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Effect of Chemical and Thermal Treatments on Browning Inhibition of Senescent Plantain (*Musa paradisiaca*) Puree for Semolinas Preparation

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Abstract Polyphenol oxidases (PPOs) from several fruits, including plantain, have been involved in the undesirable brown discoloration of food products that resulted in negative effects on colour, taste, and nutritional value. The present work was carried out to evaluate the effect of chemical and thermal treatments on browning inhibition of senescent plantain (*Musa paradisiaca*) puree for the preparation of a local semolina called "*M'Bahou*". A screening of PPOs activities from senescent plantain was carried out. The physicochemical characteristics and thermal stability of main PPOs activities were determined in order to develop methods of anti-browning. The anti-browning methods focused on chemical treatments alone or combined with heat treatment. The effectiveness of these methods was determined by measuring colour parameters of purees. Sensory analyses were carried out in order to evaluate the effect of the anti-browning treatments on organoleptic quality of "*M'Bahou*" semolina. Dopamine oxidase, pyrocatechol oxidase and pyrogallol oxidase activities were the main PPO activities from senescent plantain that were optimally active at pH range (5.6 to 7.0). At 60 ℃, their D-values ranged from 31 to 47.27 min. Hence, heat treatment at 60 ℃ for 35 min reduced browning of senescent plantain puree. Sodium metabisulfite was found to be the most potent inhibitor of PPOs from senescent plantain followed by ascorbic and citric acids. Pre-treatment of plantain puree with a combination of metabisulfite and ascorbic acid (5:50 mg and 7:30 mg) per 100 g of puree or heating combined with the addition ascorbic and citric acids (50:50 mg) indicated low browning. A comparison between sensorial characteristics of semolinas obtained from these pre-treatment methods revealed no significant difference in terms of odour, texture, and overall acceptability of semolinas. But for health reasons related to metabisulfite in Asthmatics, we would recommend the use of the lowest concentration (5:50 mg; metabisulfite/ascorbic acid) or the method which combined using of heat treatment and ascorbic acid/ citric acid for semolina preparation.

Keywords Browning index, Food additives, Polyphenol oxidase, Senescent plantain, Semolina

1. Introduction

Plantain (*Musa paradisiaca*) is an important starchy, staple and commercial crop in West and Central Africa where fifty per cent of the world's plantain crop is produced [1]. It constitutes a major source of carbohydrate for millions of African people. Plantain contains low protein and fat but rich in starch and mineral elements, especially potassium [2]. Food uses and consumption patterns of plantain are quite diverse [3, 4]. The plantain flour is used to make donuts, biscuits, bread [5, 6] and also food for children and pregnant women [7]. In Côte d'Ivoire, unripe and ripe plantain pulps are generally used to make popular foods such as *foutou* and *foufou* [8]. The production of plantain is estimated at 1,624,354 million tons [9] but, about 35 to 60% of this production is lost after harvested due to the lack of storage conditions and the inappropriate processing technologies for food [10]. After harvest, plantain exhibits a respiratory peak during natural ripening. The ethylene gas triggers the enzymatic reactions in plantain fruits causing ripening [11] and over-ripening of pulps for the most of time. After maturity, the plantain fruit evolves in 9 different stages of ripening [12]. From stage 1 to stage 7, plantain still retains its market value. Beyond stage 7, there is senescence, where there is rapid deterioration coupled with the growth of microorganisms as a result of drastic softening of the fruit tissue [13, 14]. The over-ripeness and senescence of pulp make plantain unused for common diet but some alternative products such as cakes [15], beer [16] and juice [17] were generated.

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In the eastern part of Côte d'Ivoire, a local semolina product from senescent plantain called "*M'Bahou*" is also produced to reduce post-harvest losses. The "*M'Bahou*" semolina supplements the dietary needs of rural population and is well appreciated by urban consumers. The process of "*M'Bahou*" semolina requires senescent plantain pulp that is crushed and blended with cassava flour. A long-time of treatment of the plantain pulp leads to more oxidation and production of coloured products [18]. Several users of plantain have noted the presence of a polyphenol oxidase activity [19] and the occurrence of enzymatic browning during the preparation of plantain [20]. The enzymatic browning occurring in the crushed pulp remains a critical point that impacts negatively the "*M'Bahou*" semolina. This browning makes the product dark, limits the storage and contributes to reduce its organoleptic quality [21]. It leads also to a change in flavour and a reduction in the nutritional quality [22, 23]. Several methods such as the use of sodium metabisulfite as a chemical browning inhibitor, as well as thermal processing have been used to inhibit enzymatic browning and limit its impact on the quality of plantain products [24, 25]. Ruthra *and al*. [26] evaluated the effect of the combination of citric acid, ascorbic acid and potassium metabisulfite and their combinations on minimally processed banana. They observed that potassium metabisulfite (0.2%) and combination of potassium metabisulfite and ascorbic acid were most effective in control of browning. Metabisulfite (0.1 and 0.2%) was also found to be the most effective pre-treatment for preventing browning in ripe and over-ripe banana pulp, as these concentrations were likely to maintain the original colour of banana pulp [27]. According to Koffi *and al*. [28], heating whole bananas or the puree was effective in inhibiting PPO, but the addition of 100 mg/L potassium metabisulfite was the most effective treatment in inhibiting browning. Bazaz *and al*., [29] showed that banana pulp (stage 4 of ripening) subjected to heat treatment (65℃, 30 min) and chemical treatment can be stored for long term without deterioration. All these works were generally carried out on unripe, ripe or over-ripe plantain. Investigations on the browning inhibition of senescent plantain for its valorisation are scarce.

The present study was carried out to assess the effect of chemicals and thermal treatments on browning inhibition of senescent plantain (*Musa paradisiaca*) puree for semolina preparation in Côte d'Ivoire. It should also provide helpful information about the physicochemical characteristics of PPO activities as well as the impact of these treatments on the organoleptic quality of "*M'Bahou*" semolina.

2. Materials and Methods

2.1. Materials

Plantain sample preparation

Mature plantains (*Musa paradisiaca*, variety Corn 1) were harvested in the experimental plot of Azaguié (located 50 km north of Abidjan 5° 38'N, 4° 05' W) located in Côte d'Ivoire. Plantains were allowed to ripen naturally at room temperature to senescence (stage 9).

2.2. Methods

Extraction of Polyphenol Oxidase (PPO)

A sample of senescent plantain (150 g) was crushed in a blender (Moulinex, France) and homogenized for 10 min in 300 ml of NaCl 0.9% (w/v). The resulting homogenate was centrifuged at 8000 g for 10 min at 4℃ (Refrigerated centrifuge TGL-16M, China). The collected supernatant was the crude enzymatic extract used for PPO activity assays [30].

Enzyme assay

The PPO activities were determined using various phenolic compounds consisting of dopamine, pyrocatechol, pyrogallol, guaïacol, L-Tyrosine and L-Phenylalanine as substrates. The reaction mixture adjusted to 2 mL with extraction buffer at appropriate pH, contained 0.1 mL of crude enzymatic extract and 10 mM of substrate. This reaction mixture was incubated at 25℃ for 10 min. O-quinones content starting from the dopamine and guaïacol was estimated at 480 nm and those resulting from the others phenolic compounds at 420 nm [31]. Experiments were performed in triplicate, and the results expressed as units of enzymatic activity per mg of protein. One unit of enzymatic activity (U) was defined as an increase in absorbance of 0.001 per min (standard test conditions).

Optimal pH of PPO activities

The effect of pH on the crude PPO activities was determined by measuring the oxidation of phenolic substrates in different buffers at various pH values ranging from pH 2.6 to 10.6. The buffers (100 mM concentration) used were sodium acetate from pH 3.6 to 5.6, sodium phosphate from 5.6 to 8.0 and sodium citrate from pH 2.6 to 7.0 and Tris-HCl from pH 8.6 to 10.6. The PPO activities were determined at 25℃ under the standard test conditions.

Optimal temperature of PPO activities

The effect of temperature on PPO activities was performed in 100 mM sodium phosphate buffer pH 7.6 (for dopamine oxidase and pyrogallol oxidase), in sodium acetate buffer pH 5.6 (for pyrocatechol oxidase) after 10 min incubation at temperatures ranging from 5 to 80℃ under standard test conditions [30, 32].

Effect of some chemical agents on PPO activities

To determine the effect of some food additives as effective inhibitors of PPO activities, the crude enzymatic extract was preincubated at 25℃ for 30 min with the chemical agents. Citric acid, ascorbic acid, and sodium metabisulfite were tested at different concentrations and the activity of PPO was assayed under the standard test conditions. Residual activities were expressed as percentage refers to a control without chemical agents.

Thermal inactivation and kinetic parameters ($t_{1/2}$ and D-values) determination

The thermal inactivation of PPO activities was determined at temperatures ranging from 45 to 85°C. The crude enzymatic extract in appropriate buffers [100 mM sodium phosphate pH 7.6 (for dopamine oxidase and pyrogallol oxidase) and sodium acetate pH 5.6 (for pyrocatechol oxidase)] was preincubated at different temperatures. Aliquots were withdrawn at intervals and cooled at room temperature for 10 min. The enzymatic activities of the aliquots were measured under standard conditions. The Kinetic data analysis of thermal inactivation of the PPO activities was done from the equation 1 (1). This equation is derived from the equation of first-order reactions [33].

$$\ln [At/A\circ] = -kt \quad (1)$$

Where At is the residual enzymatic activity at time t, A0 is the initial enzymatic activity, and k is the reaction rate constant (min^{-1}) at the temperature studied.

The inactivation rate constant k was estimated by linear regression analysis of the logarithm of residual activity versus treatment time.

The time where the residual activity reaches 50% known as the half-life ($t_{1/2}$), was given by the equation 2 (2):

$$t½ = \ln (2)/k \quad (2)$$

The D-value, defined as the treatment time (min) needed to reduce the initial activity of 90% was calculated according to the equation 3 (3) [34].

$$D = 2{,}303/k \quad (3)$$

Anti-browning pre-treatments

The effectiveness of thermal and chemical treatments on the browning was evaluated by measuring the colour parameters of purees from senescent plantain. The purees were obtained by two methods.

The first treatment consisted in grinding samples (100 g) of senescent plantain to which various combinations of metabisulfite and ascorbic acid (5:30, 5:40, 5:40 and 7:30, 7:40, 7:50 mg) per 100 g of puree were added.

For the second treatment, samples (100 g) of senescent plantain pulps were heated at 60°C for 35 min. Then, the pulps were crushed using a mixer (Moulinex, France) and various combinations of ascorbic acid and citric acid (40:40, 40:50, 50:40, and 50:50 mg) per 100 g of puree were added.

For each test, a blank prepared under the same conditions without chemicals and thermal treatment was used as control. The puree samples putted into petri dish were exposed to air at room temperature for 24 h.

The initial chromaticity indices (L *, a *, b *) of each puree were determined immediately and after 24 hours with a Chromameter (Colour Reader CR-10 Input: 8v/9v = 1.5A konica minolta, inc, Japan). Chroma difference (ΔC), colour difference (ΔE) and browning index (BI) were calculated as follows:

$$\Delta C = (\Delta a^{*2} + \Delta b^{*2})^{1/2} \quad (4)$$

$$\Delta E = (\Delta L^2+\Delta a^2+\Delta b^2)^{1/2} \quad (5)$$

$$BI = [100(x - 0.31)] / 0.172 \quad (6)$$

where:

$$x = (a^* + 1.75L^*) / (5.645L^* + a^* - 3.012b^*) \quad (7)$$

Parameter L* refers to the lightness of the samples, and ranges from black (L* = 0) to white (L* = 100). A negative value of parameter a*indicates green, while a positive one indicates red colour. Positive value of parameter b* indicates yellow while negative value indicates blue colour [35, 36].

Preparation of "*M'Bahou*" semolina

The "*M'Bahou*" semolina samples were obtained from three pre-treatment methods of plantain pulps or purees: (1) puree to which a combination of metabisulfite and ascorbic acid (5:50 mg) per 100 g of puree was added, (2) puree to which a combination of metabisulfite and ascorbic acid (7:50 mg) was added and (3) plantain pulps subjected to heat treatment (60°C, 35 min) and crushed to which a combination of ascorbic acid and citric acid (50:50 mg) was added. Each puree sample was blended with cassava flour (50/50), and 0.5% salt was added to the mixture. The resulting mixture was sieved (2 mm) to obtain granules which were then rolled. The granules obtained were steamed for 20 min using a couscous maker.

Sensory evaluation

In order to evaluate the effect of the anti-browning treatments of senescent plantain puree on organoleptic quality of "*M'Bahou*" semolina, sensory analyses were carried out. For this study, a 9- point hedonic test scale was used. The panelists rated three "*M'Bahou*" semolina samples from 1-9, where 9 corresponds to "like extremely" which means the most accepted product and 1 corresponds to "dislike extremely" which means least accepted [37]. Fifty (50) panelists were selected from the University of Nangui Abrogoua community. The samples were evaluated for colour, odour, texture, taste characteristics and the overall acceptability of the products was asked to panelists.

Statistical analysis

Data shown are means of triplicates for each treatment. The data generated from the sensory evaluation and other analysis (chemical and heating treatment and browning inhibition) was subjected to analysis of variance (ANOVA) to determine the significant difference, using Statistica 7.0 (Stat Soft Inc., USA). The Least Significant Difference (LSD) of the sample was also calculated with Duncan test.

3. Results and Discussion

Physicochemical characteristics and thermal stability of PPOs from senescent plantain

The results of the screening of PPO activities as presented in Figure 1 showed that diphenolic compounds were suitable substrates for PPOs from the pulp of senescent plantain. Our results were in agreement with those of Ngalani *and al.* [20], who reported that PPOs of plantain pulp (variety " French ", ripening stage 4 and 5) were able to oxidize diphenolic

substrates more than monophenolic substrates. The fact that dopamine, pyrocatechol and pyrogallol were the most suitable diphenolic substrates suggested that dopamine oxidase, pyrocatechol oxidase and pyrogallol oxidase activities were the main enzymatic activities involved in the enzymatic browning of senescent plantain pulp (variety Corn 1). To this end, browning prevention strategies during the processing of plantain pulp should focus on these enzymatic activities. Hence, their physicochemical characterization has been considered.

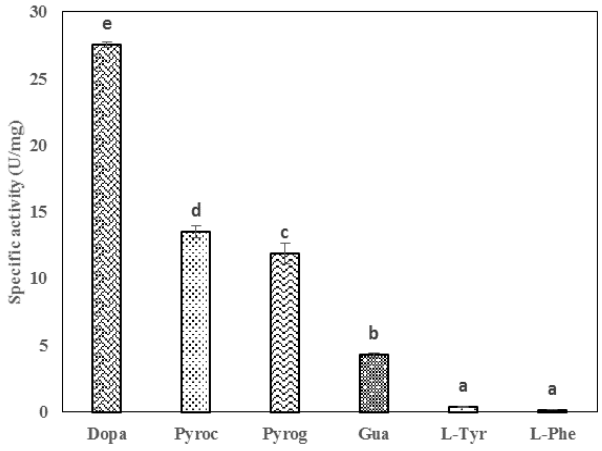

Figure 1. Screening of polyphenol oxidase (PPO) activities of the pulp from senescent plantain (*Musa paradisiaca*; Variety Corn1). Dopa: Dopamine, Pyroc: Pyrocatechol, Pyro: Pyrogallol, Gua: Guaïacol, L-Tyr: L-Tyrosine, L-Phe: L-Phenilalanine

Table 1. Some physicochemical characteristics and kinetic parameters of polyphenol oxidase (PPO) activities of the pulp from senescent plantain (*Musa paradisiaca*; variety Corn1)

Enzymatic activities	Parameters				
	T (°C)	$t_{½}$	D	Buffer and optimal pH	Optimal temperature (°C)
Pyrocathechol oxidase	50	24.15	80.24	sodium acetate pH 5.6	40
	55	18.78	62.41		
	60	14.03	46.62		
	65	7.12	23.64		
	70	5.44	18.08		
Pyrogallol oxidase	50	17.24	54.80	Sodium phosphate pH 7.0	30
	55	15.61	49.62		
	60	14.87	47.27		
	65	9.32	29.61		
	70	3.55	11.29		
Dopamine oxidase	50	21.07	70.00	Sodium phosphate pH 7.0	35
	55	18.10	60.13		
	60	9.33	31.00		
	65	4.62	15.36		
	70	2.45	8.13		

The effect of pH on PPO activities from senescent plantain pulp was examined at different pH values, ranging from 2.0 to 11.0 (Table 1). The optimal activities were found at pH 5.6 for pyrocatechol oxidase and, pH 7.0 for dopamine and pyrogallol oxidase activities. Similar results about pH profile were observed by several authors for PPOs from various plants [38]. Indeed, Chaisakdanugull and Theerakulkait [38] reported an optimal pH (7.0) for dopamine oxidase activity from banana pulp [*Musa* (AAA Group) 'Gros Michel']. The work carried out by Barthet [39] on isozymes isolated from roots of *Manihot esculenta*, indicated optimal pH values of 6.5, 6.8 and 7.5 using dopamine and pyrocatechol as substrates. Since PPO activities from senescent plantain were optimal at pH ranging from 5.6 to 7.0, their activity could be inhibited when exposed in acidic environment. According to [40], the activity of PPOs decreases in an acidic environment by protonation of the catalytic site, thereby preventing the conformation of the active site, the binding of the substrates and / or the catalysis of the reaction.

As concerned the effect of temperature on PPOs from senescent plantain, the optimal temperature, half-life ($t_{1/2}$-value) and D-value which are important parameters for enzyme thermal stability evaluation [41] were determined as shown in Table 1. The optimal temperatures of these enzymatic activities ranging from to 30 to 40°C indicated their mesophilic character. Note that mesophilic enzymes are relatively sensitive to elevated temperatures. The increase in temperature from 45 to 80°C resulted in a decrease in PPO activities, hence the decrease of $t_{1/2}$-values and D-values. At 60°C, the $t_{1/2}$-values of the studied enzymatic activities decreased to values between 9.33 and 14.87 min. These values were lower than those obtained by Ü nal [42] who reported a $t_{1/2}$-value of 54.6 min at 65°C for the PPO of Anamur banana (*Musa cavendishii*). Thus, the low $t_{1/2}$-values obtained suggested that PPOs from senescent plantain are strongly inactivated at temperatures from 60°C. With respect to D-values, a 90% reduction in activity of PPOs from senescent plantain was observed at times ranging from 31 to 47.27 min at 60°C. Similar results were obtained by Gouzi *and al.* [43] for the PPO of *Agaricus bisporus* at 60°C ($t_{1/2}$-value = 11.8 min, D-value = 39.0 min).

Regarding the effect of food additives used as inhibitors, PPOs from senescent plantain were found sensitive to citric acid, ascorbic acid, and metabisulfite (Figure 2). As concentrations of food additives increased, a decrease in PPO activities was observed, thus reflecting their inhibitory effect. A similar result was observed by Sikora and Swieca [44], Sodium metabisulfite and ascorbic acid were the most potent inhibitors of these enzymatic activities compared to citric acid. Indeed, sodium metabisulfite was effective at low concentrations. At a concentration of 100 mg/L, the inhibitory effect of sodium metabisulfite was three and four times higher than that of ascorbic (300 mg/L) and citric acids (300 mg/L), respectively. The effectiveness of sodium metabisulfite on inhibiting PPO activities has been previously described by Wuyts *and al.* [45]. Ascorbic acid and citric acid displayed also an inhibitory effect on PPOs from senescent plantain, but this seemed to be effective at high concentrations. Note that, citric acid has also been reported to effectively inhibit PPOs. Its inhibitory effect is due to the chelation of copper located at the active site of

PPO and lowering of the pH [46]. Therefore, the studied food additives could be effective as anti-browning of plantain, since PPOs activities they inhibit were reported to be involved in browning. Besides, none of these additives were able to completely inhibit the activity of these enzymatic activities. Thus their use in combination may be promising to prevent browning of senescent plantain puree.

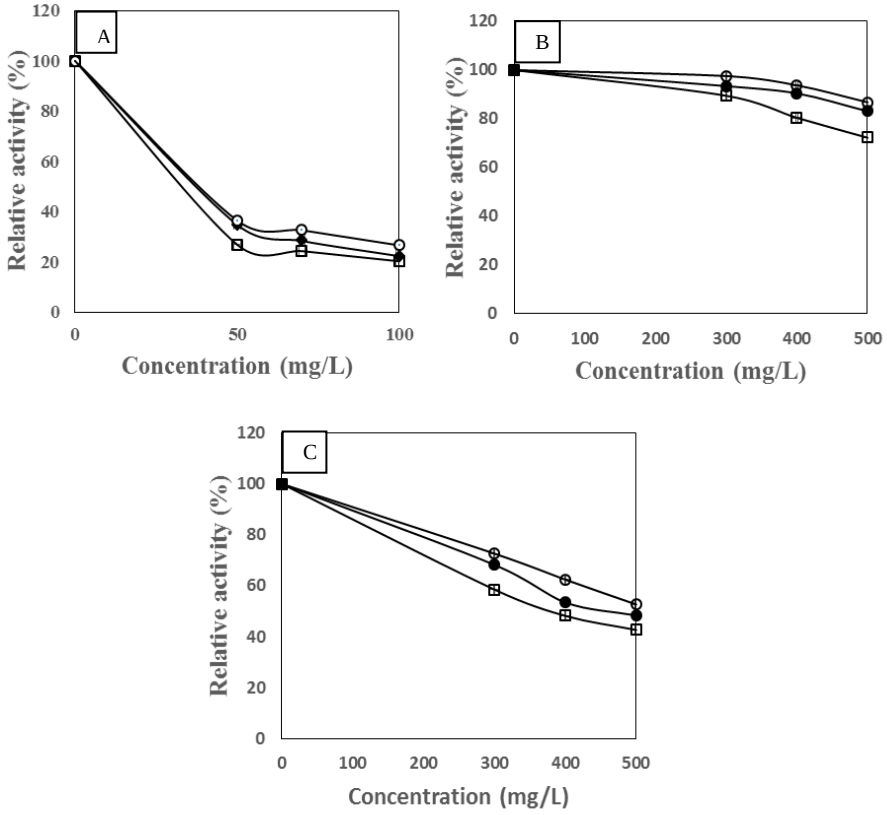

Figure 2. Effect of three food additives on Polyphenol oxidase (PPO) activities of the pulp from senescent plantain (*Musa paradisiaca*; Variety Corn1). A = sodium metabisulfite, B = citric acid, C = ascorbic acid, —●— Pyrocathecol oxidase activity, —○— Pyrogallol oxidase activity, —□— Dopamine oxidase activity

Effect of food additives and heat treatment on brown discoloration of senescent plantain puree

Based on physicochemical characteristics and the thermal stability of PPOs from senescent plantain, two methods were developed to control the browning of the puree.

First, the use of food additives alone was considered. Samples of senescent plantain puree to which food additives were added, were measured for browning immediately after preparation and 24 h of storage. Thus, ΔC, ΔL and ΔE were calculated as differences between two L* a* b* colours based on the values obtained at the two measurement times (0 and 24 h) [47]. ΔBI was also calculated as the difference between the initial BI and the BI at 24 h. The treatment of the senescent plantain puree with sodium metabisulfite and ascorbic acid in combination led to a decrease in the ΔBI, ΔC and ΔE values compared with those observed for the control after 24 h. The highest values of ΔBI (43.3 ± 0.5), ΔC (15.2 ± 0.2) and ΔE (22.2 ± 0.1) recorded for the control decreased for the puree treated with the combination of metabisulfite and ascorbique acid (7: 50 mg) per 100 g of puree to values of 4.9 ± 0.7, 4.3± 01, and 8.11 ± 1.0, respectively (Table 2). This combination (7: 50 mg) was found effective to reduce the browning of senescent plantain puree. Similar results were reported by others authors. Indeed, sweet potato '*Colorada Correntina*' minimally processed and treated with a mixture of anti-browning agents (sodium metabisulfite 2%/citric acid) was preserved [48]. Olubunmi [49] reported that browning reactions of plantain, due to catechol oxidase activity were inhibited by sodium metabisulfite (0.1%), ascorbic acid (0.2%), malic acid (1.0%), and the least sodium (> 1.0%). Tan *and al.* [50] showed that the use of ascorbic acid and/or sodium metabisulfite was significantly ($P<0.05$) effective to reduce brown discoloration of processed green coconut water. Indeed, there is evidence that enzymatic browning of fruit may be delayed or eliminated by removing the reactants, such as oxygen and phenolic compounds, by using PPO inhibitors [51] or by inhibiting secondary reactions [52]. Among the effective compounds to prevent browning, sulfites do not only act as a reducing agent, but also have ability to directly inhibit PPOs. They also interact with quinones, thereby preventing their subsequent participation in secondary reactions leading to brown pigments formation [53]. As for ascorbic acid, it has the ability of being strongly reducing. It acts on oxygen by an

oxido-reduction reaction thanks to its enediol function and it is converted into dehydroascorbic acid which has the same biological activity as the ascorbic acid [54]. However, in this study, the effectiveness of the treatments was dose dependent as shown by Lim and Wong [55] and Sarpong *and al.*, [56]. Tapre and Jain [18] reported that the clarity of the banana pulp juices was a function of the concentration of ascorbic acid used. As the concentration of ascorbic acid increased, the browning index of the clarified juices also decreased. Ascorbic acid at a rate of 470 mg/kg, was not effective in inhibiting browning [17]. This suggested that at low concentrations, these inhibitors can be rapidly consumed in the reduction process so that colour development is only delayed for a limited time but not completely suppressed [57]. Jang and Moon [58] rightly showed that high concentrations of ascorbic acid could be a good inhibitor of PPOs. Yeo *et al*. [17] reported that potassium metabisulfite (100 mg / kg) was more effective at producing a slightly coloured banana juice with a stable colour. However, the standard required for the use of metabisulfite in foods such as flakes and semolina is 100 mg / kg. In this study, it was of great importance that the combination of sodium metabisulfite/ ascorbic acid (5:50 mg) per 100 g of puree led to a decrease in ΔBI by four and a half times. In addition there was no significant difference between the variation of colour parameters of the puree to which sodium metabisulfite/ascorbic acid (7:30 mg) was added than those recorded for puree to which sodium metabisulfite/ascorbic acid (5:50 mg) was added. The increase in the level of ascorbic acid and the decrease in the level of metabisulfite were likely to reduce browning. Thus, the combination of metabisulfite and ascorbic acid (5:50 mg) is promising to control browning of senescent plantain puree. In particular, this is advantageous for senescent plantain processing because metabisulfite should be used at low concentrations as recommended by the standard. Note that, there is increasing concern regarding allergic reactions to sulfites in certain individuals, especially in asthmatics due to health-related problems. Although, it was reported that heating could eliminated the residual sulfites in foods subject to cooking, their adverse health effects led to growing interest in the use of non-sulfite anti-browning agents to replace sulfites preservatives [59, 46]. For this reason, combinations of non-sulfite anti-browning agents are also used to preserve the products.

Second, the heat treatment combined to the addition of ascorbic and citric acids was investigated as an alternative to the use of metabisulfite. Remember that the thermal stability study indicated a 90% reduction in activity of PPOs from senescent plantain at times ranging from 31 to 47.27 min at 60°C. Therefore, heat treatment of senescent plantain pulp at 60°C for a time within the range (31 to 47.27 min) would reduce browning. The mild heat treatment (40°C, 5 min) was reported as a promising approach to inhibit browning sliced button mushrooms [36]. As concern plantain processing, the choice of the optimum heat treatment time should require a perfect match between organoleptic characteristics of the semolina to be produced and the processing quality of the senescent plantain pulp to be processed. To produce quality semolina, avoid the cooking of the plantain pulp that causes starch gelatinization is need. In fact, the gelatinization of the starch leads to an aggregation of semolina in the precooking stage, thereby inducing a sticky effect that affects the disintegration of semolina. The suitable time of heat treatment (60°C) of senescent plantain pulp that reduced browning of puree was 35 min (results not shown). This time that fell within the range (31 – 47.27) was able to avoid cooking of plantain pulp intended to make puree for semolina preparation. Then, senescent plantain pulps were subjected to heat treatment (60°C, 35 min) and different concentrations of ascorbic and citric acids were added. The variation of browning index and others colour parameters of the puree from the senescent plantain samples are shown in Table 3. Plantain puree that did not receive any treatment registered the highest values of ΔBI (44.6±0.4), ΔL (22.5±0.8), ΔC (17.3±0.6) and ΔE (28.4±0.4). The ΔBI, ΔL, ΔC and ΔE values of purees decreased significantly ($p < 0.05$) as a function of concentrations of food additives used. For the combination of ascorbic acid and citric acid (50:50 mg) per 100 g of puree, low ΔBI (11.8 ± 0.1), ΔL (9.1±0.0) and ΔC (8.3±0.7) were observed, showing the effectiveness of the treatment in controlling the browning. The effectiveness of ascorbic acid in controlling browning was previously documented for slices of banana smoothies [60], while the effectiveness of citric acid as acidifier was demonstrated [48, 61, 62]. Tapre and Jain [18] showed that pre-treatment of pulp with 1000 ppm of ascorbic acid or heating at 90°C for 6 min was observed to retain the natural colour of prepared clarified juice without marked changes up to 4 h of storage at room temperature.

Based on the concentrations, ascorbic acid and citric acid were not more effective in controlling browning than metabisulfite. However, the heat treatment of senescent plantain followed by the combined addition of ascorbic acid and citric acid improved their effectiveness in preventing browning of the puree.

Sensorial analysis of semolinas

Table 4 presents the mean scores of colour, odour, texture, taste and overall acceptability of semolinas obtained by three pre-treating methods of senescent plantain puree. There was no significant difference between the odour, texture and overall acceptability of semolinas obtained from the different treatments applied to pulp or puree of senescent plantain. However, significant differences were observed in colour and taste. The slightly elevated scores (6.90 ± 1.0 and 6.94 ± 1.37) of colour for semolinas obtained from the puree to which the combinations of metabisulfite /ascorbic acid were added could be due to the effectiveness of their levels to control browning of plantain puree. Semolinas obtained from these methods were above average acceptability (6.9 ± 0.7 - 7.0 ± 0.7). Therefore, these methods can be used for the preparation of semolina without affecting the acceptability of the product.

Table 2. Initial and final values of colour parameters of senescent plantain (*Musa paradisiaca*, variety Corn 1) puree treated with the combination of metabisulfite and ascorbic acid

Food additives	(Metabisulfite : ascorbic acid)	Time (h)	L*	a*	b*	C	BI	ΔBI	ΔC	ΔE
Control		0	43.7±0.4^{b}	9.2±0.3^{d}	18.8±0.1^{c}	20.9±0.2^{f}	69.8±1.1^{e}			
		24	27.5±0.5^{A}	0.3±0.1^{A}	6.4±0.3^{A}	6.4±0.2^{A}	26.5±1.1^{A}	43.3±0.5^{f}	15.2±0.2^{d}	22.2±0.1^{g}
Concentrations (mg/100 g)	5 : 30	0	44.7±0.2^{e}	7.3±0.3^{c}	17.5±0.4^{b}	19.0±0.4^{d}	60.2±1.4^{c}			
		24	37.2±0.1^{B}	2.7±0.0^{D}	12.9±0.1^{B}	13.2±0.0^{B}	46.6±0.1^{B}	13.6±1.5^{e}	6.5±0.4^{c}	9.9±0.3^{f}
	5 : 40	0	44.7±0.2^{e}	6.4±0.1^{b}	17.3±0.2^{b}	18.5±0.2^{c}	57.9±0.8^{a}			
		24	37.6±0.2^{B}	2.6±0.1^{D}	13.1±0.3^{B}	13.3±0.3^{C}	46.5±0.9^{B}	11.5±1.4^{d}	5.7±0.3^{b}	9.1±0.2^{e}
	5 : 50	0	43.4±0.10^{a}	5.3±0.0^{a}	17.5±0.2^{b}	18.3±0.2^{b}	58.9±0.8^{b}			
		24	37.9±0.1^{B}	1.0±0.1^{B}	14.8±0.3^{D}	14.8±0.3^{E}	49.4±0.9^{D}	9.5±1.2^{c}	5.1±0.1^{b}	7.5±0.1^{b}
	7 : 30	0	43.8±0.1^{c}	6.3±0.2^{b}	16.8±0.3^{a}	17.9±0.2^{a}	57.2±0.9^{a}			
		24	37.3±0.4^{B}	2.3±0.1^{C}	13.6±0.2^{C}	13.8±0.3^{D}	48.4±0.7^{C}	8.8±1.5^{c}	5.1±0.4^{b}	8.3±0.6^{d}
	7 : 40	0	43.7±0.3^{b}	7.1±0.1^{c}	18.5±0.2^{c}	19.8±0.2^{e}	65.1±1.4^{d}			
		24	38.2±0.2^{B}	3.9±0.0^{E}	15.6±0.5^{E}	16.1±0.5^{F}	58.2±2.4^{E}	6.9±0.8^{b}	4.3±0.6^{a}	7.0±0.1^{a}
	7 : 50	0	44.1±0.1^{d}	7.0±0.0^{c}	18.4±0.2^{c}	19.7±0.2^{e}	64.0±0.7^{d}			
		24	37.3±1.2^{B}	3.9±0.0^{E}	15.4±0.2^{E}	15.9±0.2^{F}	59.2±2.0^{E}	4.9±0.7^{a}	4.3±0.1^{a}	8.1±1.0^{c}

The values followed by different letters in the same column for the same parameter and at an identical time are statistically different (α = 0.05)

Table 3. Initial and final values of colour parameters of senescent plantain (*Musa paradisiaca*, variety Corn 1) puree subjected to heat treatment (60 °C; 35 min) combined with the addition of ascorbic and citric acid

Heat treatment + additives (mg/100g)		Time (h)	L*	a*	b*	C	BI	ΔBI	Δ L	ΔC	ΔE
Control		0	47.3±0.4^{a}	5.4±0.0^{f}	22.7±0.7^{d}	23.3±0.7^{d}	71.0±2.0^{e}	44.6±0.4^{e}	22.5±0.8^{e}	17.3±0.6^{d}	28.4±0.4^{d}
		24	24.7±0.8^{A}	-5.3±0.0^{A}	9.0±0.9^{A}	10.5±0.6^{A}	26.4±0.8^{A}				
Heating (60 °C; 35 min) + (ascorbic acid : citric acid)	40 : 40	0	56.0±0.1^{d}	0.3±0.0^{c}	22.7±0.2^{d}	22.7±0.3^{c}	50.3±0.3^{c}	16.6±0.0^{d}	12.1±0.8^{c}	9.3±0.8^{c}	15.3±0.6^{c}
		24	43.9±0.6^{C}	-1.0±0.1^{D}	13.5±0.7^{B}	13.6±0.7^{B}	33.8±0.0^{C}				
	40 : 50	0	55.1±0.1^{c}	0.7±0.0^{d}	21.8±0.7^{c}	21.8±0.8^{b}	49.2±0.1^{c}	13.9±0.7^{c}	10.7±0.0^{b}	7.7±1.0^{a}	11.9±0.4^{a}
		24	44.4±0.1^{C}	-2.5±0.0^{B}	15.0±0.9^{D}	15.3±0.2^{C}	34.2±0.6^{D}				
	50 : 40	0	53.7±0.1^{b}	-2.0±0.0^{a}	21.5±0.1^{b}	21.6±0.2^{a}	45.9±0.0^{b}	13.0±0.5^{c}	9.4±0.7^{a}	8.3±0.8^{b}	12.6±0.5^{a}
		24	44.3±0.6^{C}	-0.9±0.1^{D}	13.3±0.1^{B}	13.4±0.1^{B}	32.9±0.7^{B}				
	50 : 50	0	54.7±0.1^{c}	-1.7±0.0^{b}	21.2±0.6^{b}	21.5±0.8^{a}	44.5±0.4^{a}	11.8±0.1^{a}	9.1±0.0^{a}	8.3±0.7^{b}	13.6±0.4^{b}
		24	45.6±0.1^{D}	-1.2±0.1^{C}	13.8±0.1^{B}	13.9±0.1^{B}	32.7±0.1^{C}				

The values followed by different letters in the same column for the same parameter and at an identical time are statistically different (α = 0.05)

Table 4. Mean acceptability of the quality attributes of "*M'Bahou*" semolinas obtained from three pre-treating methods of senescent plantain (*Musa paradisiaca*, variety Corn 1) pulps or purees

Quality attributes	Pre-treatment methods		
	Metabisulfite/ ascorbic acid		
	Metabisulfite/ ascorbic acid (5:50 mg)	Metabisulfite/ ascorbic acid (7:50 mg)	Heat treatment/ ascorbic acid/ citric acid (50:50 mg)
Colour	6.90 ± 1.05^{b}	6.94 ± 1.37^{b}	6.40 ± 1.18^{a}
Odour	7.04 ±1.32^{a}	7.00 ± 1.16^{a}	6.86 ± 1.14^{a}
Texture	7.20 ± 1.05^{a}	6.98 ± 1.45^{a}	6.68 ± 1.28^{a}
Taste	7.38 ± 0.78^{b}	7.02 ± 0.71^{a}	6.72 ± 1.05^{a}
Overall Acceptability	7.04 ± 0.67^{a}	6.98 ± 0.68^{a}	6.86 ± 0.70^{a}

On each line, means followed by the same letter are not significantly different (α = 0.05)

4. Conclusions

The screening of PPO activities has shown that dopamine oxidase, pyrocatechol oxidase and pyrogallol oxidase were enzymatic activities mainly involved in the browning of senescent plantain. Based on the physicochemical properties and thermal stability of these PPOs activities, treatments carried out on the senescent plantain pulp and puree led to reduce browning of plantain puree. The combined addition of metabisulfite and ascorbic acid to the puree was the most effective method of anti-browning. The thermal treatment of pulps combined with addition of ascorbic and citric acids had good effects on the organoleptic quality of the generated "*M'Bahou*" semolina, but a slightly preference was observed for semolina obtained from the puree to which the combination of metabisulfite and ascorbic acid (5:50 mg) per 100 g of puree was added. Colour, odour, texture and taste of semolina were found to be acceptable with fairly good grades. For health reasons related to metabisulfite in Asthmatics, we would advise users to use the combination of metabisulfite and ascorbic acid (5:50 mg) or the method using heat pretreatment combined with the use of ascorbic and citric acids to produce semolina of good quality. We believe that this study will enable the valorisation of senescent plantain and thus contribute to food security.

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