Original Research Article

Introducing in Côte d'Ivoire a simple technique contributing to the post-harvest management of mangoes (*Mangifera indica* L. 1753) perishability and browning by targeting polyphenol oxidases (PPOs)

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ABSTRACT

Côte d'Ivoire is the leading sub-Saharan Africa exporter of *Palmer* and *Keitt* mangoes cultivars. Despite the importance of the foreign exchange generated, these tropical fruits are confronted with enormous post-harvest losses linked to their high perishability, mainly caused by enzymatic browning. Indeed, the appearance of brown discoloration due to alterations whether engendered by mechanical, technological or natural treatment, is the cause of huge economic losses. To face this thorny problem and thereby improve export volumes, three technological treatments including steam bleaching, oven drying, and osmotic dehydration were performed on mangoes edible parts in order to inhibit polyphenol oxidases (PPOs), the key enzymes catalyzing enzymatic browning. The results showed that only oven drying at 60°C and steam bleaching at 60°C and 100°C, have had significant effect on inactivating PPOs activity after only few minutes. Osmotic dehydration maintained high rates of the enzymes activity even after 24 hours of treatment. In sum, steam bleaching at 60°C for 7 minutes was found to be the best post-harvest management technique for *Palmer* and *Keitt* mangoes because it contributed both to the preservation of the marketable quality as well as to the stability of the mango's organoleptic properties.

Keywords: Post-harvest management, Mangifera indica, steam bleaching, preservation techniques, polyphenol oxidases, Côte d'Ivoire

1. INTRODUCTION

Mango, *Mangifera indica* L. 1753, is one of the most popular tropical fruits in the world. It is widely cultivated in tropical areas as one of the most important horticultural crops with a production representing 52% of the world production [1]. Not only delicious, mangoes boast an impressive nutritional value particularly in vitamin C which aids immunity, iron absorption and cell growth and repair. Because of its beneficial effects on health, mango is considered one of the tropical fruits with high commercial value whose sale and export are very profitable. In Africa, Côte d'Ivoire is the first exporter and the first European countries supplier [2]. Among the most popular varieties, *Palmer* and *Keitt* are the Floridian mangoes mainly exported in Côte d'Ivoire [3]. However, the ripe fruits have a limited commercial life due to high perishability linked to various intrinsic and extrinsic factors. Indeed, mangoes, like many other fruits and vegetables, undergo enzymatic browning that can occur during the various stages of handling from harvest to storage when it is bruised or damaged. This physiological phenomenon is mainly caused by an enzyme, polyphenol oxidase, a copper metalloprotein that catalyzes the oxidation of phenolic compounds into highly reactive quinones that polymerize into brown pigments (melanins) giving a dark color to the fruit and leading to the loss of its nutritional value by altering organoleptic qualities [4]. So, the study of this enzyme is important for the food industry to avoid the loss of the marketable quality of fruits and vegetables [5]. In this perspective, several technological treatments mostly based on chemicals (sulphites) and physical applications are used in order to slow down or suppress browning. However, as the use of chemicals is highly controversial for health, preservation processes such as bleaching, osmotic dehydration and drying are more encouraged in the food industry. Also, the enzymatic behavior is variable depending on the environmental characteristics of the enzyme. Thus, the stability of polyphenol oxidase can be affected by a number of parameters such as the fruit variety, the physiological state and/or the climatic conditions [6]. The knowledge to the field of food preservation aimed at controlling browning in mango attributed to PPOs activity can still be updated. Although heat treatment has been well-studied and has long been known as an effective and an established technique to control browning, it is also important to specify the exact parameters required for efficient inactivation of the enzymes catalyzing browning while preserving the marketable and organoleptic qualities of fruits.The aim of our study was to kinetically characterize the impact of three well known conservation treatments (steam bleaching, drying and osmotic dehydration) on the activity of the enzymes involved in the browning of the different parts of *Palmer* and *Keitt* mangoes varieties produced in Côte d'Ivoire and intended for export.

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2. MATERIAL AND METHODS

2.1 Plant material and chemicals

The mango samples (*Palmer* and *Keitt)* used in this study were collected during may 2023 from the orchards of the village of Olléo (Korhogo) and those of Sinématiali which are both located in the north of Côte d'Ivoire at approximately 600 km from Abidjan. The fruits were picked fresh, physiologically ripe, hard and without visible lesions. All chemicals used in this study were of analytical grade and suitable for scientific research.

2.2 Samples preparation

The fruits were cleaned by immersion in trays containing tap water to remove any foreign matter and latex. Peeling, pitting and cutting were done manually with stainless steel knife and scissors. The peeling and pitting consisted respectively in separating edible and non-edible parts of the mango while cutting of the different parts was made transversally to obtain approximately 1 cm thick cubes. All these processes were done at laboratory room temperature of 25 °C.

2.3 Extraction of PPOs from the four main parts of the mangoes

The extraction was performed according to [7]method slightly modified as follow. 25g of each cut part of the mango (peel, pulp, hull and kernel) were crushed in 75 ml of sodium chloride (0.9% w/v NaCl) by using a Moulinex type blender for 5-15 min depending on the type of sample. The resulting slurry was immediately centrifuged at 4°C and 7,000 rpm for 10 min in a refrigerated centrifuge (Sigma 3-30K). The supernatant obtained after centrifugation was stored at 4 °C and used as the crude PPOs extract for further assays.

2.4 Preservation processes of mangoes edible parts (peel and pulp)

The bleaching process was carried out as previously described in [8]. Samples from peel and pulp of *Palmer* and *Keitt* mangoes were placed for 15 min in contact with water vapors obtained after heating at 60 °C or 100 °C. For the drying process, the method used was that followed by [9]. In this protocol, edible parts of *Palmer* and *Keitt* mangoes were oven dried at 60 °C for 24 hours. Samples were taken every hour to measure the enzymatic activity of the extracts.Concerning the osmotic dehydration technique, the experiments have consisted in immersing at 25 °C the mangoes samples in a 70 °Brix sucrose solution for 9 consecutive hours [10].During the three experiments, the kinetic effect of each treatment on the browning enzymes activity of the facing samples were determined. Indeed, every minute (when steam bleaching) or every hour (when oven drying and osmotic dehydrating) 25 g of each exposed sample were withdrawn for PPOs extraction as described above. Then, the residual enzymes activity was determined.

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2.5 Determination of polyphenol oxidases (PPOs) activity

The PPOs (EC 1.10.3.1) activity was spectrophotometrically measured at 420 nanometer by using pyrocatechol as substrate [11]. The reaction mixture (1.5 mL) consisted of 0.3 mL of enzymes extract, 0.9 mL of sodium phosphate buffer pH 6.6, and 0.3 mL of 0.5 M pyrocatechol. Incubation of the reaction mixture was performed at 25 °C for 10 min. Assays were made in triplicate using a UV-Visible spectrophotometer (Pioway, CHINA). One unit of the polyphenol oxidases activity was defined as the amount (in mg) of extracted proteins that catalyzes the oxidation of 1 µmol of pyrocatechol into brown colored quinone molecules per minute. All residual activities measured were expressed as a percentage relative to the maximum activity (100%) obtained from untreated enzymes samples. Enzymatic browning is an undeniable cause of agricultural loss of mangoes in Côte d'Ivoire. Depending on the variety studied and the harvesting conditions, the onset of browning caused mainly by PPOs also called tyrosinases can be more or less accelerated. In our study, the variation of the activity of the PPOs in two varieties of mangoes subjected or not to conservation treatments allowed to evaluate the potentiality of inhibition of these enzymes for a better conservation of the fruit.

3. RESULTS AND DISCUSSION

3.1 PPOs activity in the four main parts of the fruits

The PPOs activity in the essential parts of *Palmer* and *Keitt* mangoes is presented in Figures 1(a) and 1(b). This activity is mainly localized in the peel for the both varieties. Considering the *Palmer* cultivar, the activity of PPOs in the peel (100%) is respectively followed by this in the kernel (82%), then the hull (76%) and the pulp (62%) in decreasing order (Figure 1(a)). For the *Keitt* cultivar (Figure 1(b)), the PPOs also showed the same trend activities as being higher in the peel (100%) but less important in the kernel (68%), the hull (51%) and the pulp (33%). The average of the spectrophotometric absorbencies obtain in the peel was therefore used as the reference for the calculation of activity percentages of the other mango parts. The PPOs activity was detected in all parts of the both mango varieties and was much higher in the exocarp (peel). This activity was lower in the endocarp (hull and kernel) and in the mesocarp (pulp). Such results have already been observed in [12], who demonstrated that polyphenol oxidases are present in different organs of higher plants such as root, seed, leaf, peel, and fruit cortex. Moreover, [13] demonstrated that PPOs are copper metalloproteins, found in all plant organs and different cell compartments, especially the cytoplasm and pectocellulosic walls. As the *in-vitro* increase in the activity of an enzyme depends on pH, temperature and substrate concentration, it could be assert that the dissimilarity between polyphenol oxidases activity in the studied mangoes parts and cultivars is intimately connected to the variability in phenolic compounds concentration of the fruits cell vacuoles [14].

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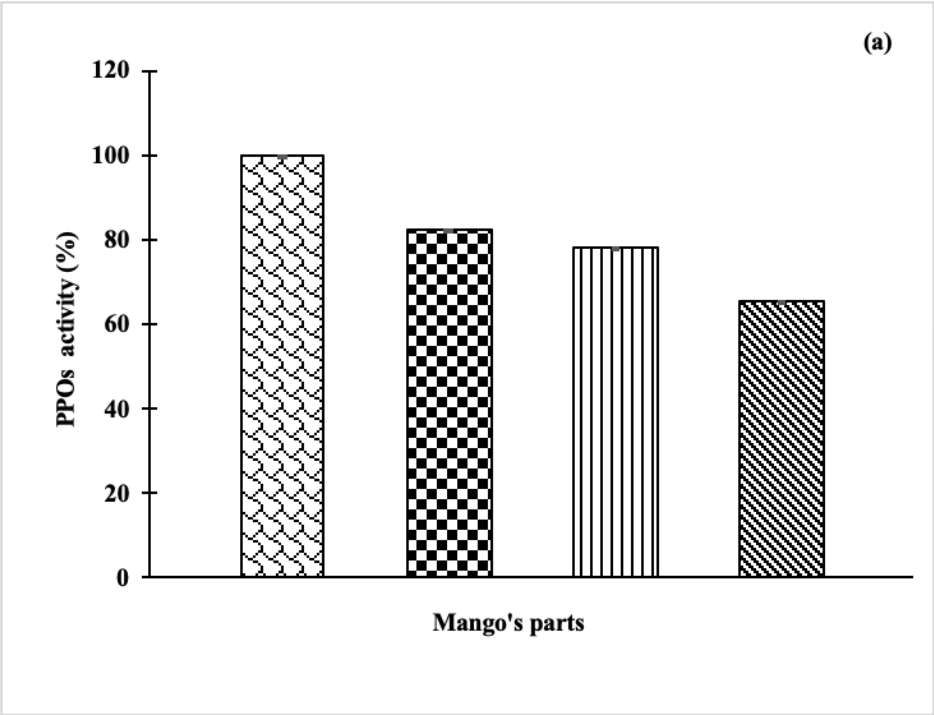
(a)
120
100
80
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40
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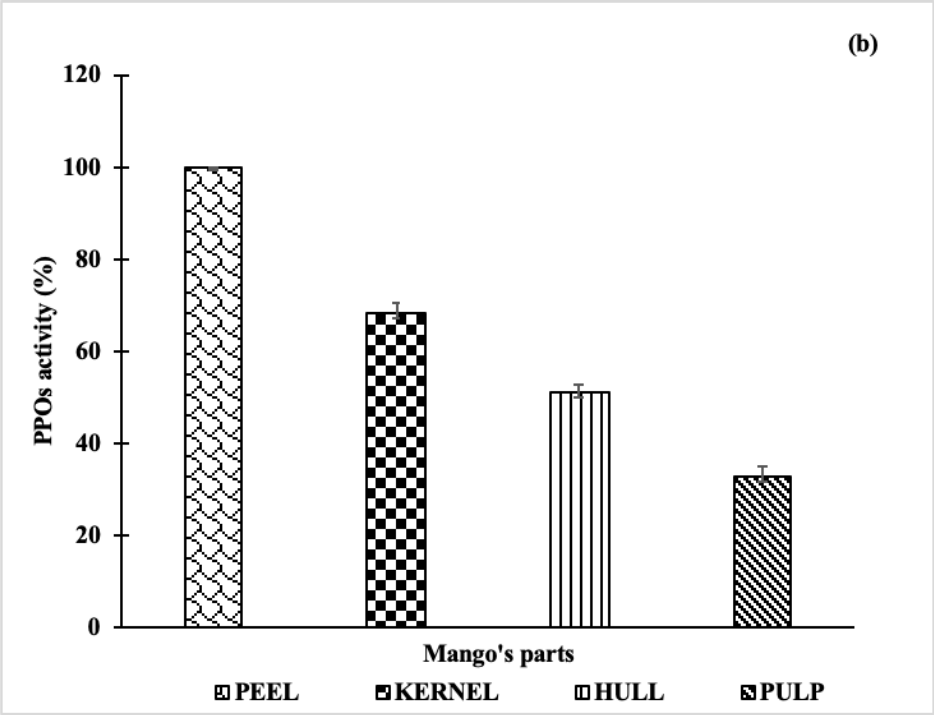
PPOs activity (%)
Mango's parts


(b)
120
100
80
60
40
20
0
PPOs activity (%)
Mango's parts
PEEL
KERNEL
HULL
PULP

3.2 Influence of steam bleaching on PPOs activity in mangoes edible parts

The inhibitory effect of steam bleaching at 60°C and 100°C on polyphenol oxidases activity from the peel and the pulp was noted in the two cultivars (Figures 2 and 3). As expected, the higher the temperature, the more rapidly inactivation of PPOs occurred. The Figures 2(a) and (b) as well as Figures 3(a) and 3(b) showed that an exposure of the edible mango's parts (peel and pulp) to water vapors even at 60 °C or 100 °C led to a considerable loss in polyphenol oxidase activity after several minutes, until it was completely inhibited. Indeed, when samples are subjected to steam bleaching at 100 °C, the PPOs activity is inhibited by more than 50% after 2-3 min in both peel and pulp of *Palmer* (Figure 2(b)) and *Keitt* (Figure 3(b)) varieties. However, PPOs located in the peel seem to be more resistant to heat because their complete inhibition was observed after 7 min of exposure to hot vapors while those contained in the pulp were totally inhibited after only 4-5 min of treatment. Considering the bleaching at 60 °C for *Palmer* (Figure 2(a)) and *Keitt* (Figure 3(a)) cultivars, the results are likely the same as in the 100 °C although the lower temperature required of course more time to completely inhibit PPOs in the peel and the pulp. In *Palmer* as in *Keitt* cultivars, the studied enzymes activities were inhibited precisely at 8 min in the pulp and at 14 min in the peel. Generally, the enzymatic activity decreases with the increase of temperature and exposure time of both varieties. The inactivation of PPO by temperature has already been reported by several authors. Indeed, according to [15], 50% of the polyphenol oxidases activity in mango was inactivated after heating for 2.1 and 4.0 min at 85 and 80 °C, respectively. In addition, [16] reported that 3 and 5 min of treatment at 90 °C were capable of 50% and 90% inactivation of the PPO extract in mango peel, respectively. Also, only 45 seconds were enough to achieve 50% of loss in polyphenol oxidase activity in the mango pulp treated at 100 °C while 1 min was sufficient for inactivating PPOs from mangoes puree extract after exposure at 85, 88, 90.5 and 93 °C ([17]. Furthermore, [18] showed that an exposure of mangoes to steam for 12 min completely inactivated PPOs in the peel and pulp, and thus corroborating our results. In sum, we can affirm that steam bleaching has a thermosensitive effect on the polyphenol oxidases activity of *Palmer* and *Keitt* cultivars under specific time and temperature conditions that could be exploited for a better preservation of these mangoes' varieties.

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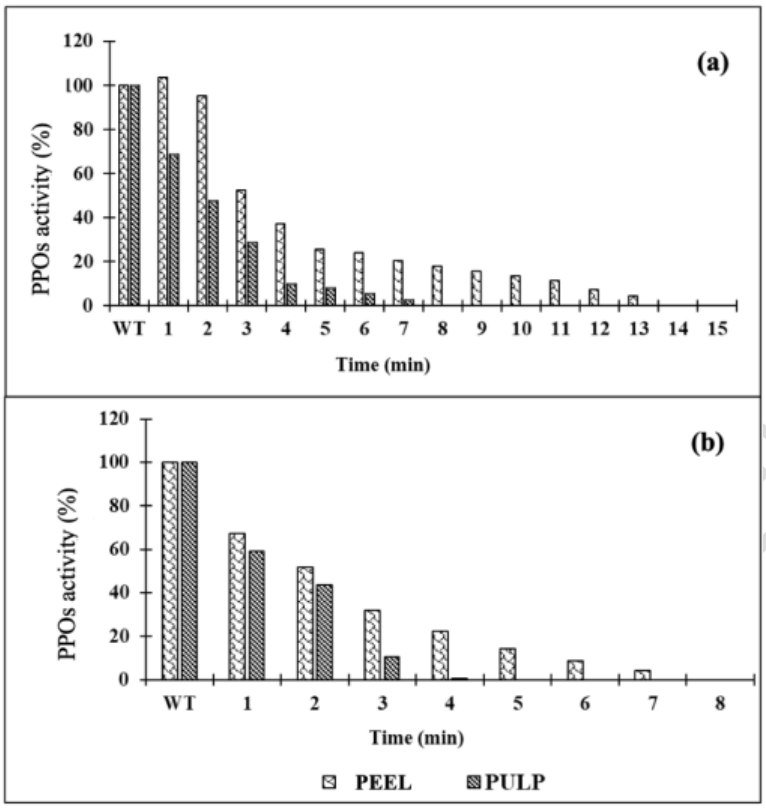

Fig.1.Influence of steam bleaching treatments at 60°C (a) and at 100°C (b) on the polyphenol oxidases activity of edible parts (peel and pulp) of Palmer cultivar

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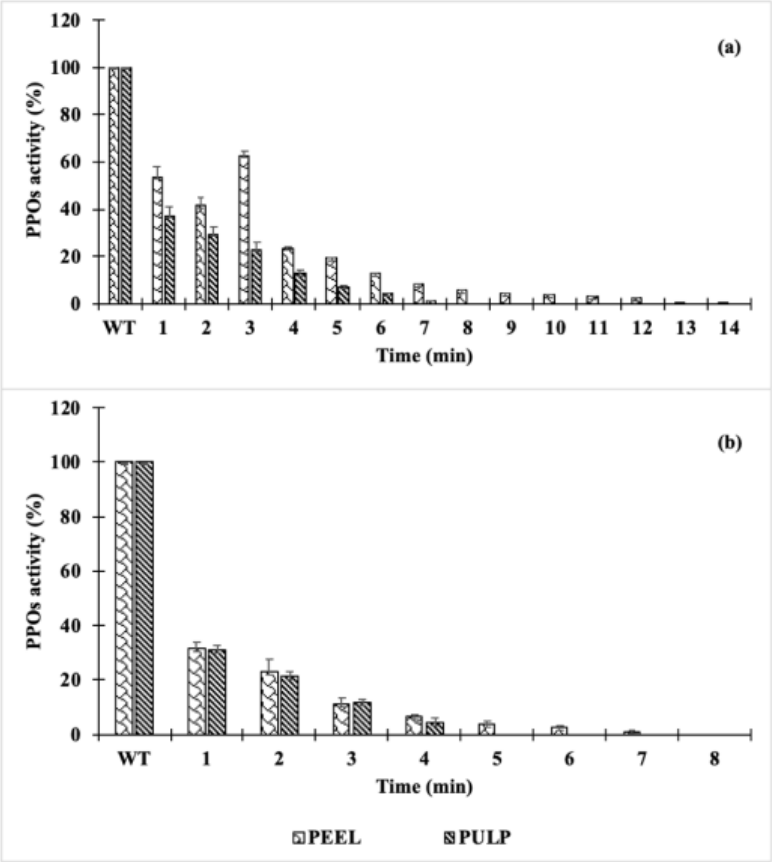

3.3 Influence of osmotic dehydration on PPOs activity on mangoes edible parts

The immersion of the edible parts of the both mango varieties in a sucrose solution prepared at 70 °Brix did not caused a considerable variation of the PPOs activity contained in these parts (Figure 4). Indeed, for the *Palmer* cultivar (Figure 4(a)), there are no statistical differences between the enzymes activity of untreated samples (WT) compared to those exposed to osmotic dehydration both in pulp and peel even after 24 h. However, the results showed an exponential increase of more than 225% and 170% in PPOs activity from the pulp after 3 h and 4 h, respectively (Figure 4 (a)). The activation observed could be the result of an auto-condensation of the excess sugars in the reaction mixture contributing to the formation of new phenolic compounds spectrophotometrically quantified at the same wavelength. For the *Keitt* cultivar, we noticed a loss in the pulp PPOs activity during the first 3 h which reached a mean of 36%. However, from 4 h of treatment, an increase in the enzymes activity was observed every hour till it reaches a rate of 120% after 9 hours of treatment (Figure 4 (b)). In the peel, a slight and constant decrease in PPOs

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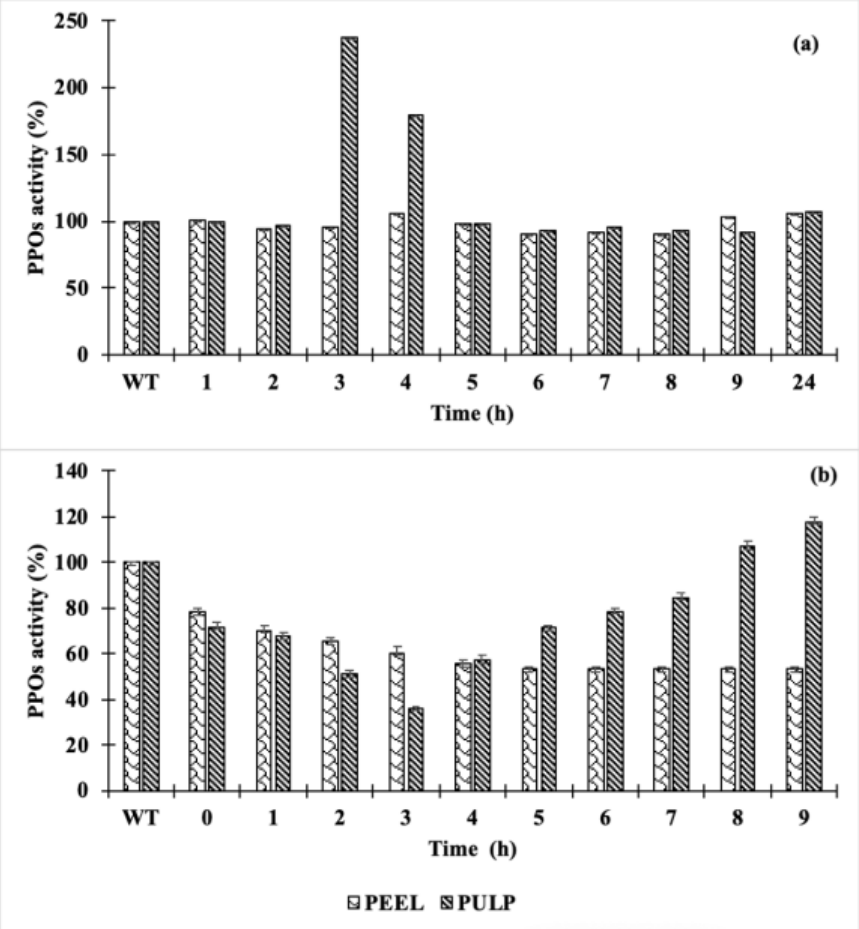
activity was observed during the first 5 h of dehydration getting down to approximately 50%. From this time, the enzymes activity remained stable throughout the experiment (Figure 4(b)). During osmotic dehydration, a significant outflow of water from mango cells occurs as a result of osmotic imbalance between the fruit cells and the high concentration (70 °Brix) of the sucrose solution used [19]. However, our results showed that this preservation technique did not significantly affect the studied enzymes as residual activity remained during the 9 h of experimentation. Such observations led [20] to postulate that in industry, osmotic dehydration is never used alone, but rather as a pre-treatment, which gives good color and sweeter taste to the final product. Nowadays, osmotic dehydration has become an essential pre-treatment method in fruit and vegetable preservation [21–23]. This treatment reduces the energy required for drying, bleaching and freezing techniques and improves the resulted products quality



[24].

3.4 Influence of drying on PPOs activity in mangoes edible parts

As expected, oven drying at 60°C of the edible parts of the *Palmer* and *Keitt* mango cultivars had a considerable influence on the activity of their PPOs (Figure 5). The results showed a progressive decrease the enzymes activity over time. Indeed, as the drying time increased, there was a progressive loss of water activity in the pulp and peel samples of the both varieties. For the *Palmer* cultivar (Figure 5(a)), the PPOs activity in dried peel samples increased significantly (from 100% to 185%) after 1 h of drying. Then, it regressed progressively till it was practically inhibited after 24 h. This increase in polyphenol oxidases activity after 1 h could be due to the progressive rise in temperature of the sample by passing through the optimum temperature range for better activation of the enzymes, thus improving catalysis. As soon as the temperature of 60°C is reached, it apparently becomes critical for the PPOs that are progressively affected to the point of almost losing their initial activity after 24 h (Figure 5(a)). Concerning the dried pulp, the results showed a continuous decrease in polyphenol oxidases activity with complete inactivation after 21 h (Figure 5(a)). Regarding the *Keitt* cultivar, the histograms depict a downward trend of the PPOs activity both in the peel and in the pulp as a function of the drying temperature (60°C) and times (Figure 5(b)). In the pulp, the inhibition of the enzymes was complete after 9 h while a residual activity of about 1% remained in the peel samples even after 24 h of drying (Figures 5(a) and (b)).Although the inactivation of the enzymes was directly correlated to the treatment temperature, this inhibition was also strongly related to water activity. In fact, oven drying which consists in removing the excess of moisture from a product by evaporation, is a phenomenon that causes a decrease in the water activity of the product. Water activity is defined in relation to a reference state, which is pure water, for which the water activity is equal to 1. It corresponds to the ratio between the water vapor pressure of the food and the vapor pressure of pure water at the same temperature. In highly hydrated food products such as mangoes, a very large part of the water is in the form of free water shared on the surface of the product or in cell pockets, and weakly absorbed water retained by capillary action in tissues [25]. According to the same authors, the decline in water activity slows down enzymatic browning. Therefore, the inactivation of PPOs during the drying process should be considered as the result of the coupled effects of time, temperature, substrate and water activity [26–28].

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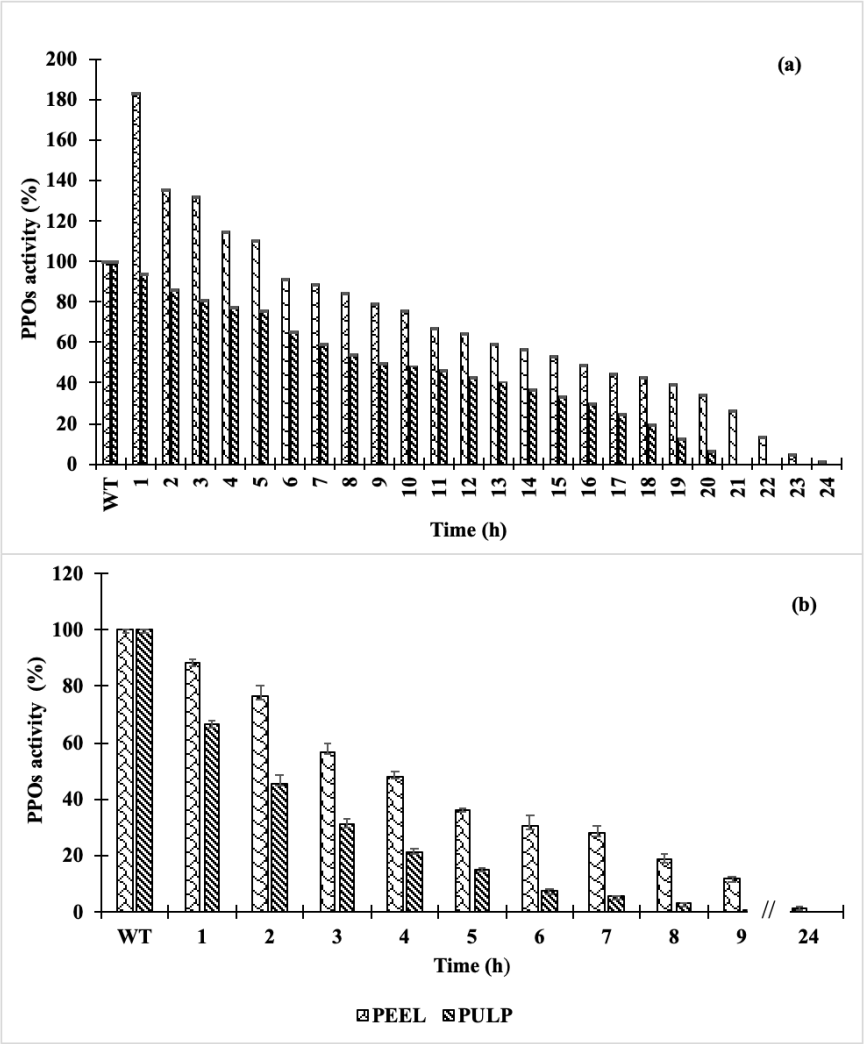

3.5

Efficiency of the three post-harvest preservation techniques

Preservation techniques generally refers to processing methods applied to protect a product from deterioration during long-term storage. Mangoes perishability is largely caused by micro-organisms deterioration and/or by the intense activity PPOs which are fruits physiologic enzymes scientifically known to be responsible for enzymatic browning [29]. In this context, all preservation processes aim at inhibiting or eliminating germs and enzymes responsible of the fruit's degradation, even at creating unfavorable conditions for the proliferation of microorganisms and/or the expression of PPOs activity. Numerous preservation treatments involving heating and refrigeration are used to protect fruit and vegetables. However, the use of various techniques such as sterilization, freezing, fermentation, salting, smoking or

the use of chemicals is also in vogue[30,31]. In this work, three treatments frequently used for preservation such as bleaching, osmotic dehydration and oven drying have been studied for their capability to influence the activity of polyphenol oxidation catalyzing enzymes (PPOs). The summary of the results shows that only steam bleaching at 60°C and 100°C, and oven drying treatments at 60°C led to a complete inhibition of PPOs activity. Osmotic dehydration (70° Brix) had not a significant influence on the activity of the enzymes even after more than 24 h of treatment. As a result, this method is more likely to be use as a pre-treatment technique before applying another method for the preservation of mangoes. Drying is one of the oldest methods of preservation [32] that allows to extend shelf life of fruit and vegetables. Although complete inhibition of PPOs activity took several hours after oven drying treatment on edibles parts of both varieties, total inhibition occurs only after few minutes of using the steam bleaching technique. As a result of comparing the influence of the three treatments on PPOs activity, steam bleaching appears to be a more efficient preservation treatment for the edible parts of *Palmer* and *Keitt* mangoes cultivars. This work allowed us to introduce steam bleaching treatment at 60°C for 7 min as the best preservation technique for mangoes post-harvest management in Côte d'Ivoire before export.

4. CONCLUSION

In this study, the objective was to subject an adequate technological treatment to guarantee a good post-harvest preservation of mangoes cultivated in Côte d'Ivoire that would allow the sustainable preservation in order to minimize losses and increase its market value by acting on the polyphenol oxidase activity. It appears from these experiments that the polyphenol oxidases of *Palmer* and *Keitt* mangoes are highly thermosensitive as their complete inhibition were observed during steam bleaching and oven drying. Osmotic dehydration does not inhibit polyphenol oxidase activity; it should therefore be used as a pre-treatment to enhance the drying or bleaching of mangoes. Finally, steam bleaching at 60°C for 7 min seemed to be the more adapted treatment for post-harvest preservation of Ivorian mangoes intend for export as it has contributed in maintaining the organoleptic properties of the edible parts and marketability of the fruits.

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