2018; Nooshkam et al., 2019). Furthermore, the antioxidant mechanisms have also been reported. However, no or little studies have been conducted focusing on the EB inhibition mechanism, where actual compounds formed during heating are identified, therefore, it was necessary to carry out this review. Table 1 displays a summary of studies conducted on the EB inhibition of various heated plant extracts and associated by-products.

Heated plant extracts obtained from onions, nectarines, grape leaf, chilli pepper, pineapple, and rice bran were found to inhibit in vitro PPO activity in potato, banana, taro, eggplant, avocado, pear,

TABLE 2 Browning inhibition by heated plant extracts expressed as residual enzyme activity and percentage inhibition

Plant extract type[a]	Content (mg/ml)	Reaction conditions	REA (%)	Inhibition (%)	References
Fresh onion extract	1.55	Potato PPO, 0.2 M Catechol, Spectrophotometric 420 nm at 25°C/60 s	79	21	Lee et al. (2002)
Heat-treated onion extract			17	78	
Fresh onion extract	3.1		44	56	
Heat-treated onion extract			10	90	
Fresh onion extract	60	Pear PPO, 0.2 M Catechol, Spectrophotometric 420 nm at 25°C/60 s	73	27	Kim et al. (2005)
Heated onion extract			46	37	
Fresh onion extract	3.1	Banana PPO, 0.2 M Catechol Spectrophotometric 420 nm at 25°C/60 s	61	41	Lee (2007)
Heated onion extract			22	78	
Fresh onion extract	3.1	Taro PPO 10 mM Catechol Spectrophotometric 420 nm at 25°C/60 s	88	12	Lee et al. (2007)
Heated onion extract			46	54	
Fresh onion extract		Taro PPO 10 mM 4-methylcatechol Spectrophotometric 410 nm at 25°C/60 s	75	25	
Heated onion extract			39	61	
Fresh onion extract		Taro PPO 10 mM pyrogallol Spectrophotometric 334 nm at 25°C/60 s	95	5	
Heated onion extract			52	48	
Fresh onion extract		Taro PPO 10 mM Catechin, Spectrophotometric 400 nm at 25°C/60 s	88	12	
Heated onion extract			45	55	
Fresh onion extract		Taro PPO 10 mM L-DOPA, Spectrophotometric 475 nm at 25°C/60 s	87	13	
Heated onion extract			43	57	
Frozen onion juice	–	Avocado PPO, 0.07 M Catechol, Spectrophotometric 420 nm at 25°C/30 s	60	40	Roldán et al. (2008)
Pasteurised onion juice			34	67	
Sterilised onion juice			40	60	
Frozen onion paste			43	57	
Pasteurised onion paste			47	53	
Sterilised onion paste			10	90	
Frozen onion bagasse			60	40	
Pasteurised onion bagasse			86	14	
Sterilised onion bagasse			27	73	
Fresh juice	–	Eggplant PPO, Catechol, Spectrophotometric 505 nm	63	37	Barbagallo et al. (2012)
Stabilised juice			46	54	
Fresh paste			71	29	
Stabilised paste			59	41	
Fresh bagasse			74	26	
Stabilised bagasse			77	23	
Fresh onion extract	3	Cassava leaf PPO, 10–100 mM, Catechol Spectrophotometric 410 nm at 20°C/360 s	62	38	Wong and Angel Lee (2014)
Heat-treated onion extract			28	72	
Non-heated red onion extract	10	Ginger PPO, 0.1 M Pyrocatechol, Spectrophotometric at 410 nm/5 min room temperature	72	28	Lim and Wong (2018)
Heated red onion extract			67	33	
Non-heated chilli pepper extract			59	41	
Heated chilli pepper extract			51	49	
Non-heated pineapple juice			75	25	
Heated pineapple juice			73	27	

(Continues)

TABLE 2 (Continued)

Plant extract type[a]	Content (mg/ml)	Reaction conditions	REA (%)	Inhibition (%)	References
Non-heated red onion extract	10	Ginger PPO, 0.1 M 4-Methylcatechol, Spectrophotometric at 410 nm/5 min room temperature	86	14	Lim and Wong (2018)
Heated red onion extract			84	16	
Non-heated chilli pepper extract	10		77	23	
Heated chilli pepper extract			71	29	
Non-heated pineapple juice	10		82	18	
Heated pineapple juice			78	22	
Non-heated red onion extract	10	Sweet potato PPO, 0.1 M Pyrocatechol, Spectrophotometric at 410 nm/5 min room temperature	61	39	Lim et al. (2019)
Heated red onion extract			59	41	
Non-heated chilli pepper extract	10		54	46	
Heated chilli pepper extract			57	43	
Non-heated pineapple juice	10		65	35	
Heated pineapple juice			80	20	
Non-heated red onion extract	1,000	Sweet potato PPO, 0.1 M 4-Methylcatechol, Spectrophotometric at 410 nm/5 min room temperature	93	7	Lim et al. (2019)
Heated red onion extract			82	18	
Non-heated chilli pepper extract	50		73	27	
Heated chilli pepper extract			74	26	
Non-heated pineapple juice	1,000		68	32	
Heated pineapple juice			93	7	

Abbreviations: L-DOPA, L-dihydroxy phenylalanine; PPO, polyphenoloxidase; REA, residual enzyme activity which is also termed relative enzyme activity.

[a]Heat treatment as specified from source.

cassava leaf, and mushrooms, as well as in vivo in potato, pear, peach, and eggplant. Moreover, most of the authors proved that heated plant extracts exhibited higher browning inhibition than their fresh (unheated) counterparts (Kim et al., 2005; Lee, 2007; Lee et al., 2002, 2007; Lim & Wong, 2018; Redondo et al., 2016).

The superiority of heated over unheated/fresh plant extracts in inhibiting EB was attributed to the development of the MR during heating, thus production of MRPs (Barbagallo et al., 2012; Kim et al., 2005; Lim et al., 2019; Redondo et al., 2016; Wong & Angel Lee, 2014). Although authors concluded thus, only the study conducted by Redondo et al. (2016) linked the formation of non-specific indicators such as acetic and formic acid to the development of the MR during heating of plant extracts. To presumptively conclude the MR, consumption of key reactants (amount of free amino group and sugars) before, and throughout the reaction can be monitored (Laroque et al., 2008). However, conclusive evidence is gathered when specific MRP's such as glucosylamine Schiff bases, Amadori/Heyns rearrangement products, reductones, furfural, hydroxymethyl furfural, and melanoidins amongst others are identified (analysed) in heated plant extracts.

In light of the abovementioned limitations with reference to identification of specific MRPs, in this study we reviewed several papers (stated above) that reported on the EB inhibition of heated plant extracts and MR model systems. Seeing that the inhibition mechanism of MRP's obtained from simple amino-sugar model systems against PPO activity is well documented. The review by Nooshkam et al. (2019) compiled a summary of studies based on sugar-amino MRPs against EB. We then also looked at papers that studied the antioxidant, antimicrobial, and anti-hyperglycaemic effect of MRPs formed during processing and storage of plant extracts (Table 1) and model systems as well as their mode of action. Using the information obtained, we then speculated and made conclusions on the formation of specific MRPs responsible for EB inhibition in heated plant extracts.

An enormous portion of work covered in this study focused more on the EB inhibitory effect of heated onions than other extracts, as this one of the vegetables that was tremendously explored.

5 | FORMATION OF MRPS IN HEATED PLANT EXTRACTS

5.1 | Onions (*Allium cepa*) extracts

Authors who studied the anti-enzymatic browning effect of aqueous extracts of fresh onions in comparison to the heated counterparts reported good inhibitory capacity, albeit it was lower than that of heated extracts (Kim et al., 2005; Lee, 2007; Lee et al., 2002, 2007;

Lim et al., 2019; Lim & Wong, 2018; Roldán et al., 2008; Wong & Angel Lee, 2014) (Table 2). The inhibitory effect of fresh onion was attributed to it being a rich source of bioactive compounds. The nutritional composition of onion is complex, onion (*Allium cepa*) contains amongst other constituents' sugars (fructose, sucrose, galactose, glucose, mannose, fructans), amino acids (S-alk(en)yl cysteine sulfoxides, arginine) flavonoids (quercetin, quercetin-3-glucoside, isorhamnetin-4-glucoside) (Sharma et al., 2015), organosulfur compounds (sulfur, allylsulfides, cycloalliin, and thiosulfinates), vitamin (ascorbic acid), selenium, and its organic compounds (Roldán et al., 2008). Quercetin one of the most abundant flavonoid found in onion (Moreno-Ortega et al., 2020) was reported to exhibit antioxidant activity via multiple mechanisms. Benkeblia (2005) investigated the antioxidant capacity of methanol extracts of selected onions, where he found them to exhibit 2,2-diphenyl-1-picrylhydrazyl (DPPH), reducing power, and hydrogen peroxide radical scavenging. With the latter reported to be comparable to that of ascorbic acid. Moreover, it was also reported to inhibit the oxidation of L-dehydroxypheylalanine (L-DOPA) by chelating copper on the enzyme active site. Therefore, its activity is unquestionable. In addition, these authors also attributed this to the phenolic bioactives in synergy with thiol compounds, a phenomenon which was also reported by (Yapi et al., 2015). Moreover, Alk(en)yl cysteine sulfoxides and other active thiol containing compounds abundant in onion also bind on the active site of PPO inactivating the catalytic ability of the enzyme, they also reduce the o-quinone to their precursor diphenols or interact with o-quinone to form stable sulfoquinones.

Moreover, numerous studies have been devoted in investigating the effect of cysteine and ascorbic acid in inhibiting enzymatic browning. Ali et al. (2015) compared ascorbic acid and cysteine and found that these compounds showed different behavior depending on concentration. At low concentration of (0.5%) both cysteine and AA acted as enzyme inhibitors, and as the concentration increased these compounds were more inclined to act as a reducing agents. However, the mode of inhibition of cysteine was of a peculiar nature where it condensated with catechol and o-quinone to form monothiolcatechol and dithiocatechol, respectively. A similar trend was reported by Ali et al. (2016). Therefore, the inhibitory exhibited by fresh onion extracts was due to the activity of the myriad of bioactives discussed above.

Heating onion extracts at temperatures ranging between 95 and 115°C for 10–31 min increased the inhibitory effect against EB (Table 1). The inhibition has been attributed to inherent components as well as their interactions to form products that exhibit the above properties. Thermal treatment has been proven to aid in loosening bound bioactive compounds which are highly concentrated in cell walls (Pérez-Burillo et al., 2019; Roldán et al., 2008). The increase in bioactive concentration, especially polyphenols is known to increase the inhibitory capacity (Roldán et al., 2008). Moreover, these authors proved that sterilizing at 115°C provoked a high phenol release compared to freezing (−18°C) and pasteurization (100°C). Similarly, Sharma et al. (2015) evaluated the effect of various heating temperatures (60–150°C) for 30 min on the flavonoid content of six onion varieties. They observed a positive effect of temperature on the total phenolic and flavonoid content of onion varieties when the maximum temperature was 120°C. However, when the temperature reached 150°C, a drastic decrease was observed. Moreover, a study by Moreno-Ortega et al. (2020) found a 12-fold reduction in total flavonoid (quercetin as major) content of three varieties of onion during production of black onion at 65–70°C for 28 days. Although the temperature applied during black onion production was less intense compared to that in the study of Sharma et al. (2015), the treatment time of 28 days was lengthy, thus affecting the final content of flavonoids of the resulting black onion.

The effect of the MR in the above-mentioned studies cannot be ignored, this is based on the presence of reducing sugars and amino acids at the applied temperatures and time. The MR is divided into three stages; initial, intermediate, and final stage. The initial stage results in the formation of Schiff bases from carbonyl amine condensation, followed by Amadori rearrangement products (ARPs). The former and latter steps of the reaction mentioned above are reversible and irreversible, respectively (Nooshkam et al., 2019). At this point of the reaction, early ARPs do not impart any sensorial (color and odor) changes. Therefore, their determination serves as a sensitive indicator for early detection of quality deterioration (Cardelle-Cobas et al., 2005). Ha et al. (2011) reported on the formation of Amadori rearrangement products (ARPs) via sugar amino model systems. Arginine-glucose heated at 80°C for 120 min and arginine-maltose 80°C for 60 min resulted in the formation of arginyl-fructose (AF) and arginyl-fructosyl-glucose (AFG), respectively. The same ARPs including fructosyl-lysine (FL) were also identified in wet and dry heated onion and garlic (Javier Moreno et al., 2006; Ha et al., 2011; Kang et al., 2018). In a study by Kang et al. (2018) onion extracts heated at 90°C for 3 and 5 hr produced approximately 6 and 13% AF, 28 and 38% total ARPs, as well as 103 and 200 μg/g quercetin, respectively. This was further corroborated by the sugar and amino acid composition and content in onion, and the importance of increasing heating time in the formation of early MRPs as well as extraction of polyphenols. These authors reported that although both ARPs and quercetin content increased with heat treatment, ARPs accounted for 38% of the total weight of the tested extracts. They suspected that the observed bioactivities were due to ARPs, but could not exclude the possible synergistic effect of quercetin.

Furthermore, AF, AFG, and FL formed via sugar amino model systems and heated onions have been reported to possess antioxidant activity via scavenging peroxyl (Javier Moreno et al., 2006) and hydrogen peroxide radicals, metal chelation, competitively inhibit α-glucosidase, sucrase, and amylase activity (Ha et al., 2011; Kang et al., 2018). This may be attributed to the strong reducing power owing to an increase in hydroxyl groups formed via conjugation (Nooshkam et al., 2019). In terms of EB, hydroxyl groups are responsible for hydrogen atom transfer (Vhangani & Van Wyk, 2013) which reverses the o-quinone back to precursor diphenols. A well-known mechanism displayed by MRPs. In addition, the hydroxyl groups could bind active site of PPO, changing the enzyme's native conformation, thus losing its activity (Yuniarti et al., 2018). Evidently,

glucose-lysine and glucose-arginine models systems known to produce FL and AF Amadori rearrangement products were found to inhibit banana PPO during a 10-day storage (Lee, 2007). Although these MRPs model systems were heated at 90°C, the longer heating time of 7 hr may have resulted in the progression of the formed ARPs to intermediate and final stages of the MR. Javier Moreno et al. (2006) reported on the antioxidant activity of ARPs in dehydrated onion and garlic during storage, however, their results further indicated that advanced products of the MR were the major contributors in terms of antioxidant activity. Which could explain the results obtained by Lee, 2007. Contradictory to the above, findings of Mogol et al. (2010) when heating glucose and various amino acids (lysine, histidine, and arginine) at 100°C for 1–6 hr also reported a significant increase in PPO inhibition as the reaction time increased, and coincidently the arginine-glucose model system was the best. Moreover, a positive correlation was established between PPO inhibition and total antioxidant activity (TAC) via ABTS and DPPH radical scavenging. This can only mean that hydrogen donation was the inhibition mechanism of MRPs against apple PPO. However, contradictory to Javier Moreno et al. (2006), MRPs elucidated in this study were only early to intermediate glycosylamines, Schiff bases, reductones, and dehydoreductones. The contradiction can be arising from differences in temperature and time of processing/storage, matrix type. In this instance we are comparing a sugar amino model system with an onion extracts. It was previously mentioned in this study that onion composition is complex with several types of sugars, amino acids, and other bioactives such as sulfur compounds and polyphenols. It may be true that onion extracts heated at 100 for 10–30 min may produce the MR that progresses to the final stage.

The above phenomenon could be used to explain why heated onion extracts in the study conducted by Lee et al. (2002) inhibited EB against potato PPO as the reaction temperature increased from 40 to 100°C (Table 3) for a duration of 10 min, similarly Kim et al. (2005) observed an increased inhibitory as the reaction time increased from 2 to 12 min at 100°C (Table 4). This is linked to various stages of the MR were high temperature short time and lower temperatures longer times results in formation of early colorless MRPs which may exhibit minimal EB inhibition properties, however, as the heating time progresses complex MRPs are formed. Kim et al. (2005) and Lee et al. (2007) attempted to elucidate the compounds formed during heating onion extracts that are responsible for inhibition EB by applying dialysing. They found that dialysis resulted in loss and reduction in inhibitory effect against EB, respectively, this then proves that low molecular weight (LMW) compounds in the extract were initially responsible for the inhibition. Low molecular weight compounds are formed during early to intermediate stages of the MR, we, therefore, speculate the ARPs are responsible for EB inhibition, which is in agreement with Mogol et al. (2010).

Progression of the MR from the initial stage involves evolution of ARPs to form intermediate MRPs. Formation of furoylmethyl amino acids has been reported in literature, these compounds are formed via acid hydrolysis of corresponding ARPs (Cardelle-Cobas et al., 2005; Javier Moreno et al., 2006). Cardelle-Cobas et al. (2005)

TABLE 3 The effect of heating temperature on the browning inhibitory of plant extracts

	Assay conditions				
Plant extract type	Onion extract 3.1 mg/ml heated at a constant time of 10 min. Potato PPO,	Onion extract 3.1 mg/ml heated at a constant time of 10 min. Pear PPO, 0.2 M	Onion extract 3.1 mg/ml heated at a constant time of 10 min. Banana PPO,	Rice bran protein extract 8 mg/ml heated at a constant time of 10 min. Potato PPO,
PPO type	0.2 M Catechol, spectrophotometric	Catechol, spectrophotometric	0.2 M Catechol, spectrophotometric	0.2 M Catechol, spectrophotometric
Substrate	420 nm at 25°C/60 s (Lee et al., 2002)	420 nm at 25°C/60 s (Kim et al., 2005)	420 nm at 25°C/60 s (Lee, 2007)	420 nm at 25°C/60 s (Kubglomsong & Theerakulkait, 2014)
Wavelength				
Temperature (°C)	**REA**	**REA**	**REA**	**REA**
40	88	–	88	84
50	80	78	88	–
60	70	79	88	84
70	68	76	84	–
80	60	62	72	90
90	48	52	58	–
100	17	35	45	–

Abbreviations: PPO, polyphenol oxidase; REA, residual enzyme activity, also referred to as relative enzyme activity.

TABLE 4 The effect of heating time on the browning inhibitory of plant extracts

	Assay conditions		
Plant extract type PPO type Substrate Wavelength	Onion extract 3.1 mg/ml heated at 100°C Pear PPO, 0.2 M Catechol, Spectrophotometric 420 nm at 25°C/60 s (Kim et al., 2005)	Onion extract 3.1 mg/ml heated at 100°C Taro PPO, 0.2 M Catechol Spectrophotometric 420, nm at 25°C/60 s (Lee et al., 2007)	Grape leaf extract 90 mg/ml heated at 100°C Lettuce PPO, 1 mM Catechol Spectrophotometric 420 nm at 25°C/60 s (Altunkaya, 2014)
Time (min)	**REA**	**REA**	**REA**
0	100	89	100
2	76	73	–
4	70	65	70[a]
6	62	57	–
8	52	51	–
10	38	47	60
12	36	45	–
14	–	41	–
16	–	35	58[b]
18	–	–	–
20	–	–	56

Abbreviations: PPO, polyphenol oxidase; REA, residual enzyme activity, also referred to as relative enzyme activity.

[a]5 min heating time.

[b]15 min heating time.

assessed MRPs formed during the initial stages of the MR in dehydrated onion and garlic. They compared the furoylmethyl amino acid content of fresh freeze-dried and commercial dehydrated onion and garlic stored at 50°C at an aw of 0.5 for 6 days. Furoylmethyl amino acids were only detected in commercial dehydrated samples, this occurrence is attributed to the high temperature applied during drying that promote the MR, evident to this is the significance differences in total reducing sugar levels of fresh freeze-dried versus commercial dehydrated onions. Of the three types, 2-furoylmethyl-arginine was the highest in onion followed by 2-furoylmethyl-lysine and then 2-furoylmethyl-γ-aminobutyric. Garlic moreover contained more 2-furoylmethyl-lysine followed by 2-furoylmethyl-arginine, and all garlic samples were devoid of 2-furoylmethyl-γ-aminobutyric acid. When comparing onion and garlic samples, the latter although containing a higher protein level, their average total reducing sugar was 24 times less than that of onion, evidently the 2-furoylmethyl amino acid levels were also lower, approximately 40- and 100-fold less for 2-furoylmethyl-lysine and 2-furoylmethyl-arginine, respectively.

Furthermore, the evolution of early stage ARP's to intermediate products in *Allium sp* was tracked via a 14 days' storage at 50°C at an a_w 0.44 by Javier Moreno et al. (2006). As the storage days increased from 0 to 10 days, the total 2-furoylmethyl-amino acids (comprising of three derivatives mentioned in the study of Cardelle-Cobas et al., 2005 above) content in onion and garlic increased. However, from Day 12 to 14 the total 2-furoylmethyl-amino acids decreased for onion, whilst it continued to increase for garlic. The decrease in 2-furoylmethyl-amino acids was reported as a consequent of progression to final stage of the MR, with 2-furoylmethyl lysine exhibiting the highest reduction. The differences as reported by Cardelle-Cobas et al. (2005) in sugar and amino acid content may have caused the continued increase in 2- furoylmethy-amino acid in garlic whilst it decreased in onion, which further reveals the rate of the MR taking place in these products.

With reference to antioxidant activity, the increase in 2-furoylmethyl-amino acids in onion coincided with its ability to scavenge peroxyl radicals. Moreover, the decrease observed between Day 10 and 14 did not result in decrease in peroxyl radical scavenging activity, instead it increased continually. This finding not only conclude that compounds formed due to acid hydrolysis of ARPs exhibit antioxidant activity, but also that the main contributors are the MR final stage melanoidins. To further confirm this, the brown color intensified throughout the 14-day storage as shown by the increase in BI at 420 nm. Numerous studies have been done pertaining increase in BI in heated plants extracts (Ahmad and Langrish, 2012; Bae et al., 2014; Redondo et al., 2016) serving as an indicator of the MR, particularly formation of melanoidins. Melanoidins, end products of the Maillard reaction, have been previously described as strong scavenger of active oxygen species and metal chelators. Initial stages of the Maillard reaction might exhibit a moderate effect on the antioxidant activity of stored dehydrated onion, while advanced products behave as potent oxygen radical scavengers. This could then explain the effect of heated plant extracts where EB inhibition increased with increasing heating time or temperature.

Furans; 5-Hydroxymethyl furfural (HMF) and 2-furaldehyde are two key MR intermediates formed through enolization, dehydration, and cyclization reactions of 2-furoylmethyl amino acids from hexoses and pentoses, respectively (Nooshkam et al., 2019). Perez-Burillo et al. (2019) studied the effect of different home cooking

methods on the antioxidant capacity, furosine, and HMF formation in vegetables. They reported that all culinary techniques with the exception of steaming showed significant higher furosine than the raw counterpart. However, HMF was not detected in raw, boiled and steamed vegetables, which onions was part of this. They attributed this to these cooking techniques not providing enough energy to progress the reaction further. Furosine levels correlated positively with ferric reducing antioxidant power (FRAP), hydroxyl radical scavenging, and total antioxidant capacity, while HMF correlated with FRAP and hydroxyl radical scavenging. These authors also attributed the high antioxidant activity to the synergy between the MR as well as other bioactives whose availability was increased due to cellular breakdown during heating, a phenomenon that has been mentioned countless times in this review. The results above can be used to explain the superior EB inhibition exhibited by sterilized followed by pasteurized and frozen onion by-products in the study conducted by Roldán et al. (2008), where both increased polyphenol and MR could be taking place.

5.1.1 | Addition of sugar and amino acid in onion extracts

To ascertain the formation of MRPs during heating of onion extracts, several authors added glucose and glycine to the onion extract, and applied the same heating parameters (Lee et al., 2002, 2007; Wong & Angel Lee, 2014). According to results obtained, the EB inhibition of fresh onion extract did not differ significantly from those of fresh onion with added glucose or glucose-glycine. However, when the heat was applied, the EB inhibition increased from onion extract > onion-glycine or onion-glucose > onion-glucose-glycine as depicted by decreased in relative enzyme activity (Table 5). Similar results were reported by Lee et al. (2007) and Wong and Angel Lee (2014). In the above study the involvement of the MR is apparent since addition of glucose and glycine augmented the reaction via increase in reactant concentration, and thus EB inhibitory. However, the anomaly with regards to heated glucose-glycine model system not exhibiting EB inhibition might be due to the effect of the concentrations of 0.1% at 100°C for 10 min only which might not produce sufficient energy to drive the MR. However, the time dependent EB inhibitory of heated glucose-glycine model systems have been reported in literature. Lee and Park (2005) reported a relative enzyme activity of 20.6% when a 27:11% concentration of glucose:glycine model system heated at 90°C for 7 hr. Similarly, Kim et al. (2012) reported a decrease in enzyme activity when EB glucose-glycine solution at 90° were heated for 1–11 hr.

5.2 | Pineapple (*Ananas comosus*) juice extracts

Rattanathanalerk et al. (2005) studied the effect of thermal processing on the loss of quality attributes of pineapple juice. They found that heating of pineapple juice at temperatures ranging from 55 to 95°C had a marked effect on the browning and HMF content. The results indicated that both browning and HMF increased linearly with time, and the higher levels were found at higher heating conditions. The authors further compared the activation energy obtained for HMF and BI at 420 nm over time, it was observed that the activation energy found for HMF formation was lower than that of brown pigment formation. The results implied that HMF occurred at a higher rate than color development. This further indicates HMF as an intermediate MRP which forms and reach peak levels before the brown color associated with dark final stage intensifies.

With reference to EB inhibition, Lim and Wong (2018) and Lim et al. (2019) applied pineapple juice to against ginger and sweet potato PPO (Table 1). In the former study, there was no significant difference observed between the EB inhibition of fresh (21.5) and heated pineapple juice (17.76). Meanwhile, in the latter study EB inhibition of fresh juice (31.88%) was stronger than that of the heated extract (7.42%). In both cases the substrate used was 4-methylcatechol (Table 2). The low EB depicted by heated pineapple juice in the latter study might be attributed to the destruction of sensitive inherent bioactive components, such as malic acid (Wessels et al., 2014) and in particular bromelain a proteolytic enzyme that is known as a PPO inhibitor is easily denatured during heating (Mohd Ali et al., 2020). Moreover, according to Gardner et al. (2000) pineapple juice was ranked seventh amongst other juices in terms of total phenolic content (358 μg/mg of GAE) and vitamin C (4.4 μM) content, with the latter contributed 0.8% toward antioxidant capacity, meanwhile, Mohd Ali et al. (2020) reported 14% vitamin C content. These results also show that pineapple juice inherently contains lesser bioactives, and these could easily be destroyed during heating. Moreover, the Maillard pathway/route linked with the pH value in the range of pineapple juice (3.52–5.2) is associated with the formation of HMF via 1,2 enolization of ARPs at pH ≤ 7. We are, therefore, linking the results reported above by Rattanathanalerk et al. (2005) concerning HMF formation and those by Lim et al. (2019). However, the heating conditions (time) in the study of Lim and Wong (2018) as well as Lim et al. (2019) would not be sufficient to produce a considerable amount of HMF compared to (Rattanathanalerk et al., 2005). Moreover, MRPs with reducing activity and antioxidant activity are associated with reductone MRPs formed at pH ≥ greater than 7 (Vhangani & Van Wyk, 2013). Thus, heating pineapple juice could have resulted in destruction of natural bioactives without any considerable development of the MR based on the pH and low heating times of 15 min at 95–100°C. The result of this is the decrease in EB inhibition of the heated plant extract, without also omitting the effect of factors substrate type and concentration of the bioactives.

5.3 | Red chilli peppers (*Capsicum annuum* L.)

Red pepper fruit (*Capsicum annuum* L.) contains considerable amounts of a wide array of phytochemicals mainly vitamins C, A, and E, as well as phenolic and carotenoids compounds with known antioxidant properties. In the study of (Rufián-Henares et al. (2013), red peppers

were vacuum dehydrated at 60–90°C for 8–45 min. The ascorbic acid content of fresh red pepper was reported at 93.2 mg/100 g dry matter, and reduced to 4 and 2.7 mg/100g after drying at 60 and 90°C, respectively. Consequently, the drying process also resulted in decrease in antioxidant activity (DPPH, ABTS, and FRAP), and this then correlated with an increase in furosine (2-furoylmethyl-lysine)

TABLE 5 Inhibitory capacity of glucose-glycine added onion extracts

Assay conditions	Model system type	REA (%)	Inhibition %	References
Potato PPO, 0.2 M Catechol, Spectrophotometric 420 nm at 25°C/60 s	Fresh glucose	97	3	Lee et al. (2002)
0.1% glucose	Fresh glycine	94	6	
0.1% glycine	Fresh glucose-glycine	92	8	
1.55 mg/ml Onion extract	Fresh onion extract	79	21	
	Fresh onion extract—glucose	73	27	
	Fresh onion extract—glycine	80	20	
	Fresh onion extract—glucose-glycine	83	17	
Potato PPO, 0.2 M Catechol, Spectrophotometric 420 nm at 25°C/60 s	Heat-treated glucose [a]	88	12	
0.1% glucose	Heat-treated glycine [a]	97	3	
0.1% glycine	Heat-treated glucose-glycine [a]	92	8	
1.55 mg/ml Onion extract heated at 100°C/10 min	Heat-treated onion extract	17	83	
	Heat-treated onion extract—glucose	4	96	
	Heat-treated onion extract—glycine	6	94	
	Heat-treated onion extract—glucose-glycine	3	97	
Taro PPO, 10 mM Catechol, Spectrophotometric 420 nm at 25°C/60 s	Fresh glucose	96	4	Lee et al. (2007)
25 mM glucose,	Fresh glycine	90	10	
25 mM glycine	Fresh glucose-glycine	92	8	
3.1 mg/ml Onion extract	Fresh onion extract	88	12	
	Fresh onion extract—glucose	84	16	
	Fresh onion extract—glycine	81	19	
	Fresh onion extract—glucose-glycine	77	23	
Taro PPO, 10 mM Catechol, Spectrophotometric 420 nm at 25°C/60 s	Heat-treated glucose	92	8	
25 mM glucose	Heat-treated glycine	74	26	
25 mM glycine	Heat-treated glucose-glycine	60	40	
3.1 mg/ml Onion extract heated at 100°C/10 min	Heat-treated onion extract	46	54	
	Heat-treated onion extract—glucose	42	58	
	Heat-treated onion extract—glycine	37	63	
	Heat-treated onion extract—glucose-glycine	32	68	
Cassava leaf PPO, 10–100 mM, Catechol Spectrophotometric 410 nm at 20°C/360 s	Fresh glucose[b]	82	18	Wong and Angel Lee (2014)
3 mg/ml Onion extract	Fresh glycine[b]	74	26	
	Fresh glucose-glycine	67	33	
	Fresh onion extract	62	38	
	Fresh onion extract—glucose	28	72	
	Fresh onion extract—glycine	27	73	
	Fresh onion extract—glucose-glycine	15	85	
Cassava leaf PPO, 10–100 mM, Catechol Spectrophotometric 410 nm at 20°C/360 s	Heat-treated glucose[b]	51	49	

(Continues)

TABLE 5 (Continued)

Assay conditions	Model system type	REA (%)	Inhibition %	References
3 mg/ml Onion extract heated at 100°C/10 min	Heat-treated glycine[b]	43	57	
	Heat-treated glucose-glycine	40	60	
	Heat-treated onion extract	53	47	
	Heat-treated onion extract—glucose	19	81	
	Heat-treated onion extract—glycine	14	86	
	Heat-treated onion extract—glucose-glycine	13	87	

Abbreviations: L-DOPA, L-dihydroxy phenylalanine; PPO, polyphenoloxidase; REA, residual enzyme activity which is also termed relative enzyme activity.

[a]Heat treatment as specified from source.

[b]Glucose and glycine concentration not specified.

levels. It is worth to note that no furosine was detected in fresh peppers, therefore, this is a clear indication that the MR took place. In addition, Rufian-Henares et al. (2013) in their study also conducted activation energy where they concluded that the activation energy of furosine was almost double that of antioxidant activity decrease, and three times higher than that of ascorbic acid loss; therefore, a higher thermal load was required to initiate the Maillard reaction compared to the loss of antioxidant compounds.

With reference to antioxidant, a large amount of phenolic and flavonoid compounds as well as ascorbic acid contribute to antioxidant properties in chili pepper, which are capable of inhibiting the formation of undesired brown pigments. In the study of Lim and Wong (2018) and Lim et al. (2019) heated chilli exhibited high browning inhibition than the fresh counterpart against ginger PPO, whereas no significant differences were observed against sweet potato PPO. However, the EB inhibition of the latter was 45.97 and 43.13% which was the highest amongst all tested natural inhibitors (Table 2). The inhibition exhibited above might to attributed to the furosine level. The antioxidant activity of furosine in glucose-model systems, garlic and onion extracts has been discussed in this review. Furosine has been found to scavenge peroxyl radicals, an indicative of hydrogen donation, thus the potential to act as a reducing agent (Javier Moreno et al., 2006).

5.4 | Thinned nectarines (*Prunus persica*)

Thinning involves removing excess fruit to reduce over bearing that often leads to inferior quality attributes such as small size and deformed shape (Moon et al., 2020). Another significant reason to thin fruit is preventing obtaining a heavy crop in 1 year and almost no crop in the next year, and lastly to reduce limb breakage that occurs when too much fruit is left and the fruit begins to size (Guo et al., 2020). Thinned fruits have no use and are usually discarded, however, these young fruits can serve as potential high value-added plant resources due to their physiochemical profile and bioactive capacity (Redondo et al., 2016). A study by Guo et al. (2020) proved that thinned nectarines contained higher total polyphenol, flavonoids, sugars, and amino acids compared to their ripe counterpart. As a result, they exhibited higher antioxidant activity as depicted by DPPH, FRAP, and ABTS. When Redondo et al. (2016) applied microwaves to heat thinned nectarine extracts, they concluded that the EB capacity was due to the formation of MRPs. In this case there was no doubt that the MR would form based on analysis conducted by Guo et al. (2020) where the sugar (glucose, fructose and sucrose) content ranged from 5% to 10%, amino acid (1.4%–1.7%) as well as the microwave power (500–1,500 W) and treatment time (90 s). When the microwave power increased, there was an increase in absorbance at 420 nm as indicated by a progressively intensifying brown color. This was an indication of the formation of intermediate MRPs as well as evolution to the final stage since carbonation was also perceived, a phenomenon associated with prolonged heating. Moreover, other main intermediate stage by-products such as acetic and formic acid were identified and quantified, and these also increased with an increase in microwave power. The development of these acids was accompanied by reduction in pH, which is an indication of the presence of sugar degradation products and Schiff bases, moreover, lower pHs may indicate progressively greater extents of MR. Previous. (Ahmad & Langrish, 2012). Ahmad and Langrish (2012) also obtained similar results with reference to browning intensity, pH reduction, and carbonization when heating mandarin peels using microwaves. Intermediate stage products are known for their high reducing power, a mechanism synonymous with EB inhibition. The intermediate compounds of MRPs are reported to be capable of donating hydrogen atom as indicated by the ability to reduce quinone to DOPA.

Moreover, MRPs produced at 1,500 W exhibited the highest inhibitory at 32% residual PPO activity, this further explains the notion that final stage MRPs are responsible for EB, and that are superior than intermediates products. The reductone moiety present in the melanoidin structure has been reported to exhibit reducing, chelating properties, and scavenging properties (Lee & Park, 2005). As mentioned, polyphenols inherent in the extract can also contribute to this inhibition. In addition, nectarine MRPs could inhibit EB at low concentrations and dialysis further proved that EB inhibition was irreversible since enzyme activity was not recovered. The PPO activity of extract was the same ($p > .05$), 20%, after and before dialysis, demonstrating that MRPs are mixed type inhibitors.

Contrary to most findings above, studies conducted by Kubglomsong and Theerakulkait (2014) and Lim et al. (2019), where heating resulted in reduced ability of extracts to inhibit enzymatic browning. Kubglomsong and Theerakulkait (2014) found that heating rice bran protein extracts at 80°C for 10 min resulted in a significantly reduced enzyme inhibition compared to an unheated extract. They attributed the reduction to denaturation of the active protein at high temperature. Proteins, peptides, and amino acids have been proven to alter the enzyme activity by chelating copper at the active site and/or by reacting with the ortho-quinone preventing further polymerization to form melanin. Similarly, Lim et al. (2019) also observed a decrease in EB inhibition of heated pineapple juice against sweet potato PPO (Table 2). The fresh pineapple juice exhibited an inhibition of 32% which reduced to 7% after heating at 100°C for 10 min. This was probably due to the destruction of bioactive compounds in pineapple juice such as malic and citric acid, which play major roles as acidulants (Wessels et al., 2014), both acids inhibit PPO activity by lowering the pH value or chelating the copper at the enzyme active site, ascorbic acid moreover also contributed to lower pH value and reduction of ortho-quinone back to precursor diphenol.

Any research that requires proving antioxidant, anti-inflammatory, of a new food additive or compound requires that the researchers investigate the safety aspect, that's if it is intended for consumption (Roldán et al., 2008). Additional to that, the effectiveness of the test compound has to be compared to other well-known/established antioxidants. Most studies involving heated plant extracts also compared their anti-browning potential to that of commercial anti-browning agents such as cysteine, ascorbic acid, citric acid, and sulfites (Lee et al., 2007; Wong & Angel Lee, 2014; Lim & Wong, 2018). Although the concentrations between heated plants extracts and antioxidants differed and could not be compared at molecular level, heated plant extracts exhibited anti-browning better than some known anti-browning agents (Table 6) (Lim et al., 2019; Lim & Wong, 2018).

Since MRPs were implicated as compounds responsible for the inhibitory effect of heated plant extracts, it was noteworthy to also investigate whether frequently discussed variables known to play a role during the Maillard reaction were affecting the anti-browning capabilities of heated plant extracts. As a result, common factors such as reaction temperature, time, color formation, reactant concentration, and reactant type associated with the MR are discussed in this review.

6 | MAILLARD REACTION RELATED FACTORS

6.1 | Non-specific Maillard indicators as BI, color, and pH of heated plant extracts

Development of a brown color is a non-specific index used to assess the extent and the rate to which the MR has taken place (Laroque et al., 2008; Vhangani & Van Wyk, 2013). In the early stages of the MR, the carbonyl group reacts with the amino group giving rise to colorless compounds which do not absorb in the visible spectrum. Further progress of the MR involves the production of high molecular weight compounds, termed melanoidins with a characteristic absorbance maximum at 420–460 nm (Vhangani & Van Wyk, 2013).

Most authors studying the Maillard reaction frequently determine the browning intensity/index spectrophotometrically or the color difference by means of coordinates using the Hunter Lab scale. In terms of common Maillard reaction model systems involving sugar-amino or sugar-protein, this is a well adopted means used as a quantitative indicator. The brown color associated with MRPs formed during the MR is known to intensify as the reaction temperature and time increases. Several authors have reported on similar findings in both food and model systems (Lee, Dae, et al., 2016; Vhangani & Van Wyk, 2016). With reference to heated plant extracts little emphasis was placed on browning, however, those who investigated this phenomenon produced similar findings.

Redondo et al. (2016) observed an intensified brown color when the absorbance (420 nm) increased from 0.7 to 1.8 at 500 W and 1,500 W of microwave heating thinned nectarines, respectively. In another study where MRPs were formed during microwave extraction of polyphenols from mandarin peels, the browning intensity of the extract was increased as the microwave power increased as well (Ahmad & Langrish, 2012). Moreover, both these authors also perceived carbonation at highest powers. This is a common occurrence during the MR, known to be associated with over or prolonged heating.

The decrease in pH as a result of acid formation is another commonly used indicator of the MR, therefore, lower pH may indicate progressively greater extents of MR. Redondo et al. (2016) and Ahmad and Langrish (2012) also measured the change in pH over time, as well as the formation of acetic and formic acid. Redondo et al. (2016) reported a pH decrease from 6.4 to 5 for control (0 W) and 1,500 W, respectively. Consequently, the concentration of formic and acetic acids increased from 26.0 and 0.2 mmol/kg at 500 W, to 56.0 and 6.2 mmol/kg at 1,000 W and to 82.2 and 27.2 mmol/kg at 1,500 W, respectively. Moreover, Ahmad and Langrish (2012) when investigated the importance of the MR in their study, the findings were in accordance to the results of Redondo et al. (2016) with reference to pH reduction as the extraction temperature was increased. Ahmad and Langrish (2012) observed that the unheated extract pH was initially 6.5 and then decreased to 4.8, 4.5, and 4.1 as the heating temperatures increased from 50, 100, and 150°C, respectively. In both instances the decrease in pH and formation of acid correlated with the intensity of the resulting brown color.

6.2 | Effect of reaction temperature and time on the inhibitory effect of plant extracts

The MR reaction takes places during processing and storage, however, with storage the progression is slower and brown color may

take long before it is perceived, compared to heating at higher temperatures for shorter periods where color development is immediate (Lee et al., 2017). Therefore, temperature and time plays a big role during the MR. Time in this instance is viewed as min/hours/days of heating or incubating to produce MRPs. Increase in temperature is known to increase the rate of the MR, and is associated with

TABLE 6 Enzymatic browning inhibition of heated plant extracts and chemical anti-browning agents

Assay conditions	Heated plant extracts versus anti-browning agents	Concentration mg/ml	REA	% Inhibition	References
Potato PPO and 0.2 M catechol	Fresh onion extract	3.1	44	56	Lee et al. (2002)
	Heated onion extract	3.1	10	90	
	Ascorbic acid	0.02	57	43	
	Citric acid	0.02	80	20	
	Sodium pyrosulfite	0.02	0.3	99.7	
	Potassium sorbate	0.02	77	23	
Pear PPO and 0.2 M catechol	Fresh onion extract	60	73	27	Kim et al. (2005)
	Heated onion extract	60	46	54	
	Ascorbic acid	0.05	1	99	
	Citric acid	0.05	97	3	
	Potassium sorbate	0.05	83	13	
	Cysteine	0.05	0.2	99.8	
Banana PPO, 0.2 M Catechol Spectrophotometric 420 nm at 25°C/60 s	Fresh onion extract	3.1	61	39	Lee (2007)
	Heated onion extract	3.1	22	78	
	Citric acid	19.21	80	20	
	Potassium sorbate	12.02	68	32	
	Sodium chloride	5.8	88	12	
	Ascorbic acid	1.76	1	99	
	Sodium pyrosulfite	1.96	0.3	99.7	
Taro PPO, 10 mM Catechol, Spectrophotometric 420 nm at 25°C/60 s	Fresh onion extract	3.1	88	12	Lee et al. (2007)
	Heated onion extract	3.1	46	54	
	L-ascorbic acid	0.02	0	100	
	L-cysteine	0.01	0	100	
	Glutathione	0.03	0	100	
	Dithiothreitol	0.02	0	100	
	Sodium pyrosulfite	0.02	0	100	
	Citric acid	0.02	92	8	
	Potassium sorbate	0.02	84	16	
	Sodium chloride	0.01	98	12	
	EDTA	0.03	84	16	
Cassava leaf PPO, 10–100 mM, Catechol Spectrophotometric 410 nm at 20°C/360 s	Fresh onion extract	3	62	38	Wong and Angel Lee (2014)
	Heated onion extract	3	53	47	
	L-ascorbic acid	0.18	29	71	
		0.53	22	78	
		0.88	21	79	
	L-cysteine	0.12	39	61	
		0.36	23	77	
		0.61	19	81	
	Citric acid	0.19	85	15	
		0.58	71	29	
		0.96	64	37	

(Continues)

TABLE 6 (Continued)

Assay conditions	Heated plant extracts versus anti-browning agents	Concentration mg/ml	REA	% Inhibition	References
Ginger PPO, 0.1 M Pyrocatechol[a], Spectrophotometric at 410 nm/5 min room temperature	Non-heated red onion extract	10	72	28	Lim and Wong (2018)
	Heated red onion extract	10	67	33	
	Non-heated chilli pepper extract	10	59	41	
	Heated chilli pepper extract	10	51	49	
	Non-heated pineapple juice	10	75	25	
	Heated pineapple juice	10	73	27	
	L-cysteine	0.12	88	12	
		0.36	79	21	
		0.61	72	28	
	Sodium chloride	0.06	89	11	
		0.17	80	20	
		0.29	72	28	
	Sodium metabisulfite	0.19	96	4	
		0.38	50	50	
		0.95	45	55	
Sweet potato PPO, 0.1 M Pyrocatechol/4-methylcatechol[b], Spectrophotometric at 410 nm/5 min room temperature	Non-heated red onion extract	1,000	61	39	Lim et al. (2019)
	Heated red onion extract	1,000	59	41	
	Non-heated chilli pepper extract	50	54	46	
	Heated chilli pepper extract	50	57	43	
	Non-heated pineapple juice	1,000	65	35	
	Heated pineapple juice	1,000	80	20	
	L-ascorbic acid	0.18	42	58	
		0.53	39	61	
		0.88	36	64	
	L-cysteine	0.12	42	58	
		0.36	37	63	
		0.61	35	65	
	Sodium chloride	0.06	96	4	
		0.17	97	3	
		0.29	88	12	

Note: Concentration of all anti-browning agents was converted into mg/ml.

Abbreviation: REA, residual enzyme activity which is also termed relative enzyme activity.

[a]Results based on substrate which exhibited highest inhibition.

[b]Highest browning inhibition for plant extracts was exhibited by pyrocatechol and for anti-browning agents 4-methylcatechol was the best.

formation of brown melanoidic compounds known to possess antioxidant activity (Bai et al., 2017). Although time and temperature are known to influence the MR in terms of color formation and antioxidant activity. One should also take cognizant of the influence of the reactant type. There are model systems where color formation was not perceived, that is, model system stayed colorless, however, compounds with antioxidant activity were formed. The enzyme inhibitory effect of heated plant extracts increased markedly when heat treatment was applied, and most authors observed a stronger inhibitory when the heating temperature was increased in vitro and in vivo (Table 3). When Lee et al. (2002) heated onion extract at a 10°C increment from 40 to 100°C, the residual enzyme activity (REA) of potato PPO decreased as the temperature increased from 88% to 18% for 40 and 100°C, respectively. Similar inhibitory against taro PPO was reported in a study of by Lee et al. (2007) with when the heating temperature of onion extracts was increased. With regards to increasing heating time, Kim et al. (2005) studied onion extracts and proved that increasing heating time from 2 to 12 min at a constant temperature of 100°C increased the inhibitory effects of the onion extracts on pear PPO. Longer exposure time during heating

reduced the REA from 78% to 39%, which was in agreement to Lee et al. (2007) who also observed a reduction in REA with 16 min of heating resulting with 35% REA, which relates to greater enzyme inhibition (Table 4). Altunkaya (2014) also reported a decrease in REA on lettuce PPO as the heating time of grape leaf extract (GLE) increased at 100°C. Compared to the onion extract, the GLE exhibited lower browning inhibition. For instance, after heating for 15 min the GLE reduced the enzyme activity by 42%, meanwhile, the onion extract at a similar time averaged at 63%. The slight differences in performance are governed by various factors such as type of plant, its concentration and substrate type. Although there are little discrepancies, these results still further prove that Maillard reaction products formed during heating are responsible for the increased inhibition, and yielded constant results regardless of variables.

Redondo et al. (2016) applied microwave heating on thinned nectarines at increasing power, the mushroom PPO catalytic efficiency was reduced, this was evident through decreased ability to bind with substrate. The Km increased from 0.9, 1.73 and 2.05 for 500, 1,000 and 1,500 W microwave power, respectively, proving that an increase in heat energy increased the formation of MRPs. Ahmad and Langrish (2012) studied the impact of the MR during microwave extraction of antioxidants from mandarin peels. Although their study did not investigate the anti-enzymatic browning, they looked at the impact of heating on the antioxidant activity. They reported that the amount of antioxidants extracted increased at higher temperatures starting from 50, 100, 150, 200, and 250°C. However, the color of the extract intensified to dark brown and acquired a pungent odor at higher temperatures. They attributed this finding to high molecular weight Maillard reactions products, in particular the brown color nitrogenous melanoidins formed through a series of condensation and polymerization reactions. They further detected the formation of acrylamide at temperatures of 180°C and above. Darkened extracts may be associated with formation of carcinogenic compounds such as acrylamide as well as color that would not be desirable when incorporated in food products. For this reason, Roldán et al. (2008) recommended pasteurization of onion by-products although sterilization was proved as the process that produced compounds which exhibited better enzyme inhibitory capacity. This is an important factor in the optimization of the extraction process for antioxidants.

6.3 | Effect of the matrix type on the inhibition of browning by heated plant extracts

Maillard reaction products have been produced and extracted from various matrices; some of these include coffee, bread, simple sugar-amino, or sugar-protein models systems. Most of these extractions are semi-solid, solid, liquid type and when the MRPs are studied, they are made in solution form (Anese et al., 1999). It is significant to investigate the inhibitory effect of MRPs produced from plant extracts of different matrices to identifying where these bioactive compounds are located.

A study by Barbagallo et al. (2012) which investigated the in vitro and in vivo anti-browning effect of fresh and heated onion (100°C for 15 min) by-products against eggplant PPO, proved that heated onion juice exhibited the strongest inhibition at 45.8% and 40% residual enzyme activity (REA) and relative browning index (RBI), respectively. Furthermore, the inhibition followed in the order of heated paste (58.8%) (50%), fresh juice (62.7%) (58%), fresh paste (71.1%) (64%), fresh bagasse (73.7%) (66%), and heated bagasse (76.8) (70%) and this last more than bagasse, despite the heat-treatment for REA and RBI, respectively (Table 2). Moreover, observing the differences between matrices, it was quite evident how effective onion by-products are in inhibiting browning, and that this progressively increased with the increase of the water content, since the juice resulted more effective than paste and bagasse. Barbagallo et al. (2012) suggested that active compounds responsible for inhibition are inherent in the onion, namely; alk(en)yl cysteine sulfoxides combined with Maillard reaction products that form during heating could be of a hydrophilic nature. A study by Roldán et al. (2008) who investigated similar matrices from two different onion cultivars (recas and figueres) found that among the different heat treatment of pasteurization (100°C for 11–17 min) and sterilization (115°C for 17–31 min), the latter exhibited the highest inhibition against avocado PPO in the form of paste at 10.29% REA, bagasse (27.32%), and juice (40.36%), which contradicts the pattern observed by Barbagallo et al. (2012). However, with reference to pasteurized recas by-products, onion juice exhibited highest browning at REA of (45.8%) followed by paste (58.8%) and bagasse (76.2%) (Table 2). The excellent inhibition displayed by onion by-products subjected to sterilization might be due to the synergic effect of thiol and phenolic compounds, Maillard reaction and caramelization products (Lee et al., 2002). Moreover, onion contains a myriad of polyphenols (anthocyanins, isohamnetin, kaempferol, and quercetin) (Lee, Seo, et al., 2016) which may participate during the MR. To support this notion, Delgado Andrade and Morales (2005) found that phenolic compounds (ferulic, chlorogenic, caffeic, and p-coumaric acid) inherent in coffee participated in the Maillard reaction during roasting to form water soluble coffee melanoidins. The reaction was possible due to the phenols contributing their carbonyl groups in the Maillard reaction to form phenolic-bound melanoidins known to possess excellent antioxidant and metal chelation properties (Vhangani & Van Wyk, 2016). With reference to the anomaly between the superior browning inhibition of juice versus paste. In conjunction with anti-browning studies, Roldán et al. (2008) also reported that bioactive compounds were highly concentrated in the paste or bagasse by-products that contained more cell walls than in the juice. Thus, high temperatures of sterilization increased the release of bioactive compounds from the cell walls of onions. A probable explanation would be linked to the progression of the MR and possible caramelization during sterilization compared to pasteurization. Sterilization is more likely to result in faster polymerization of LMW compounds to form nitrogenous HMW, as a result, these HMW polymers may have been trapped in the solid matter of the paste matrix than free flowing in the liquid, thus, resulting in

these HMW melanoidins in the paste and imparting anti-browning properties. Moreover, the differences in PPO type, eggplant versus avocado for Barbagallo et al. (2012) and Roldán et al. (2008), respectively might have an effect on how the onion extracts react.

6.4 | Effect of added sugar and amino acid on the inhibition of browning by heated plant extracts

Numerous studies have been conducted regarding enzymatic browning involving sugar-amino acid Maillard model systems. Lee and Park (2005) reported a great reduction in enzyme activity when applying glucose and various amino acid (valine, glycine, serine, cysteine, asparagine, lysine, arginine, and histidine) produced at 90°C for 7h on potato PPO in vitro. Amongst the amino acids arginine exhibited highest inhibition at 0.1% REA. Billaud et al. (2005) investigated MRPs resulting from sulfur-related compounds with several sugars against apple, mushroom and eggplant PPO. Furthermore, Xu et al. (2017) went a step further and correlated the browning inhibition capacity with the antioxidant ability of MRPs derived from different amino acids and sugars. These authors concluded MRPs derived at 115°C for 200 min exhibited the highest antioxidant capacity and significantly inhibited EB of mushroom tyrosinase.

Results reported by the afore-mentioned authors sparked an interest in investigating the browning inhibition of heated plant extracts. Since the browning inhibition of sugar-amino MRPs are known, in order to determine the mechanism of the inhibitory effect of the heated plant extracts, sugars and amino acids were added before heating extracts. Heating of plant extracts with added sugar and amino acid increased the browning inhibition much greater than heated plant extracts or fresh plant extracts, in most cases, with a few exceptions, the enzyme inhibition increased in the order from plant extract-amino acid, plant extract-sugar to plant extract-sugar-amino models systems (Lee et al., 2002, 2007; Wong & Angel Lee, 2014) (Table 5).

Lee et al. (2002) studied the inhibitory effect of heated onion extract with added sugar and amino acid against potato PPO. The REA for the onion-glucose-glycine, onion-glucose, onion-glycine, onion and glucose-glycine heated at 100°C for 10 min was 2.6%, 4.1%, 5.9%, 17%, and 92%, respectively. The heated onion-glucose-glycine added model system exhibited four times browning inhibition than the heated onion only (Table 5). Similarly, when Lee et al. (2007) applied the same matrix on taro the results also showed that glucose-glycine added onion extract exhibited the highest browning inhibition at REA of 31.5%, followed by onion glycine (37%), onion-glucose (41.8%), onion extract only (46%), and glucose-glycine 60%. The glucose-glycine added heated onion extract exhibited 1.5 times the browning inhibition than heated onion only. The reduced inhibitory effect when comparing the above studies may be attributed to the differences in enzymes in question, *viz* potato and taro PPO. Moreover, the concentration of the onion, sugar, and amino acid was different in both studies; Lee et al. (2002) applied the onion at concentration of 1.55 mg/ml with glucose and glycine at 0.1%, however, Lee et al. (2007) had double the onion concentration at 3.1 mg/ml, glucose at 0.45% and glycine at 0.18%. An increase in reactant concentration is known to heighten the MR, in the latter study both onion, glucose, and glycine were increased, evident to this the glucose-glycine model system resulted in REA of 60.3% compared to the former study at 92%. In another study conducted by Matmaroh et al. (2006) who demonstrated that an increase in reactant concentration from 0.75 to 30 mM of fructose-glycine model systems increased the browning inhibition against black tiger shrimp PPO by 38 and 80%, respectively. Thus, an increase in concentration of the sugar and amino acid increased the inhibitory capacity of MRPs (Lee & Park, 2005). In addition, to obtain conclusive explanation by comparing similar model systems to those studied by the above-mentioned authors. The REA of 39, 60, and 92% exhibited by sugar-amino acid model systems was based on heating at 100°C for 10 min, the low inhibition of browning might be attributed to lower reactant concentration and shorter heating times. Considering that there is well documented evidence of high PPO inhibitory effect of heated glucose-glycine model systems. Authors Lee and Park (2005) and Lee (2007) observed slightly lower REA of 20 and 14.4% against potato and banana PPO, respectively when reacting 1.5 M of each glucose and glycine model systems at 90°C for 7 hr. In another study by Kim et al. (2012) where 1 M of each glucose and glycine were also heated at 90°C for 7 hr resulted with a REA of 80%. The high inhibition in browning by these glucose glycine model systems was attributed to the increased reactant concentration and long exposure time of 7 hr compared to heating at 100°C for 10 min. The anomaly regarding the study with the 80% REA could be due to lowered concentration, as well as the effect of different PPO employed.

Wong and Angel Lee (2014) were the latest authors to investigate the browning inhibition of heated onion extracts with added glucose-glycine against cassava PPO. They obtained similar findings where heated onion extract with added glucose-glycine with a REA of 12.82% proved superior than all model systems. The REA increased to 14.1% for onion-glycine, 19.23% for onion-glucose, 39% for glucose-glycine, and 53% for the heated onion only. Although the glucose and glycine concentration was not specified by these authors, results suggest that the inhibition exhibited by heated sugar and amino acid alone surpassed that of the heated onion only, which is contrary to results obtained by Lee et al. (2002, 2007), where the heated onion extract surpassed the heated glucose-glycine. The latter authors concluded that onion ingredients might have been necessary for the inhibitory effect of heated onion. Fresh onions are inherently rich in flavonoids and alk(en)yl cysteine sulfoxides, with sulfur compounds of low molecular weight known to inhibit PPO activity. One flavonoid occurring in onion that may exert substantial inhibitory effect against PPO activity is quercetin (Lee, Seo, et al., 2016) known to inhibit oxidation of L-DOPA by chelating copper in the enzyme (Bernaś & Jaworska, 2015; Lee, Seo, et al., 2016). In addition, numerous authors proved the dominance of sulfur compounds and sulfur amino

acid in inhibiting browning. In model systems where various amino acids and sugars were reacted, sulfur containing amino acids exhibited higher browning inhibition than their counterparts (Billaud et al., 2005; Lee, 2007; Phonpala et al., 2009; Xu et al., 2017). Moreover, the stronger inhibitory action shown by the heated onion amino acid-sugar model system was as a result of the synergistic effect between compounds inherent in the onion reacting with the in combination with MRPs resulting from reactivity of the sugar and amino together.

6.5 | Effects of plant extract concentration on the inhibition of browning by heated plant extracts

The effect of increasing reactant concentration during the MR is a well know phenomenon. An increase in reactant concentration, that is, sugar and amino in the case of the MR has been correlated with an increase in browning intensity (color), antioxidant, and antimicrobial activity of MRPs (Benjakul et al., 2005). However, with reference to heated plant extracts varying concentrations, in this case the increase thereof did not always have a positive correlation to enzyme inhibition. Lee et al. (2002) investigated onion extracts at a concentration of 1.55 and 3 mg/ml heated at 100°C for 10 min. The increase in concentration had a significant effect on browning inhibition of potato PPO in vitro, since the REA decreased from 17% to 9.6% as the concentration increased. A similar trend was observed for the fresh onion extracts. However, comparison of different authors who applied heated onion extracts at constant concentration of 3 ± 0.1 mg/ml on various PPO yielded versatile results. Lee (2007), Lee et al. (2007), Wong and Angel Lee (2014), found that heated onion extract exhibited a REA of 38%, 46.1%, and 53% when applied to banana, taro and cassava leaf PPO, respectively, which compared to findings of Lee et al. (2002) translates to reduced enzyme inhibition (Table 2). The lack of conclusion in terms of concentration might be attributed to the versatility in test PPO of different studies. Enzymes are known to be affected by certain factors such as temperature, pH, plant species, substrate specificity etc (Lee et al., 2007). For instance, a study by Duangmal and Owusu Apenten (1999) compared the activity of taro and potato PPO. They observed that both taro and potato-PPO had similar substrate specificity for catechol; however, potato PPO had temperature maxima at 25°C, than 30°C of taro PPO. This then explains why taro exhibited less enzyme inhibition at higher concentration (3.1 mg/ml) compared to 1.5 mg/ml of potato-PPO, which was then favored at the test temperature of 25°C applied by Lee and Park (2005) and Lee et al. (2007).

In vivo studies by Kim et al. (2005) applying heated plant extracts of increasing concentrations of 40, 60, 80, and 100 mg/ml was found to increase greatly the inhibition of browning of pear juice. They concluded that as the onion extract concentration increased, the lag period lengthened and the extent of browning decreased.

In another in vivo study Lee, Seo, et al. (2016) investigated the browning index of unheated and heated (96°C, 1 hr) apple juice supplemented with onion solutions of increasing concentrations (0.5, 1.25, and 2.5%). The authors reported that addition of onion consistently reduced the estimated browning index in unheated apple juice as the concentration increased. The browning index decreased from 0.58 to 0.3 at 420 nm. Likewise, the heated counterpart exhibited a similar trend from 0.36 to 0.28, however, in comparison to unheated samples it exhibited significantly lower browning index as indicated by increased L-value. However, contrary to other studies where heating is usually applied on the onion extract separately, and then addition into the juice. In this study heating was applied to the juice with incorporated onion. This approach might have an inflated the effect of added onion, since heating on its own denatures the enzyme.

7 | ENZYMATIC BROWNING RELATED FACTORS

7.1 | Effect of substrate type on the inhibition of browning by heated plant extracts

One of the important properties of enzymes is the specificity they exhibit relative to the reactions they catalyze. Few enzymes exhibit absolute specificity; that is, they will catalyze only one particular reaction. Other enzymes will be specific for a particular type of chemical bond or functional group. In general, there are four distinct types of specificity, namely: absolute; group; linkage and stereo chemical specificity. When evaluating anti-enzymatic activity, it is always advisable to employ a variety of substrates to conclude the level of inhibitory. To conclude the effectiveness of MRPs or heated plant extracts as anti-browning agent, we have to follow suite. Most authors investigating MRPs or heated plant extract's effectiveness in enzyme inhibition utilized catechol as substrate with an exception of Roldan Wong and Angel Lee (2014), Lim and Wong (2018) and Lim et al. (2019)

Lee et al. (2007) investigated the effect of fresh and heated (110°C for 10 min) onion extract on taro PPO using several substrates at a constant concentration of 10 mM. In terms of specificity, taro PPO was most effective in the order from 4-methylcatechol with REA of 39%, LDOPA (43%), catechin (45%), catechol (46.2%), and pyrogallol red (52%), and showed poor or no substrate specificity for hydroquinone and resorcinol. Regardless of the substrate type, the heated onion extract always exhibited stronger inhibitory effect on taro polyphenol oxidase than did the fresh onion extract.

Lim and Wong (2018) studied the effect of unheated and heated onion (95°C for 15 min), chilli pepper and pineapple extracts using 4-methycatechol and pyrocatechol as substrates to inhibit browning of ginger PPO. Heated and unheated chili pepper extracts showed the greatest inhibition percentage on ginger PPO for both 4-methylcatechol and pyrocatechol as compared to onion and pineapple extracts. However, when comparing unheated with heated extracts, the latter exhibited significantly stronger inhibition in the order from pineapple (27%), onion (33%) to chilli (48%). With reference to

substrates, ginger PPO showed a stronger affinity to bind toward pyrocatechol as shown by a lower Km (4.39) and Vmax (8,928.27), compared to 4-methylcatechol at Km (6.35) and Vmax (12,658.23). Moreover, greater enzyme inhibition was exhibited by all heated extracts with pyrocatechol was as a substrate, where the superiority of chilli pepper was more evident based on its increased at Km 6.1, this was solely based on its ability to reduce the binding and formation of the ginger PPO-pyrocatechol complex.

Similarly, in a subsequent study Lim et al. (2019) repeated the above study, however, the enzyme of choice was sweet potato PPO. Their findings were in accordance to those obtained by Lim and Wong (2018), where they concluded that sweet potato PPO exhibited a greater affinity toward pyrocatechol compared to 4-methylcatechol. In addition, greater enzyme inhibition was also exhibited by all extracts when pyrocatechol was used as a substrate, however, in this instance unheated chilli pepper showed the highest percentage browning inhibition at 46%, which was still considerably lower than the findings of Lim et al. (2019) who reported heated chilli pepper as the highest inhibitor at 48% against ginger PPO. Interestingly, heated onion extract was found to exhibit a higher inhibition percentage on sweet potato PPO than fresh onion extract for both substrates. These results were in accordance with the findings by Kim et al. (2005), Lee et al. (2007) and Lim and Wong (2018), whereby heated onion extract exhibited a stronger inhibitory effect on PPO activity than did fresh onion extract in the study of pear, taro, and ginger PPO.

7.2 | The effect of known anti-browning versus heated extracts on the inhibition of browning

Kim et al. (2005) applied anti-browning agents alongside heated onion extracts as reference anti-browning agents against pear PPO. Based on residual enzyme activity (REA) percentages, cysteine and ascorbic acid exhibited the highest browning inhibition followed by heated and fresh onion extracts (60 mg/ml), potassium sorbate, and citric acid at 0.16%, 0.68%, 46%, 73%, 83%, and 97%, respectively (Table 6). Similar findings were reported by Lee (2007) against banana PPO, Lee et al. (2007) against taro PPO and Wong & Angel Lee (2014) against cassava leaf PPO, where ascorbic acid (0%–21%) and cysteine (0%–19%) exhibited the highest reduction of enzyme activity, in some cases it was close to complete reduction followed by heated and fresh onion extracts. Ascorbic acid, cysteine and sulfites are known for their excellent inhibitory effect owing to their multiple mode of action in preventing enzymatic browning. Yapi et al. (2015) reported an inhibition percentage of 95% for sodium bisulfite, 87% for L-cysteine and 86% for L-ascorbic acid against yam PPO. The mechanism of ascorbic acid inhibition involves the reduction of the enzymatically formed o-quinone back to diphenol. However, ascorbic acid offers limited inhibition of browning since it is most likely oxidized to dehydroascorbic acid and further degraded to 2,3-diketo-gluconic acid (Altunkaya, 2014). Citric acid (64%–97%), potassium sorbate (67%–84%), and sodium chloride (88%–98%) moreover, were the least effective inhibitors as reported by all the authors in question. Granting that concentrations applied by all these authors for both onion extracts (3–60 mg/ml) and anti-browning agents (0.04–100 mM) were variable the results were consistent.

With reference to authors who investigated plant extracts other than onion extracts, Altunkaya (2014) investigated the effect of fresh and heated grape leaf extracts in conjunction with ascorbic acid and citric acid against lettuce PPO. Ascorbic acid exhibited the highest inhibition, followed by citric acid, heated, and then fresh grape leaf extract.

In a study conducted by Lim and Wong (2018) L-cysteine, sodium chloride, and sodium metabisulfite anti-browning agents at increasing concentrations were applied against ginger PPO using 4-methylcatechol and pyrocatechol. The ginger PPO had a greater catalytic effect against pyrocatechol than 4-methylcatechol. Greater inhibition was observed when 4-methyl catechol was used as a substrate compared to pyrocatechol. Moreover, the inhibition percentage increased as concentration of inhibitors increased from 1 to 5 mM, with sodium metabisulfite exhibiting the greatest inhibition of ginger PPO at 46.36% at 1 mM. Furthermore, it had the lowest IC_{50} and Ki value as compared to sodium chloride and L-cysteine, this signified its strongest binding affinity to ginger PPO. The findings of this study are contradictory to what has been previously discussed. In this case L-cysteine has been found to exhibit similar inhibition to sodium chloride, whereas in findings by Lee (2007) and Lee et al. (2007), sodium chloride was the least effective amongst six anti-browning agents, where cysteine ranked first (Table 6).

When comparing anti-browning agents with plant extracts, in this study all unheated and heated plant extracts (10 mg/ml) exhibited browning inhibition comparable to L-cysteine and sodium chloride, with heated chilli pepper exhibiting browning inhibition of 47.97% which was close to that of sodium metabisulfite (3 mM) at an inhibition of 50%.

In a subsequent study of Lim et al. (2019) similar to Lim and Wong (2018) where L-ascorbic acid, L-cysteine, and sodium chloride at (1, 3, and 5 mM) were applied against sweet potato PPO. L-cysteine showed the greatest inhibition as the concentration increased with 5 mM exhibiting an inhibition of 70%. The inhibition decreased in the order from L-cysteine, L-ascorbic acid, and sodium chloride. These results are in accordance with the findings of Lim and Wong (2018) who reported that sodium chloride was the least effective anti-browning agent. Moreover, all heated and unheated plant extracts surpassed sodium chloride's inhibitory capacity (Table 6). However, plant extracts were surpassed heated onion, fresh, and heated chilli pepper exhibited similar results equivalent to L-ascorbic acid at 1 and 3 mM. When pyrocatechol was used as a substrate.

7.3 | Effect of Dialysis on the inhibition of browning by heated plant extracts

Dialysis is performed to establish whether the inactivation of PPO by MRP is reversible or irreversible. Dialysis allows dissociation of

the enzyme-inhibitor complex. Studies by Kim et al. (2005) and Lee et al. (2007) proved that inhibition of enzyme activity by heated onion extract was reversible. By observing an increase in enzyme activity post dialysis, they suggested that low molecular weight compounds could have been responsible for inhibition of PPO enzyme. Evident to this in terms of the capabilities of MRPS are the results of our previous study which found that low molecular weight MRPs exhibited stronger antioxidant activity than HMW and WM (Vhangani & Van Wyk, 2016). Contrary to this Wong and Angel Lee (2014) and Redondo et al. (2016) investigated heated onion extracts and thinned nectarines proved that the enzyme inhibition was irreversible. This anomaly further proves the complexity of MRPs and this could have been settled if the type of products formed during heating were known.

8 | CONCLUSION

Authors who reported an increase in browning inhibition due to heating of plant extracts linked this action to the Maillard reaction. The antioxidant activity of Maillard reaction products have been proposed by the common assays, and some mechanisms of action identified. For instance, Amadori rearrangement products are key intermediates of the early stage of the Maillard reaction that has been proven to possess metal chelating, reducing and oxygen-scavenging properties. Melanoidins moreover are also excellent metal chelators. In this review we conclude that most authors agreed that all MRPs formed throughout the MR possess EB inhibition, however, the final stage MRPs surpassed that of early and intermediate stage. Moreover, studies involving sugar-amino, sugar-protein model systems and real food systems have been devoted on identifying specific Maillard reaction products. Thus, more research on heated plant extracts should focus on identifying actual compounds formed in the matrices during early, intermediate and final stages of the Maillard reaction, by doing so further conclusive evidence will then validate the involvement of the Maillard reaction in heated plant extracts. Moreover, optimization of heating parameters should be applied to be able to produce MRPs which exhibit maximum antioxidant activity with brown color intensity.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

AUTHOR CONTRIBUTION

LN Vhangani: Funding acquisition; Investigation; Project administration; Resources; Visualization; Writing-original draft; Writing-review & editing. **Jessy Van Wyk:** Supervision; Validation.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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