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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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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Côte d'Ivoire is the leading exporter of *Palmer* and *Keitt* mangoes in sub-Saharan Africa. Despite the importance of the foreign exchange generated, these tropical fruits suffer enormous post-harvest losses due to their high perishability, mainly caused by enzymatic browning. In fact, the appearance

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of brown discolouration due to alterations, whether caused by mechanical, technological or natural treatment, is the cause of huge economic losses. To address this thorny problem and thereby improve export volumes, three technological treatments including steam bleaching, oven drying and osmotic dehydration were carried out on mango edible parts to inhibit polyphenol oxidases (PPOs), the key enzymes that catalyse enzymatic browning. The results showed that only oven drying at 60°C and steam bleaching at 60°C and 100°C had a significant effect on inactivating PPOs activity after only a few minutes. Osmotic dehydration maintained high levels of enzyme activity even after 24 hours of treatment. In conclusion, steam bleaching at 60°C for 7 minutes was found to be the best post-harvest management technique for Palmer and Keitt mangoes, as it contributed to both the preservation of marketable quality and the stability of organoleptic properties of the mango.

Keywords: Post-harvest management; mangifera indica; steam bleaching; preservation techniques; polyphenol oxidases; Côte d'Ivoire.

1. INTRODUCTION

The 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, accounting for 52% of world production [1]. As well as being delicious, mangoes have an impressive nutritional value, particularly in vitamin C, which supports immunity, iron absorption and cell growth and repair. Because of its health benefits, the mango is considered one of the tropical fruits with high commercial value, whose sale and export is very profitable. In Africa, Côte d'Ivoire is the first exporter and the first supplier to European countries [2]. Among the most popular varieties, *Palmer* and *Keitt*, the Floridian mangoes are 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 [4]. In fact, mangoes, like many other fruits and vegetables, are subject to enzymatic browning, which can occur during the various stages of handling, from harvesting to storage, if they are bruised or damaged. This physiological phenomenon is mainly caused by an enzyme, polyphenol oxidase, a copper metalloprotein that catalyses the oxidation of phenolic compounds into highly reactive quinones that polymerise into brown pigments (melanins), giving the fruit a dark colour and leading to a loss of nutritional value by altering organoleptic qualities [5]. The study of this enzyme is therefore important for the food industry to prevent the loss of marketable quality of fruit and vegetables [6]. 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 [7]. 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.

2. MATERIALS AND METHODS

2.1 Plant Material

The mango samples (*Palmer* and *Keitt)* used in this study were collected in May 2023 from the orchards of the village of Olléo (Korhogo) and those of Sinématiali, both located in the north of Côte d'Ivoire, approximately 600 km from Abidjan. The fruits were harvested fresh, physiologically ripe, hard and without visible lesions.

2.2 Chemicals and Reagents

All chemicals used in this study were of analytical grade and suitable for scientific research. The

main products and reagents used in this study were Catechol, sodium chloride (NaCl), hydrochloric acid (HCl), sodium hydroxide (NaOH) and sodium hypochlorite, all purchased from the Sigma Aldrich company, USA.

2.3 Sample 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.

2.4 Extraction of PPOs from the Four Main Parts of the Mangoes

The extraction was carried out according to the method of [8], slightly modified as follows. 25 g of each cut part of the mango (peel, pulp, skin and kernel) were crushed in 75 ml of sodium chloride (0.9% w/v NaCl) using a Moulinex 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 crude PPOs extract for further assays.

2.5 Preservation Methods for Mango Edible Parts (Peel and Pulp)

The bleaching process was carried out as previously described in [9]. Samples of peel and pulp of *Palmer* and *Keitt* mangoes were placed in contact with water vapour obtained after heating at 60 °C or 100 °C for 15 min. The drying process was carried out according to [10]. In this protocol, the edible parts of Palmer and Keitt mangoes were dried in an oven at 60 °C for 24 hours. Samples were taken every hour to measure the enzymatic activity of the extracts. For the osmotic dehydration technique, the experiments consisted of immersing the mango samples in a 70 °Brix sucrose solution at 25 °C for 9 consecutive hours [11]. During the three experiments, the kinetic effect of each treatment on the activity of browning enzymes in the samples was determined. In fact, every minute (for steam bleaching) or hour (for oven drying and osmotic dehydration), 25 g of each exposed sample was taken for PPOs extraction as described above. The residual enzyme activity was then determined.

2.6 Determination of Polyphenol Oxidases (PPOs) Activity

The activity of PPOs (EC 1.10.3.1) was measured spectrophotometrically at 420 nanometres using pyrocatechol as substrate [12]. The reaction mixture (1.5 mL) consisted of 0.3 mL enzyme extract, 0.9 mL sodium phosphate buffer pH 6.6 and 0.3 mL 0.5 M pyrocatechol. The reaction mixture was incubated at 25 °C for 10 min. Assays were performed in triplicate using a UV-visible spectrophotometer (Pioway, CHINA). One unit of polyphenol oxidase activity was defined as the amount (in mg) of extracted proteins catalysing the oxidation of 1 µmol pyrocatechol to brown quinone molecules per minute. All residual activities measured were expressed as a percentage relative to the maximum activity (100%) obtained from untreated enzyme samples. Enzymatic browning is an undeniable cause of agricultural losses 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 accelerated to a greater or lesser extent. In our study, the variation in the activity of PPOs in two varieties of mangoes subjected or not to preservation treatments allowed us to evaluate the potential of inhibiting these enzymes for better preservation of the fruit.

3. RESULTS AND DISCUSSION

3.1 PPOs Activity in the Four Main Parts of the Fruits

The activity of PPOs in the main parts of *Palmer* and *Keitt* mangoes is shown in Fig. 1(a) and 1(b). This activity is mainly localised in the peel of both cultivars. For the *Palmer* cultivar, the activity of PPOs in the peel (100%) is followed by that in the seed (82%), then in the skin (76%) and in the pulp (62%) in decreasing order (Fig. 1(a)). For the Keitt cultivar (Fig. 1(b)), the PPOs also showed the same trend activity, being higher in the peel (100%) but less important in the kernel (68%), hull (51%) and pulp (33%). Therefore, the average of the spectrophotometric absorbencies

obtained in the peel was used as a reference for calculating the activity percentages of the other mango parts. PPOs activity was detected in all parts of both mango varieties and was much higher in the exocarp (peel). This activity was lower in the endocarp (skin and kernel) and in the mesocarp (pulp). Such results have already been observed by [13] who showed that polyphenol oxidases are present in different organs of higher plants such as root, seed, leaf, peel and fruit cortex. In addition, [14] showed that PPOs are copper metalloproteins found in all plant organs and in different cell compartments, especially in the cytoplasm and pectocellulose walls. Since the in vitro increase in the activity of an enzyme depends on pH, temperature and substrate concentration, it could be asserted that the dissimilarity between the polyphenol oxidase activities in the studied mango parts and cultivars is closely related to the variability in the concentration of phenolic compounds in the fruit cell vacuoles [15].

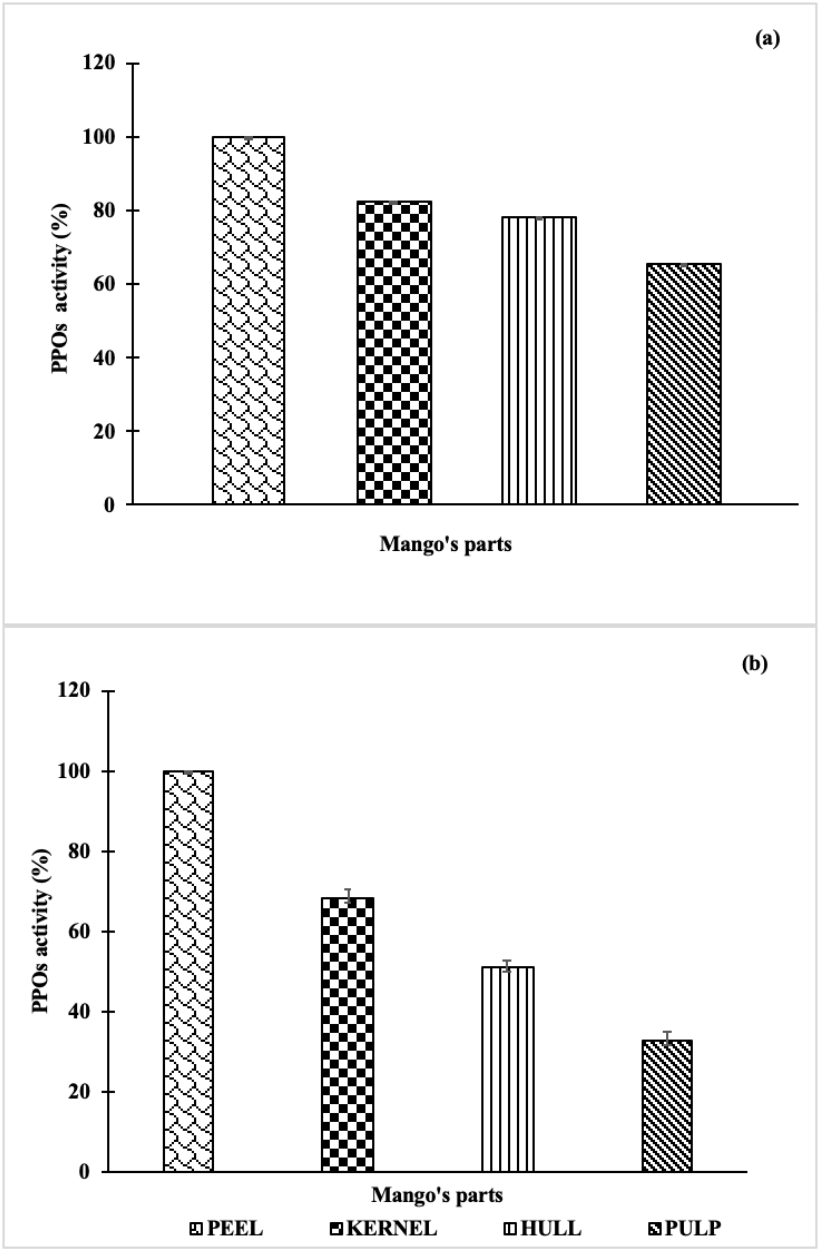

Fig. 1. Polyphenol oxidases activity of the peel, the pulp, the hull and the kernel extracts from Palmer (a) and Keitt (b) mango cultivars produced in Côte d'Ivoire

3.2 Influence of Steam Bleaching on the Activity of PPOs in Mango Edible Parts

The inhibitory effect of steam bleaching at 60°C and 100°C on the activity of polyphenol oxidases from peel and pulp was observed in both cultivars (Fig. 2 and 3). As expected, the higher the temperature, the faster the inactivation of PPOs. Fig. 2(a) and (b) and 3(a) and (b) show that exposure of the edible parts of the mango (peel and pulp) to water vapour, even at 60 °C or 100 °C, resulted in a significant loss of polyphenol oxidase activity after a few minutes, until it was completely inhibited. Indeed, when samples are subjected to steam bleaching at 100 °C, the activity of PPOs is inhibited by more than 50% after 2-3 minutes in both the peel and pulp of the Palmer (Fig. 2(b)) and *Keitt* (Fig. 3(b)) varieties. However, PPOs located in the peel seem to be more resistant to heat, as their complete inhibition was observed after 7 min of exposure to hot vapours, whereas those located in the pulp were completely inhibited after only 4-5 min of treatment. Considering the bleaching at 60 °C for *Palmer* (Fig. 2(a)) and *Keitt* (Fig. 3(a)), the results are probably the same as for 100 °C, although the lower temperature obviously required more time to completely inhibit PPOs in the peel and pulp. In both *Palmer* and *Keitt* cultivars, the enzymatic activities studied were inhibited exactly at 8 min in the pulp and 14 min in the peel. In general, the enzymatic activity of both varieties decreased with increasing temperature and exposure time. The inactivation of PPOs by temperature has been reported by several authors. Indeed, according to [16], 50% of the polyphenol oxidase activity in mango was inactivated after heating for 2.1 and 4.0 min at 85 and 80 °C, respectively. In addition, [17] reported that 3 and 5 minutes of treatment at 90 °C were able to inactivate 50% and 90% of the PPOs extract in mango peel, respectively. Also, only 45 seconds was sufficient to achieve 50% of the loss of polyphenol oxidase activity in mango pulp treated at 100 °C, while 1 minute was sufficient to inactivate PPOs from mango puree extract after exposure at 85, 88, 90.5 and 93 °C [18]. Furthermore, [19] showed that exposure of mangoes to steam for 12 min completely inactivated PPOs in the peel and pulp, confirming our results. In conclusion, we can affirm that steam bleaching has a thermosensitive effect on the polyphenol oxidase activity of *Palmer* and *Keitt* cultivars under specific time and temperature conditions, which could be exploited for better preservation of these mango cultivars.

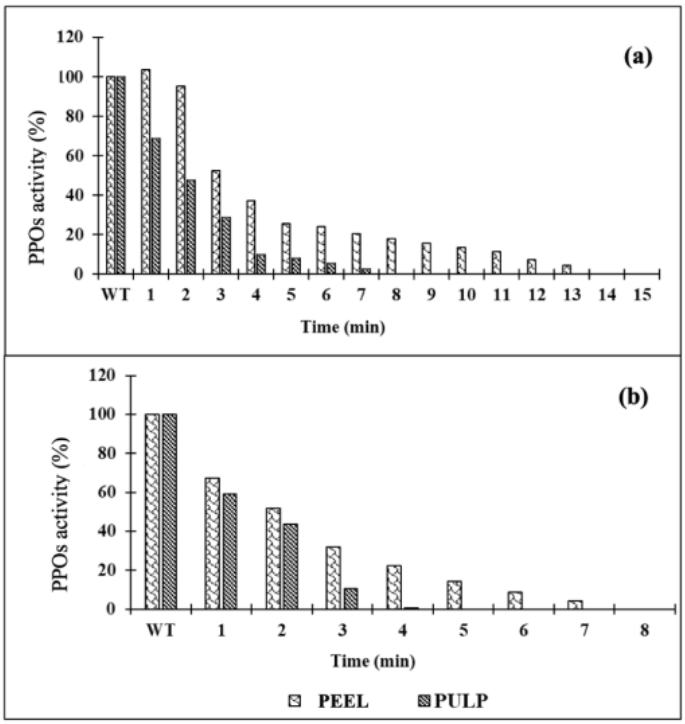

Fig. 1. Influence of steam bleaching treatments at 60°C (a) and at 100°C (b) on the polyphenol oxidases activity of edible parts (peel and pulp) of Palmer cultivar

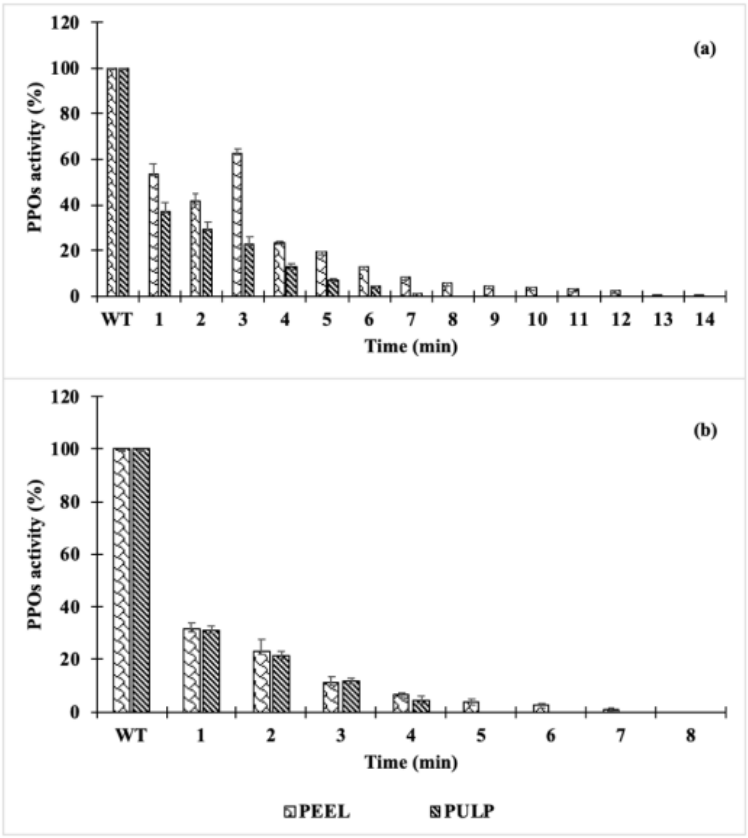

Fig. 3. Influence of steam bleaching treatments at 60°C (a) and at 100°C (b) on the polyphenol oxidases activity of edible parts (peel and pulp) of Keitt cultivar

3.3 Influence of Osmotic Dehydration on the Activity of PPOs on Mango Edible Parts

The immersion of the edible parts of both mango varieties in a sucrose solution prepared at 70 °Brix did not cause any significant variation in the activity of the PPOs contained in these parts (Fig. 4). In fact, for the *Palmer* variety (Fig. 4(a)), there are no statistical differences between the enzyme activity of untreated samples (WT) compared to those exposed to osmotic dehydration, both in the pulp and in the peel, even after 24 h. However, the results showed an exponential increase of more than 225% and 170% in the PPOs activity from the pulp after 3 h and 4 h, respectively (Fig. 4(a)). The activation observed could be the result of auto-condensation of the excess sugars in the reaction mixture, contributing to the formation of new phenolic compounds, which were quantified spectrophotometrically at the same wavelength. For the *Keitt* cultivar, we observed a loss of PPOs activity in the pulp during the first 3 hours, which reached an average of 36%. However, after 4 hours of treatment, an increase in enzyme activity was observed every hour until it reached 120% after 9 hours of treatment (Fig. 4 (b)). In the peel, a slight and constant decrease in the activity of PPOs was observed during the first 5 h of dehydration, reaching approximately 50%. From this time, the enzyme activity remained stable throughout the experiment (Fig. 4(b)). During osmotic dehydration, a significant outflow of water from the mango cells occurs as a result of the osmotic imbalance between the fruit cells and the high concentration (70 °Brix) of the sucrose solution used [20]. However, our results showed that this preservation technique did not significantly affect the enzymes studied, as residual activity remained throughout the 9 h experiment. Such observations led [21] to postulate that in industry, osmotic dehydration is never used alone, but rather as a pre-treatment that gives good colour and sweeter taste to the final product. Today, osmotic dehydration has become an essential pre-treatment method in fruit and vegetable preservation [22–24]. This treatment reduces the energy required for drying, bleaching and freezing techniques and improves the quality of the resulting products [25].

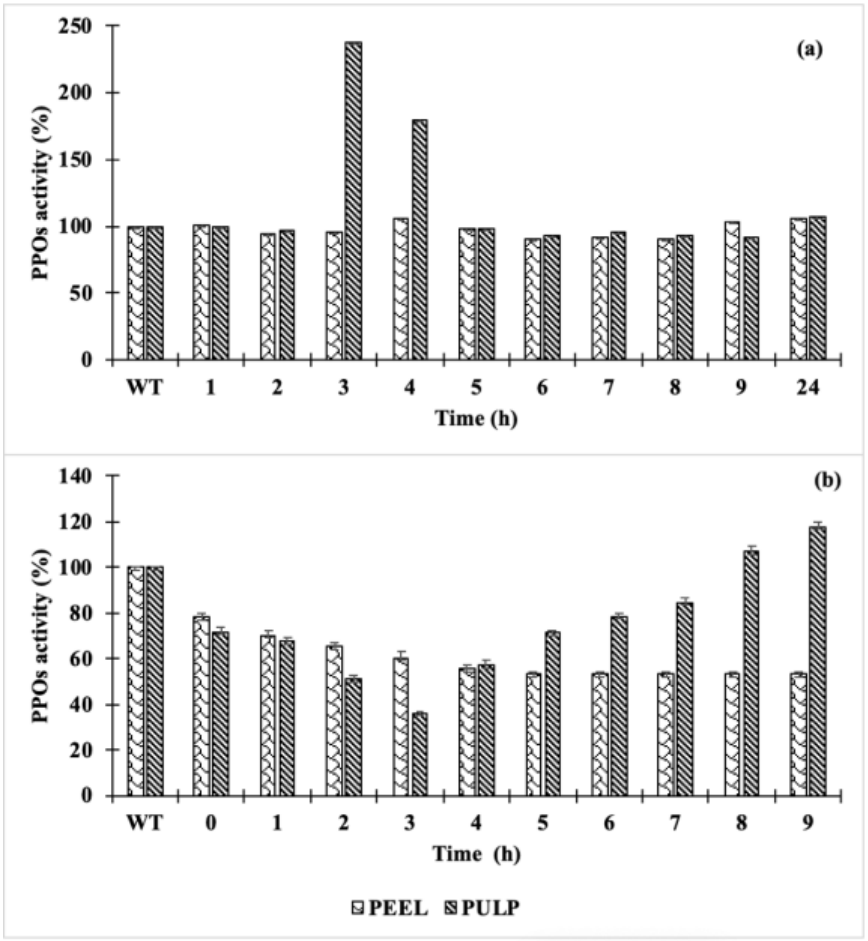

Fig. 4. Influence of osmotic dehydration on the polyphenol oxidases activity of edible parts (peel and pulp) of Palmer (a) and Keitt (b) mango cultivars

3.4 Influence of Drying on PPOs Activity in Mango Edible Parts

As expected, oven drying at 60°C of the edible parts of the *Palmer* and *Keitt* mango cultivars had a significant effect on the activity of their PPOs (Fig. 5). The results showed a progressive decrease in enzyme activity over time. In fact, with increasing drying time, there was a progressive loss of water activity in the pulp and peel samples of both cultivars. For the Palmer variety (Fig. 5(a)), the activity of PPOs in dried peel samples increased significantly (from 100% to 185%) after 1 h of drying. This increase in polyphenol oxidase activity after 1 h could be due to the progressive increase in temperature of the sample, passing through the optimum temperature range for better activation of the enzymes, thus improving catalysis. Once the temperature of 60°C is reached, it apparently becomes critical for the PPOs, which are progressively affected to the point of almost losing their initial activity after 24 h (Fig. 5(a)). For the dried pulp, the results showed a continuous decrease in polyphenol oxidase activity with complete inactivation after 21 h (Fig. 5(a)). For the *Keitt* cultivar, the histograms show a decreasing trend of PPOs activity in both peel and pulp as a function of drying temperature (60°C) and time (Fig. 5(b)). In the pulp, the inhibition of the enzymes was complete after 9 h, whereas a residual activity of about 1% remained in the peel samples even after 24 h of drying (Fig. 5(a) and (b)). Although the inactivation of the enzymes was directly related to the treatment temperature, this inhibition was also strongly related to the water activity. In fact, oven drying, which consists of removing excess 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, pure water, for which the water activity is equal to 1. It corresponds to the ratio between the water vapour pressure of the food

and the vapour pressure of pure water at the same temperature. In highly hydrated foods such as mangoes, a very large proportion of the water is in the form of free water distributed on the surface of the product or in cell pockets, and weakly absorbed water retained by capillary action in the tissues [26]. According to the same authors, the decrease in water activity slows down enzymatic browning. Therefore, the inactivation of PPOs during the drying process should be considered as a result of the coupled effects of time, temperature, substrate and water activity [27–29].

3.5 Effectiveness of the Three Post-Harvest Preservation Techniques

Preservation techniques generally refer to processing methods used to protect a product from deterioration during long-term storage. The perishability of mangoes is largely caused by microbial spoilage and/or the intense activity of PPOs, which are physiological enzymes of the fruit that are scientifically known to be responsible for enzymatic browning [30]. In this context, all preservation processes are aimed at inhibiting or eliminating germs and enzymes responsible for fruit deterioration, even creating unfavourable conditions for the proliferation of microorganisms and/or the expression of PPOs activity. Many preservation treatments involving heating and cooling are used to protect fruit and vegetables. However, the use of various techniques such as sterilisation, freezing, fermentation, salting, smoking or the use of chemicals is also in vogue [31,32]. In this work, three treatments commonly used for preservation, such as bleaching, osmotic dehydration and oven drying, were evaluated for their ability to influence the activity of polyphenol oxidation catalysing enzymes (PPOs). The summary of the results shows that only steam

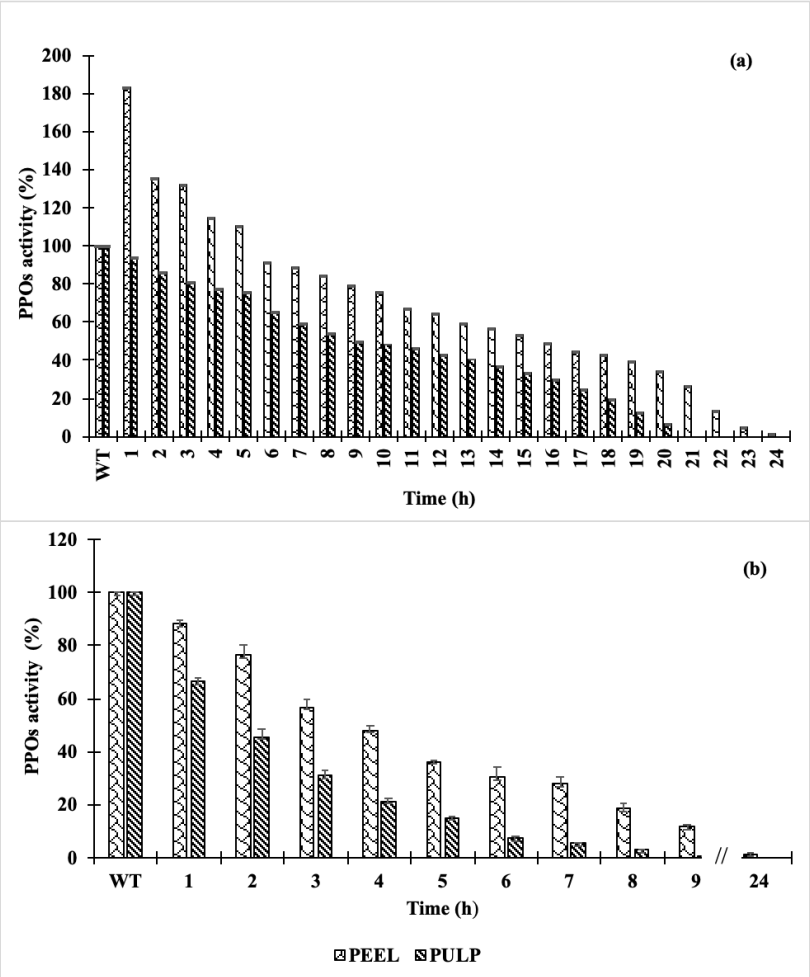

Fig. 5. Influence of oven drying at 60 °C on the polyphenol oxidases activity of edible parts (peel and pulp) of Palmer (a) and Keitt (b) mango cultivars

bleaching at 60°C and 100°C and oven drying at 60°C resulted in complete inhibition of PPOs activity. Osmotic dehydration (70° Brix) had no significant effect on enzyme activity, even after more than 24 hours of treatment. As a result, this method is more likely to be used as a pre-treatment technique before another method is applied to preserve mangoes. Drying is one of the oldest preservation methods [33] used to extend the shelf life of fruits and vegetables. Although complete inhibition of PPOs activity took several hours after oven drying treatment on edible parts of both varieties, total inhibition occurs only after a 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. This work allowed us to introduce the steam bleaching treatment at 60°C for 7 min as the best preservation technique for post-harvest management of mangoes in Côte d'Ivoire before export.

4. CONCLUSION

The aim of this study was to find an appropriate technological treatment to ensure good post-harvest preservation of mangoes grown in Côte d'Ivoire, which would allow sustainable conservation to minimise losses and increase their market value by acting on the polyphenol oxidase activity. These experiments indicate that the polyphenol oxidases of *Palmer* and *Keitt* mangoes are highly thermosensitive, as their complete inhibition was observed during steam bleaching and oven drying. Osmotic dehydration does not inhibit polyphenol oxidase activity and should therefore be used as a pre-treatment to improve drying or bleaching of mangoes. Finally, steam bleaching at 60°C for 7 minutes seemed to be the most suitable treatment for post-harvest preservation of Ivorian mangoes intended for export, as it helped to preserve the organoleptic properties of the edible parts and the marketability of the fruit.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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