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ENHANCING THE ENZYMATIC BROWNING INHIBITION CAPACITY OF *MORINGA OLEIFERA* SEED EXTRACT VIA THE MAILLARD REACTION

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ABSTRACT

The antioxidant and anti-browning activity of heated plant extracts have been attributed to the formation of Maillard reaction products (MRPs) via the Maillard reaction (MR). The inhibitory effect of heated *Moringa oleifera* (MO) seed extract on banana polyphenol oxidase (PPO) was investigated. The Plain MO seed extracts and those with added glucose and glycine (1.5 mM each) were heated at 100°C for 15, 30, 60 and 120 min. The pH and brown colour development decreased and increased significantly ($P < 0.05$) with increased reaction time, respectively, with heated moringa glucose-glycine HMGGL for 120 min exhibiting the highest pH reduction (2.58) and darkest extracts at an L* value of 8.11. This phenomenon is associated with progression of the MR. With reference to enzymatic browning, heated MO seed extracts exhibited stronger inhibitory effect against banana PPO activity in vivo and in vitro than the unheated counterpart. Evident to this are the higher inhibition percentages and lower ΔE values. Among model systems, the highest in vitro browning inhibition was exhibited mostly by longer heating times of 60 and 120 min. Model system HMGGL 120 min proved to be superior at 96% inhibition, which was comparable to known synthetic commercial antioxidants such as ascorbic acid (AA) at 99%, as well as ethylenediaminetetraacetic acid (EDTA) and citric acid (CA), both at 100% inhibition. In vivo enzymatic browning inhibition followed a similar trend, where the brown pigment (melanin) intensified as shown by an increase in ΔE as the storage time increased from 0.5 to 24 h. The model system UMGGL exhibited highest inhibition of brown melanin ($p < 0.05$). Although it was the best amongst other model systems, it was surpassed by synthetic antioxidants AA, EDTA and CA, which were ranked amongst the top three in inhibiting brown pigment formation in vivo. To further illustrate the effect of MR augmented MO seed extracts on enzyme activity inhibition, UMGGL 60 and 120 at 5 and 24 h storage surpassed the inhibitory effect of AA. At the said storage times, AA lost its inhibitory potential against pigment formation. This was due to oxidation of AA to form dehydroascorbic acid, which lacks inhibitory potential. This study proved that heating MO plant extracts increases their enzymatic browning inhibition potential, furthermore, the inhibitory capacity was heightened when reacted via the MR.

Key words: Anti-Browning, polyphenol oxidase, Maillard reaction products, Plant extracts, *Moringa oleifera*

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INTRODUCTION

Appearance is one of the key sensory attributes considered by consumers when selecting a food product. Among them, colour is a first critical determinant. Browning reactions that occur during handling, processing and storage, affect the acceptability, and thus commercial value of products. Of the different types of browning reactions, enzymatic browning is one of the well-known chemical reactions [1]. It is responsible for deterioration or enhancement of food quality. Enzymatic browning (EB) reactions are usually adverse in cases where they lead to discolouration, off-flavour formation and loss of nutritional value of fresh fruits, vegetables, nuts, tubers and crustaceans [2,3]. In some cases, it is beneficial in the development of characteristic colour and flavour associated with coffee and cacao beans, tea, black olives, dried fruits and wine. Whether EB reactions are desirable, or not, some level of control is required [1].

The browning reaction mechanism is well characterised and involves the oxidation of phenolic compounds by a class of oxidoreductases metallo-enzymes [4]. To combat this reaction, various interventions were introduced. Many papers have reported several physical and chemical methods for controlling or inhibiting enzymatic browning [1]. These methods are applied based on inactivating the enzymes , removing essential components on the enzymes, or complexing with intermediate end-products[4].

Chemical inhibitors are the most preferred choice due to cost and high performance. However, commonly used inhibitors such as sulphites have increased regulatory scrutiny due to association with initiating adverse reactions in asthmatic individuals [3] and, consequently, their application on fresh-cut food products has been banned by the U.S. Food and Drug Administration. Sulphites also reduce the uptake of 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 naturally occurring; however, these are less effective than sulfiting agents, since ascorbic acid is quickly consumed in the process. Moreover, consumer awareness has resulted in demand for clean labelling, and this has driven the food industry into looking for natural alternative anti-browning agents to synthetic food additives [4]. However, 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 odour and colour of compounds that make them up [2].

Numerous studies have been devoted to the search for other natural alternative inhibitors. Some of those proposed to have had the inhibitory effects are honey, cysteine [4], glutathione [6], natural aliphatic alcohols, kojic acid [1] and Maillard reaction products (MRPs) [7].

Numerous papers have been published with reference to the use of plant extracts to inhibit EB. Several authors proved that plant extracts obtained from garlic, green tea, sage, oregano [8], onion [9,10] could inhibit EB of apple, yam and banana. These authors attributed the inhibition to the presence of polyphenolic compounds, thiol, volatile sulfur compounds and peptides inherent in these extracts. Plant extracts have been found to competitively and non-competitively inhibit enzyme activity, by acting as a chelating agent or reducing agents. Upon witnessing the effect of plant extracts on EB, several authors decided to investigate the effect of heating these extracts as a solution to supress the inherent strong odour and flavour characteristics [2]. Moreover, the heating of plant extracts has been proven to increase functionality. Numerous studies have been devoted to investigating the effect of heating on the antioxidant activity of food commodities, especially those from fruit and vegetable origins. Heated plant extracts obtained from onion and nectarine were found to inhibit in vitro

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polyphenol oxidase (PPO) activity in potato, banana, taro, eggplant, avocado, pear, cassava leaf and mushrooms, as well as in vivo in potato, pear, peach and eggplant. Moreover, heated extracts proved to exhibit higher anti-browning activity than their fresh counterparts [4, 11–14]. Most authors attributed the increased inhibition of EB of heated plant extracts to the MR.

Therefore, the aim of this work was to study the effect of heating moringa seed extracts on the enhancement of the antioxidant and anti-enzymatic browning activity of banana.

MATERIALS AND METHODS

Materials

Moringa seeds were obtained from Super Nutri (Graafreinent, South Africa). Semi ripe bananas were bought at a local supermarket in Bellville (South Africa). Glucose, glycine, ascorbic acid (AA) and citric acid (CA) were obtained from Merck (Modderfontein, South Africa). Catechol was obtained from Sigma (Aston Manor, South Africa). The water used in the study was ultrapure water purified with a Milli-Q water purification system (Millipore, Microsep, Bellville, South Africa).

Methods

Preparation of defatted Moringa seed extract

Defatted *M. oleifera* seed flour was prepared according to the method of Singh *et al.* [15] with slight modifications. Dehulled seeds were milled into flour (100 µM) and dried at 50 – 55° C for 4 h. The flour was defatted with n-hexane and the fat content was determined by Soxhlet method [1]. Defatted flour and water in a 1:3 (w/v) ratio were homogenised for 10 min using a polytron and centrifuged at 15 000g for 20 min. The supernatant was referred to as the moringa seed extract. Fifty mL aliquots of the extract were heated at 100°C for 15, 30, 60 and 120 min with subsequent cooling in ice water.

Proximate analysis

Proximate analysis of the full-fat and defatted *M. olefeira* flour was conducted. Moisture content, crude fat and ash content were determined using AOAC methods [16]. Nitrogen content was conducted following the Duma method and total carbohydrate was calculated by difference.

Preparation of Moringa Maillard reaction products (MRPs)

Preparation of Moringa seed extract with added glucose, glycine or glucose-glycine was prepared according to the method of Lee and Park [17] with slight modifications. The Moringa seed extract supernatant was added with either glucose, glycine or glucose-glycine to produce a solution of 1.5 M concentration of each sugar and amino acid. The resulting solutions were divided into 50 mL aliquots and heated at 100°C for 15, 30, 60 and 120 min. The resulting solutions were cooled in ice water followed by pH measurement.

Extraction of polyphenol oxidase

Banana PPO was extracted following the method described by Mogol *et al*. [18] with slight modifications. PPO was extracted by homogenising a mixture of banana (1:1 w/v) and pre-chilled 50 mM phosphate buffer (pH 6.6) for 3 min. The homogenised sample was centrifuged at 15 000 g for 20 min at 4°C and the supernatant referred to as crude PPO extract.

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Colour measurements of the Moringa oleifera extracts

Colour of the unheated (control) and heated moringa seed extract was measured. The colour of the extracts was evaluated by measuring CIE Lab parameters L* (brightness, 100 = white, 0 = black), a* ($^+$ red; $^-$ green) and b* ($^+$ yellow; $^-$ blue) by means of a spectrophotometer (CM-5, Konika Minolta, Japan) measuring the colour spectra using a D65 day light source, large viewing area and the observer at 10° angle.

Enzymatic browning inhibition

Inhibition of in vitro PPO activity

Banana PPO activity assay was based on the spectrophotometric method [13]. The assay mixture contained 0.1 mL of banana PPO extract, 0.9 mL of a 50 mM phosphate buffer (pH 6.6) and 1 mL of sample extract (fresh or heat-treated moringa seed extract) incubated for 5 min at 25°C. After this incubation, 1 mL of 0.2 M catechol was added to the assay mixture. The increase in absorbance at 420 nm at 5 seconds intervals was measured for 1 min. The control contained of 0.1 mL PPO extract and 1.9 mL of 0.1 M phosphate buffer. Inhibition of enzyme activity was calculated as follows:

$$\%\text{IEA} = \frac{(A420\ control - A420\ treatment) \times 100}{A420\ control}$$

Where: $A_{420\ nm\text{-}control}$ is the absorbance of the control
$A_{420\ nm\text{-}treatment}$ is the absorbance of the sample

Inhibition of in vivo PPO activity

Anti-browning treatment of bananas was done following the procedure of Mogol *et al*. [18] with slight modifications. Semi ripe bananas cut uniformly were dipped in extract for 1 min after which they were removed and placed in a Petri dish (60mm x 15mm). Changes in colour development were measured with a spectrophotometer (CM-5, Konika Minolta, Japan), CIE Lab parameters L*, a*, b* using a D65 day light source, at 10° observer.

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\ ]^{0.5}$$

Where: ΔL^* is the difference in lightness/darkness value,
Δa^* is the difference on redness/greenness,
Δb^* is the difference on yellowness/blueness.

Statistical analysis

Statistical analysis was performed using SPSS 26.0 for Windows®. Descriptive statistical analyses determined the mean and standard deviation of triplicates. Significant differences among means were determined by Duncan's multiple range tests. The level of confidence required for significance was selected at $p \leq 0.05$.

RESULTS AND DISCUSSION

Proximate composition of *Moringa oleifera* seed flour

The proximate composition of crude (CMSF) and defatted moringa seed flour (DMSF) is presented in Table 1. Numerous studies focused greatly on the chemical composition of the moringa seeds, which might be due to the use of moringa as a source of nutrients [19]. The results of CMSF obtained in this study was within ranges similar to those reported in previous studies [20–22]. The slight variation might be due to different cultivars, geographical locations, which in turn dictates the environment the plant grows under. The defatting step resulted in significant ($p < 0.05$) changes in the chemical composition of the flour. The fat content

decreased from 32% to 1%, with a significant decrease in energy ($p < 0.05$). The ash, protein and carbohydrate content increased significantly. Consequently, a reduction ($p < 0.05$) in moisture content was observed from 5.36 to 3.9%, which might be due to extra moisture escaping during the lengthy step of evaporating hexane in the fume hood. The increase in carbohydrate and protein content will then enhance formation of MRPs.

pH and colour measurements of Moringa seed extracts

The pH and brown colour have been used as an indicator of the MR. Most authors who studied properties of the MR such as anti-browning, anti-microbial, antioxidant and functionality [24] of MRPs performed browning in the form of absorbance at 294 or 420 nm or colour measurements using the L*, a*, b* coordinates and pH monitoring [24]. The pH and colour of resulting model systems is depicted in Table 2.

Control samples, which are the unheated plain moringa (UMP), unheated moringa glucose (UMG), unheated moringa glycine (UMGL) and unheated moringa glucose-glycine (UMGGL) exhibited varying initial pH values; this might be due to the effect of added sugar and amino acids. Although most model systems did not exhibit significant differences in pH as the heating time increased (Table 2), exclusively UMGGL model systems exhibited a significant decrease in pH throughout all the heating times, with the highest reduction (pH 2.85) observed after 120 min of heating. The defatted Moringa seed flour resulted in an increase in the carbohydrates and protein content, therefore any heat applied would then result in the MR, and addition of sugar and amino acid would further heighten this reaction. The significant ($p < 0.05$) reduction in pH is attributed to the MR where acetic and formic acids are formed as by-products of the reaction. Previous studies [4, 25] not only monitored the reduction in pH, they further quantified the content of acetic and formic acid resulting from microwave assisted extraction of polyphenols from mandarin peels, and microwave heating of thinned nectarines extracts, respectively. Their results further proved that the afore-mentioned acids were formed, and that their content increased exponentially as the microwave power increased.

Additionally, authors who studied the MR regardless of the system type have reported on this phenomenon. For instance, Vhangani *et al.* [26] studied sugar-amino model systems and Yu *et al.* [27] studied soybean peptide-sugar model systems and both authors observed decreases in pH. In this study, an approach similar to that of Lee [13] was followed, whereby glucose and glycine were added to the extract before heating to confirm MR involvement.

To further ascertain the occurrence of the MR during heating, colour changes of the moringa extracts were monitored. As previously mentioned, the brown colour has been used as a measurable attribute of the MR. In the present study the browning was monitored by observing the colour coordinates L*, a* and b*.

Heating of all moringa extracts for 15, 30 and 60 min did not have a significant effect ($p > 0.05$) on colour of plain and glucose added model systems. However, after heating for 120 min, a decrease and increase in L and b, respectively was observed when compared to time zero. This phenomenon is characteristic of the MR, whereby exposure to longer heating times plays a major role in the reaction.

Glycine and glucose-glycine containing moringa extracts on the other hand showed significant enhancements of the MR compared to the afore-mentioned. This might be attributed to the presence of free and added sugar in the moringa extract which could readily react with glycine. In addition, inherent chemical nature of glycine being the most reactive amino acid could be at play [26]. Based on this, a significant decrease and increase in L* and b* values, respectively

was observed throughout heating, as shown in Table 2. Moreover, with these model systems, increase in redness was apparent, which is associated with progression of the MR.

The lack of noticeable results with reference to pH and brown colour formation in the plain heated moringa extracts can be attributed to added water, which resulted in dilution of the reactants. The effect of reactant concentration on the progression of the MR is known [28]. Moreover, the effect of added glucose in enhancing the MR was less than that of glycine. In a previous study, this was proved when ribose-glycine and fructose-glycine models systems surpassed ribose-lysine and fructose-lysine in terms of browning intensity, which was indicative of the extent of the progression of the MR [26]. On close observation, we speculate that amino acids in the moringa seed could purely exist as proteins, and thus the sugar (glucose) had less free amino acids to participate in the MR. Evident to this is the difference in browning (L*) between UMG and HMG, which were only observed from heating at 60 min, whereas between UMGL and HMGL, differences were observed from the onset of heating at 15 min. On the other hand, this proves that carbohydrates may exist as both monomers and polymers, thus the reaction of free sugars with the added glycine is evident (Table 2).

Glucose-glycine enhanced model systems on the other hand resulted in a drastic pH and colour change due to increased reactant concentration, and thus availability to partake in the MR. Moreover, visually, the glucose-glycine added model systems, especially those heated at 60 and 120 min resulted in formation of very dark solutions. This occurrence is referred to as carbonation, and this is common during the MR associated with excessive or prolonged heating, as previously observed in previous studies [4, 25]. However, when these extracts are aimed at being applied in food, they might not be useful due to their perceived colour. Thus, studies related to heated extracts or the MR should be optimised to strike a balance between obtaining compounds with exceptional functionality and colour that would be acceptable for consumers.

Anti-enzymatic browning activity of heated Moringa plant extracts

***In vitro* enzymatic browning inhibition**

Compounds that are deemed inhibitors of enzymatic browning do so via chelating of the iron metal found on PPO, acting as acidulants by reducing the pH to below the optimum 5-7 required, reducing the formed benzoquinone to its precursor diphenol, exerting competitive inhibition of the phenolic substrates and stabilization of quinones to prevent their polymerization [23].

The *in vitro* PPO inhibition of moringa seed extract is shown in Table 3. All unheated moringa extracts with the exception of UMG exhibited considerably lower PPO inhibition than the heated ones. A possible explanation associated with low inhibition by UMP might be due to the presence of phenolic compounds, which could have served as substrates for the banana PPO, thus augmenting the reaction and resulting in low inhibition. Heating of plain moringa increased the PPO inhibition capacity significantly, from 0 to 15 min until it plateaued at 30 min, and then suddenly increased at 60 min, followed by another plateau at 120 min. Similarly, during heating, some polyphenols are degraded and formation of low molecular weight MRPs is initiated, which may result in increased PPO inhibition. These results are in accordance with previous reports where heated onion and thinned nectarine extracts exhibited stronger enzymatic browning inhibition than their fresh/unheated counterparts [4, 5,10–12,14]. These authors attributed the increased inhibitory effect on the formation of products during the MR.

Contrary to observations of low PPO inhibition by UMP, UMGL and UMGGL, the UMG model systems exhibited the highest inhibition comparable to most heated model systems. It

has been reported that glucose lowered the water activity, thus reducing enzyme ability to catalyse the reaction [17]. Consequently, heating did not have a significant ($p > 0.05$) effect on all model systems with added glucose. However, they still exhibited a fairly high PPO inhibition effect.

Heating of UMGGL model systems exhibited the high PPO inhibition considering that at 15 min inhibition of 81% was reached compared to other systems at the same time interval. The highest inhibition amongst model systems was observed for UMGGL 120 at 96%. Our findings are consistent with findings of other authors who also observed a similar trend. Plant extracts augmented with both sugar and amino acid exhibited the highest inhibition against PPO than the unheated counterparts [5,10].

Authors such as Redondo *et al*. [4] further classified the type of inhibition as either competitive or non-competitive. However, this study was limited. Nevertheless, it was speculated that the mode of action of the model systems involved a hurdle effect combining acidulation, metal chelation and reduction. These extracts exhibited 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH-RS), which involves donating a hydrogen, an important characteristic in EB prevention, where quinones are reduced back to diphenols when a hydrogen is donated (results not shown). The reduction in pH is consistent with the PPO inhibition. Heated extracts also showed excellent metal chelation capabilities. The reduction to below pH 5 are known to reduce enzyme activity. The lowest point reached by extracts was at pH 2.85, which is highly acidic, thus acidulation also becomes a possible mechanism.

Commercial antioxidants such as CA, ethylene diamine tetraacetate (EDTA) and AA prepared at 0.01% exhibited the highest PPO inhibition, which is expected since these compounds have multiple modes of action in inhibiting PPO activity (Table 3). EDTA is a well-known metal chelator and its results correspond with those obtained from the metal chelation (MC) assay (results not shown). CA acts both as an acidulant and a metal chelator. Ascorbic acid (AA) acts as an acidulant, oxygen scavenger and reduces benzoquinones back to their precursor diphenol. Thus, agreeing with the total inhibition of PPO. In addition, it was reported that CA and AA at a concentration of 0.1 mM resulted in 20 and 40% inhibition of potato PPO activity, respectively [11]. Meanwhile when studying taro PPO activity, CA, EDTA and AA at the same concentration resulted in 8, 16 and 100% inhibition, respectively. Differences in inhibition rates might be affected by the substrate type and concentration as well as type and source of enzyme, for example banana, versus potato versus Taro.

Inhibition of *in vivo* enzymatic browning.

In vivo anti-browning was conducted on banana slices of equal thickness and diameter dipped in moringa seed extract model systems for a minute, and then placed in a capped Petri dish. The colour coordinates L*a*b* were recorded at time 0, 0.5, 1, 5 and 24 hrs with a view to calculate the colour difference. Storage conditions, which these samples were subjected to did not mimic the proper storage condition applied to fresh cut fruits in the food industry. In essence, our storage conditions accelerated oxidation.

The total colour difference (ΔE) of heated plant extracts is depicted in Table 3. According to literature reports, ΔE of 1 is the threshold at which a trained observer would notice the difference between two objects, whereas ΔE between 4 and 8 is deemed acceptable, and above 8 is deemed unacceptable and likely to be rejected by consumers [29]. The ΔE of most model systems differed significantly ($p < 0.05$), and decreased with an increase in heating time, with a few exceptions. Banana slices immersed in the control samples (UMP, UMG, UMGL and UMGGL) showed a rapid onset of the brown colour during storage, whereas, those immersed

in heated plant extracts showed reduced intensity of the brown colour formation. This is indicated by the increase in ΔE values reaching detectable browning levels. Moreover, browning of all extracts increased as the storage time increased, with 24 h exhibiting the highest browning. Visually there was a clear indication of browning on the surface of the banana slices (results not shown). The results reported here are in agreement with the results of a previous study of taro PPO [14].

Among the extracts, HMP exhibited the lowest inhibition compared to extracts with added glucose and glycine. However, heating improved its browning inhibition considerably. With reference to added glucose and glycine, HMGGL was among the top five competing with commercial antioxidants in inhibition of browning in vivo throughout storage. These results are in agreement with those reported for the in vitro assay. Due to the complexity of these results, the best performing extracts are highlighted in Table 3.

Ascorbic acid (AA) exhibited the lowest ΔE at 0.5 and 1 hr followed by EDTA and CA, respectively. However, as the storage time increased, AA's ability to inhibit browning started to diminish. The reduction in inhibition might be attributed to its oxidation resulting into formation of dehydroascorbic acid. This is evidently visible at 24 h storage, where a ΔE of 20.72 was attained, which was higher than most heated plant extracts. On the other hand, CA and EDTA, inhibited browning consistently throughout the duration of storage. The *in vivo* inhibition of EB for both CA and EDTA also coincided with the in vitro findings.

CONCLUSION

Heating of the *Moringa oleifera* (MO) seed extracts increased their anti-browning capacity. Extracts heated with added sugar and amino acid proved to be the best in inhibiting enzymatic browning. In some instances, these heated MO model system's inhibitory properties were comparable or surpassed that of synthetic antioxidants like AA, EDTA and CA. Since the heated MO plant extracts effectively inhibited the browning of banana, they have a potential as natural inhibitors of browning in various plants and vegetables. However, caution must be taken when introducing MRP's as means to prevent browning, especially since high temperatures and long heating times result in much darker model systems, which might not be pleasant for consumption purposes.

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Table 1: Proximate composition of crude and defatted *M. oleifera* seed flour

Composition g/100g	Crude flour	Defatted flour
Moisture content	5.36 ± 0.63[a]	3.90 ± 1.56[b]
Ash	4.63 ± 0.31[a]	9.34 ± 2.30[b]
Crude fat	32.41 ± 3.53[b]	0.96 ± 0.09[a]
Protein	37.46 ± 3.42[a]	55.82 ± 8.4[b]
Carbohydrates	20.14 ± 0.50[a]	29.98 ± 5.99[b]
Energy	2126 ± 0.72[b]	1463 ± 0.29[a]

* Data represented as mean ± standard deviation (n = 2). ANOVA and Duncan's multiple range tests were performed. [abc] Means with different letter superscripts on the same row denotes significant differences ($p < 0.05$). Moisture content, ashcrude fat, protein and carbohydrates were reported as %, and energy was reported in KJ.

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Table 2: pH and colour of heated *M. oleifera* seed water extracts

Sample	Time (min)	pH	L*	a*	b*
UMP	0	4.60 ± 0.13^{fg}	68.02 ± 4.74^{k}	0.51 ± 0.06^{abc}	6.00 ± 0.19^{ab}
HMP	15	3.94 ± 0.07^{cd}	67.42 ± 0.01^{k}	-1.39 ± 1.05^{ab}	6.67 ± 0.03^{abc}
HMP	30	3.82 ± 0.04^{c}	66.40 ± 0.81^{jk}	-1.15 ± 1.4^{ab}	7.68 ± 0.12^{bcd}
HMP	60	3.77 ± 0.05^{c}	64.26 ± 1.61^{ijk}	-1.34 ± 2.39^{ab}	8.59 ± 0.60^{cde}
HMP	120	3.81 ± 0.01^{c}	62.82 ± 0.59^{hij}	-1.74 ± 2.90^{a}	9.87 ± 0.36^{def}
UMG	0	4.33 ± 0.04^{ef}	65.39 ± 1.91^{jk}	0.76 ± 0.06^{abc}	5.57 ± 0.34^{ab}
HMG	15	3.79 ± 0.01^{c}	64.13 ± 1.56^{ijk}	-1.18 ± 0.03^{ab}	7.23 ± 0.03^{bc}
HMG	30	3.71 ± 0.02^{c}	61.32 ± 1.35^{ghi}	-0.87 ± 0.17^{ab}	9.04 ± 0.36^{cde}
HMG	60	3.70 ± 0.17^{c}	57.84 ± 0.63^{efg}	-0.39 ± 0.09^{ab}	10.90 ± 0.00^{ef}
HMG	120	3.64 ± 0.04^{c}	49.90 ± 1.10^{d}	-0.05 ± 0.47^{abc}	12.00 ± 0.89^{f}
UMGL	0	5.03 ± 0.047^{h}	65.28 ± 0.99^{jk}	0.59 ± 0.11^{abc}	4.31 ± 0.49^{a}
HMGL	15	4.61 ± 0.042^{fg}	61.49 ±1.81^{ghi}	0.77 ± 0.07^{abc}	7.36 ± 0.53^{bcd}
HMGL	30	4.55 ± 0.35^{fg}	56.13 ± 0.5^{ef}	1.05 ± 0.09^{bc}	11.97 ± 1.69^{f}
HMGL	60	3.93 ± 0.03^{cd}	48.17 ± 0.39^{d}	2.43 ± 0.25^{c}	21.51 ± 0.71^{g}
HMGL	120	3.31 ± 1.91^{b}	42.77 ±0.42^{c}	4.87 ± 0.00^{d}	43.80 ± 1.38^{i}
UMGGL	0	5.14 ± 0.16^{h}	64.26 ± 2.00^{ijk}	-0.65 ±1.77^{ab}	5.56 ± 0.35a^{b}
HMGGL	15	4.23 ± 0.73^{de}	59.36 ± 2.61^{fgh}	0.15 ± 0.63^{abc}	20.02 ± 1.89^{g}
HMGGL	30	3.90 ± 0.25^{c}	42.94 ± 2.41^{c}	6.00 ± 1.56^{d}	31.81 ± 3.04^{h}
HMGGL	60	3.29 ± 0.01^{b}	18.95 ± 0.84^{b}	19.33 ± 0.99^{e}	46.63 ± 1.78^{j}
HMGGL	120	2.85 ± 0.11^{a}	8.11 ± 1.43^{a}	33.43 ± 1.41^{f}	56.05 ± 0.92^{k}

Unheated plain moringa (UMP), heated plain moringa (HMP), unheated moringa glucose (UMG), heated moringa glucose (HMG), unheated moringa glycine (UMGL), heated moringa glycine (HMGL), unheated moringa glucose-glycine (UMGGL) & heated moringa glucose-glycine (HMGGL). L*, lightness; a*, red/green; b* yellow/blue. Data are expressed as mean ± standard deviation of the two replicate. ^{ab} Means with different letter superscripts within and between columns denotes significant differences ($p < 0.05$)

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Table 3: Enzymatic browning inhibition of heated *M. oleifera* seed water extracts

Sample	Time (min)	In vitro PPO	In vivo PPO			
		% Inhibition	ΔE 0.5 hrs	ΔE1 hrs	ΔE 5 hrs	ΔE 24 hrs
UMP	0	21 ± 0.77^{a}	9.64 ± 0.14^{r}	13.66 ± 0.66^{r}	18.15 ± 0.04^{l}	31.17 ± 1.53^{s}
HMP	15	32 ± 14.84^{b}	8.38 ± 0.04^{q}	11.26 ± 0.19^{p}	15.77 ± 0.31^{k}	26.74 ± 0.83^{r}
HMP	30	35 ± 5.65^{ab}	5.72 ± 0.03^{n}	9.24 ± 0.07^{n}	14.79 ±1.00^{i}	20.26 ± 1.21^{n}
HMP	60	43 ± 4.95^{cd}	4.65 ± 0.06^{j}	7.65 ± 0.0^{k}	11.49 ± 0.06^{e}	18.60 ± 0.77^{j}
HMP	120	50 ± 3.54^{de}	4.23 ± 0.29^{h}	6.84 ± 0.12^{ij}	11.36 ± 0.02^{e}	17.78 ± 1.18^{g}
UMG	0	61 ± 2.12^{fg}	5.94 ± 0.04^{o}	9.18 ± 0.04^{n}	15.03 ± 0.03^{ij}	20.55 ± 0.92^{o}
HMG	15	65 ± 3.01^{gh}	5.23 ± 0.05^{m}	8.01 ± 0.11^{l}	12.33 ± 0.05^{fg}	18.7 5± 0.04^{k}
HMG	30	66 ± 0.71^{gh}	4.39 ± 0.13^{i}	6.72 ± 0.23^{hi}	11.16 ± 0.06^{e}	17.94 ± 0.07^{h}
HMG	60	65 ± 1.92^{gh}	5.0 ±1.71^{l}	7.74 ±.03^{k}	12.15 ± 0.14^{fg}	18.07 ± 0.04^{i}
HMG	120	68 ± 1.41^{ghi}	4.43 ± 0.09^{i}	7.00 ± 0.13^{j}	12.00 ± 0.01^{f}	18.06 ± 0.06^{i}
UMGL	0	35± 2.51^{ab}	**6.92 ± 0.26^{p}**	12.8 ± 1.31^{q}	15.30 ± 0.09^{jk}	22.41 ± 0.01^{q}
HMGL	15	68 ±1.41^{ghi}	5.09 ± 0.05^{m}	9.98 ± 0.03^{o}	13.82 ± 0.03^{h}	18.96 ± 0.08^{l}
HMGL	30	73 ± 0.87^{hij}	4.83 ± 0.04^{k}	8.57 ± 0.00^{m}	14.02 ± 0.08^{h}	19.89 ± 0.02^{m}
HMGL	60	72 ± 4.95^{ghij}	4.78 ± 0.01^{k}	8.22 ± 0.81^{l}	12.55 ± 0.06^{g}	18.69 ± 0.06^{jk}
HMGL	120	79 ± 2.12^{ijk}	4.17 ± 0.04^{h}	6.56 ± 0.04^{h}	10.38 ± 0.16^{d}	17.62 ± 0.00^{f}
UMGGL	0	53 ± 5.41^{ef}	5.97 ±0.04^{o}	**11.16 ± 0.57^{p}**	14.81 ± 0.05^{i}	**20.63 ± 0.03^{op}**
HMGGL	15	81 ± 4.39^{jkl}	4.04 ± 0.06^{g}	6.2 ± 0.10^{g}	10.29 ± 0.61^{d}	17.57 ± 0.04^{f}
HMGGL	30	87 ± 2.29^{klm}	3.22 ± 0.01^{f}	5.84 ± 1.11^{f}	10.01 ± 0.04^{d}	17.40 ± 0.08^{e}
HMGGL	60	91 ±1.27^{lm}	**3.06 ± 0.07^{e}**	**5.53 ± 0.13^{e}**	**10.01 ± 0.01^{d}**	**16.36 ± 0.05^{d}**
HMGGL	120	96 ±1.42^{mn}	**2.80 ± 0.06^{d}**	**5.26 ± 0.7^{d}**	**9.42 ± 0.04^{c}**	**16.10 ± 0.04^{c}**
AA		**99 ± 0.09^{n}**	**1.30 ± 0.03^{a}**	**3.75 ± 0.91^{a}**	**11.01 ± 0.01^{e}**	**20.72 ± 0.04^{p}**
EDTA		**100 ± 0.00^{n}**	**1.79 ± 0.01^{b}**	**4.79 ± 0.04^{c}**	**8.37± 0.04^{b}**	**14.47 ± 0.31^{b}**
CA		**100 ± 0.00^{n}**	**2.30 ± 0.01^{c}**	**4.47 ± 0.0^{b}**	**5.92 ±0.08^{a}**	**12.24 ± 0.02^{a}**

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