J. Agric. Food. Tech., 7(9)1-9, 2017

ISSN 2090 – 424X
Journal of Agriculture and Food Technology
www.textroad.com

© 2017, TextRoad Publication

Modulation of Phenolic Metabolism Under Effect of Storage Time and Temperature During Postharvest Preservation of Pineapple (*Ananas Comosus* L. Merrill)

Coulibaly S.[1*], Yapo S.E.S.[2], Kouadio Oi Kouadio S[1]., Tuo S.[3] and Kouakou T.H.[1]

[1]Université Nangui Abrogoua, UFR des Sciences de la Nature, Laboratoire de Biologie et Amélioration des Productions Végétales, 02 BP 801 Abidjan 02, Côte d'Ivoire
[2]Université Jean Lorougnon Guédé, UFR Agrophysiologie, BP 150 Daloa, Côte d'Ivoire
[3]Université Félix Houphouët Boigny, UFR Biosciences, Laboratoire de Physiologie Végétale, 22 BP 582 Abidjan 22, Cote d'Ivoire

ABSTRACT

The influence of storage time and temperature during post-harvest preservation of two pineapple varieties was been studied through phenolic compounds metabolism. The total phenol content was determined as well as the enzymes entering its metabolism. Thus, biosynthesis enzymes such as PAL and TAL, oxidation enzymes such as PPO and antioxidant enzymes such as CAT and AsPerox have been assayed after storage at different time and temperatures. The results show that MD2 is more resistant to the phenomenon of enzymatic browning because it is rich in ascorbic acid. Indeed, CAT and AsPerox activity in MD2 was highest. This shows a greater detoxification in MD2 compared to Smooth Cayenne Furthermore, storage at 10°C for 14 days is better for reducing the internal browning of pineapple due to cold stress when transported to Europe. At this temperature, pineapples are more resistant to shock at 25°C which is the temperature of their sale.

KEY WORDS: Pineapple, *Ananas comosus* L., Internal browning, Smooth Cayenne, Total phenol, Enzyme, MD2

INTRODUCTION

Pineapple (*Ananas comosus* [L.] Merrill) is grown for its edible fruit. It is the third largest tropical fruit crop and represents a major source of income for many countries [1]. Countries such as Thailand, Costa Rica, Brazil, Philippines and Indonesia are the largest pineapple producers while Costa Rica, Philippines, Panama, Ecuador and Honduras are the main countries exporters [2]. Pineapple is produced in more than 80 countries, including Côte d'Ivoire, and thus occupies a prominent place in international trade [3]. This country, with nearly 33,976 tons of fresh pineapple exported, is the second largest exporting countries in Africa after Ghana and seventh in the world [4]. However, in recent years, the Ivorian pineapple sector is in "crisis" with its exports falling from 210,000 tons in 2003 to 33,000 tons in 2014 and 30,000 tons currently [5]. This drop in production of pineapple in Côte d'Ivoire is an opportunity to preserve the fruit after the harvest. Indeed, during export, pineapples are conserved in freezing containers at 8°C. Pineapples harvested in the field are first washed and packaged at the packing stations. They are pre-cooled before being transported to the port. They are then placed in refrigerated containers to ensure better storage because of the transport time which can last two weeks. As soon as they arrive, pineapples are taken to purchasing centers and then sold in supermarket shelves to reach consumers. The duration of the maritime transport of pineapples which approaches the two weeks causes the internal browning of pineapple fruit [6]. This browning leads to a reduction in the quality and therefore a decrease in the commercial value of pineapple. This browning entails a drop in quality and therefore a decrease of the pineapple commercial value. During postharvest storage, pineapple browns easily and consequently loses much of its commodity value. Such knowledge is of great importance in developing storage strategies designed to maintain pineapple quality in postharvest storage and thus extend its shelf life and pineapple commercial value.

A few years ago, pineapple production was dominated by Smooth Cayenne because of the high yields and organoleptic qualities enjoyed by consumers around the world [2]. Currently, because of the high sensitivity of Smooth Cayenne fruit to internal browning during export storage, this variety has been outclassed by the hybrid pineapple, Sweet or MD2, developed by the US multinational Del Monte Foods which has set up his plantations in Costa Rica. This pineapple is sweeter and has properties that allow it to withstand shocks and cold storage during export; thus making the Smooth Cayenne is less interesting and much less profitable [7, 8]. Currently, because of the high sensitivity of Smooth Cayenne to internal browning of fruit during storage during export, this variety has been outclassed by the hybrid pineapple, Sweet or MD2, developed by the US multinational Del Monte. Foods has planted its plantations in Costa Rica [7, 8]. This pineapple also has a very

*** Corresponding Author:** Coulibaly Souleymane, Laboratoire de Biologie et Amélioration des Productions Végétales, UFR des Sciences de la Nature, Université Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire, Tel: (+225) 20 30 42 00, E-mail: yohousoul@gmail.com

good yield, it's sweeter and has properties that allow it to withstand shocks and cold storage during export. It is therefore less sensitive to internal browning, which has favored the expansion of its culture for export, and this to the detriment of the Smooth Cayenne [9, 10]. Internal or enzymatic browning is the set of phenomena that contribute to the appearance of brown colorations following the alteration of fruits or vegetables. Enzymatic browning is due to endogenous phenolic compounds oxidation, catalyzed by oxidases [11]. In addition, it has been shown that in response to heat shock due to cold and which is the cause of internal browning of fruits a variation in the activity of the enzymes of biosynthesis and catabolism of phenolic compounds was observed in many plants [12-14]. In this case, an implication of the metabolism of phenols in the fight against oxidative stress is thus proven.

The purpose of this study was to evaluate the effect of storage time and temperature on pineapple quality after postharvest preservation. This paper reports the changes in PAL, TAL, PPO, CAT and AsPerox activities correlating to phenolic compounds accumulation of in fruits. Modulations in phenolic compounds metabolism were related to internal browning of cold-preserved pineapple.

MATERIAL AND METHODS

Plant material

Two varieties of pineapple (*Ananas comosus* L.) for export, Smooth Cayenne and MD2 were used. Fruits were harvested at export maturity in the experimental plot located in Nangui Abrogoua University of Abidjan (Côte d'Ivoire, West Africa). Fruits were selected according to uniformity of size and color. blemished and damaged fruits were discarded.

Methods

Pineapple fruits were distributed into four groups (30 fruits in each group) to be stored at different temperatures for experimentation. The storage temperatures were 4.0, 10, 16 and 22 °C for 4, 7 and 14 days respectively with group 1, 2 and 3. After that time, each group of fruit was stored at 22°C which is the usual temperature of pineapple marketing in Europe [15] for one week of storage. The fourth group was the control sample was stored at 25°C for each above time. Three fruits were taken at random from each group in order to determine the changes in phenolic metabolism during fruit storage at different temperatures and their impact on fruit commercial value.

Extraction and quantification of total phenols

Fifty milligrams of freeze-dried pineapple pulp were extracted with 10 mL of methanol overnight at 4°C in a blender. The homogenate was centrifuged for 10 min at 5,000 rpm and supernatant samples was collected as crude total phenols extract to be analyzed.

Total phenolic content (TPC) for the pineapple fruit was assayed using the Folin Ciocalteu method as described by Kouakou *et al*. [16] with several modifications. Briefly, an aliquot 0.9 mL of distilled water was added to aliquots of 0.1 mL of crude extracts and 1.0 mL of Folin-Ciocalteu reagent (diluted 10 times), and 1.0 mL of sodium carbonate ($NaCO_3$) at 17% (w/v). After incubation at room temperature for 60 min in the dark, the absorbance was done at 765 nm in using a spectrophotometer for quantification of phenols. Gallic acid (0-100 µg/mL) was used for calibration of a standard curve ($y = 0.038x + 0.192$, $r^2 = 0.998$, where y is absorbance and x is the concentration of gallic acid). The results are expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/ g DW). Three replicates were conducted for each sample.

Extraction of enzymes

Enzymes extraction was carried out under cold conditions by grinding 0.5 g of freeze-dried pineapple pulp in 5 ml of buffer in presence of 0.5% PVP. During grinding, 0.1 ml of solution composed with 0.5% PEG 6000, 0.25% sodium thiosulfate, 15% glycerol, 1.0 mM EDTA and 15 mM mercaptoethanol was added. After centrifugation at 5,000 rpm for 20 min at 4 °C, the supernatant was collected. Then, 1.0% DOWEX 2 was added to crude enzyme extract and all, stirred and incubated for 30 min at 4 °C, was centrifuged as before to remove resin. The resulting supernatant was used as a crude enzyme extract for assays.

Phenylalanine ammonia lyase activity analysis

Phenylalanine ammonia lyase (PAL, EC 4.3.1.24) activity was determined according to the method of Cheng *et al*. [18]. The reaction mixture contained 1 ml of L-phenylalanine 100 mM, 1.9 ml of sodium borate buffer 0.2 M (pH 8.8) and 0.1 ml of enzyme extract, in a total volume of 3.0 ml. After incubation for 1 h at room temperature, the reaction was stopped by addition of 100 µl of HCl 1N. The kinetic of cinnamic acid formation was monitored at 470 nm. PAL activity was expressed in µkat per minute per gram of dry matter (µkat/min/g dw), considering that the molar extinction coefficient of cinnamic acid is equal to 19,600 M^{-1} cm^{-1}. One unit of

PAL was defined as the amount of enzyme required for the formation of 1.0 µM of cinnamate in 1.0 min under the assay conditions. Assay was performed in triplicate.

Tyrosine ammonia lyase activity analysis

Tyrosine ammonia lyase (TAL, EC 4.3.1.23) was determined using the method of Berner *et al*. [19]. The reaction mixture consisted of 1.0 ml of L-tyrosine 100 mM and 0.1 ml of enzyme extract was adjusted to 3.0 ml with sodium borate buffer 0.2 M (pH 8.5). After incubation for 30 min at room temperature, the reaction was stopped by addition of 100 µl of HCl 1N. TAL activity which is proportional to *p*-coumaric acid formation was monitored at 310 nm. TAL activity was expressed in µkat per minute per gram of dry matter (µkat/min/g dw), considering that *p*-coumaric acid's molar extinction coefficient as 17,600 M^{-1} cm^{-1}. One unit of TAL was defined as the amount of enzyme required for 1.0 µM of *p*-coumaric acid formation in 1.0 min under the assay conditions.

Polyphenol oxidase activity analysis

The measurement of Polyphenol oxidase (PPO, EC 1.14.18.1) activity was carried out according to the method described by Coseteng and Lee [20], with certain modifications. The reaction mixture contained 1.0 ml of pyrocatechol 10 mM, 1.9 ml of sodium phosphate buffer 0.1 M (pH 6.5) and 0.1 ml of enzymatic extract, in a total volume of 3.0 ml. After incubation for 10 min at room temperature, oxidation of pyrocatechol to *o*-quinone was monitored at 420 nm. PPO activity was expressed in µkat per minute per gram of dry matter (µkat/min/g dw), considering that the molar extinction coefficient of formed product as 1,400 M^{-1} cm^{-1}. One unit of PPO was defined as the amount of enzyme required for the formation of 1.0 µM of *o*-quinone in 1.0 min under the assay conditions. Assay was performed in triplicate.

Catalase activity analysis

Catalase (CAT, EC 1.11.1.6) activity was performed to the method of Aebi [21] modified by Dorival and Ann [22]. The reaction mixture consisted of 0.5 ml of hydrogen peroxide 30 mM, 2.4 ml of sodium phosphate buffer 0.1 M (pH 7.0) and 0.1 ml of enzyme extract. After incubation for 10 min at room temperature, CAT activity which is proportional to the decrease in absorbance due to hydrogen peroxide (H_2O_2) depletion was recorded spectrophotometrically at 240 nm. CAT activity was expressed in µkat per minute per gram of dry matter (µkat/min/g dw), considering that H_2O_2 molar extinction coefficient is equal to 43.6 M^{-1} cm^{-1}. One unit of CAT was defined as the amount of enzyme required for 1.0 µM of H_2O_2 formation in 1.0 min under the assay conditions.

Ascorbate peroxydase activity analysis

Ascorbate peroxydase (AsPerox, EC 1.11.1.11) activity was assayed according to the method of Nakano and Asada [23] with certain modifications. The reaction mixture contained 0.5 ml of ascorbic acid 5 mM, 2.4 ml of potassium phosphate buffer 0.05 M (pH 7.0) and 0.1 ml of enzymatic extract, in a total volume of 3.0 ml. The loosely stoppered tubes were thoroughly mixed. After incubation for 15 min at room temperature, the reaction was stopped by addition of 0.5 mL of hydrogen peroxide 30 mM. AsPerox activity is proportional to the decrease in absorbance due to ascorbic acid oxidation was monitored at 290 nm.AsPerox activity was expressed in µkat per minute per gram of dry matter (µkat/min/g dw), considering that the molar extinction coefficient of formed product as 2.8 M^{-1} cm^{-1}. One unit of AsPerox was defined as the amount of enzyme required for 1.0 µM of of formed product in 1.0 min under the assay conditions. Assay was performed in triplicate.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using Statistica software (release 7.1). Means comparison was performed using Newman Keuls test at threshold $\alpha = 0.05$. Thus, when $P \geq 0.05$, there is no significant difference between the means. On the other hand, when $P < 0.05$, the difference between means is significant. Data are the mean of three repetitions.

RESULTS AND DISCUSSION

The results showed that total phenol content (TPC) of both pineapple varieties decreases with storage temperature. Thus, at room temperature (25°C), TPC was significantly higher. It then decreases gradually to the lowest values at 10°C. It's also worth mentioning that TPC of pineapple pulps at10°C was same to that obtained at 4°C. With respect to preservation, TPC was increased with storage time of pineapple regardless of temperature. However, at 10°C, TPC was decreased with increasing storage time. Moreover, at this temperature, TPC was identical in the two pineapple varieties. In addition, results showed that at harvest and at room temperature (25°C) TPC no significant difference was observed in these two varieties. TPC increased with the storage temperature, whereas storage time had little influence on phenol evolution in pineapples (Table 1).

This variation in TPC was positively correlated with PAL activity which varies with storage temperature. Thus, at room temperature, PAL activity in pineapples was lower than that stored at 22°C but

greater than that stored at 16°C. The lowest activity of PAL was observed at 10°C. With regard to storage time, the activity of this enzyme increases in both fruit. However, at 10°C, PAL activity decreases with shelf life. PAL was more active in MD2 than the Smooth Cayenne. With storage at 10°C for 14 days, activity of enzyme remains substantially equal to those of day 0 at 2.31 and 2.65 µkat/min/g dw, respectively for smooth Cayenne and MD2 (Table 2).

Table 1. Effect of temperature and storage time on total phenol content in pineapple fruit

Temperature (°C)	Storage time (days)	Total phenols content (mg/g dw)	
		Smooth Cayenne	MD2
4	4	1.12 ± 0.05 b	1.10 ± 0.04 b
	7	1.13 ± 0.06 b	1.09 ± 0.08 b
	14	1.19 ± 0.02 bc	1.12 ± 0.06 b
10	4	1.11 ± 0.37 b	1.10 ± 0.05 b
	7	1.11 ± 0.19 b	1.11 ± 0.01 b
	14	0.82 ± 0.08 ab	0.88 ± 0.02 ab
16	4	1.31 ± 0.05 c	1.53 ± 0.07 bc
	7	1.47 ± 0.02 d	1.55 ± 0.08 bc
	14	1.51 ± 0.08 d	1.62 ± 0.07 c
22	4	1.72 ± 0.04 e	1.72 ± 0.01 d
	7	1.73 ± 0.07 e	1.72 ± 0.09 d
	14	1.75 ± 0.03 e	1.78 ± 0.03 de
25	0	0.70 ± 0.19 a	0.75 ± 0.01a
	4	1.72 ± 0.02 e	1.72 ± 0.08 d
	7	1.75 ± 0.03 e	1.76 ± 0.05 de
	14	1.82 ± 0.01f	1.86 ± 0.03 e

In a column and on a line, the values followed by the same letter are not significantly different (Newman-Keuls test at 5%). Values are the mean of triplicate; ±SD: standard deviation; dw: dry weight.

Table 2. Effect of temperature and storage time on PAL activity in pineapple fruit

Temperature (°C)	Storage time (days)	Activity of PAL (µkat/min/g dw)	
		Smooth Cayenne	MD2
4	4	4.23 ± 0.02 b	4.23 ± 0.04 b
	7	4.12 ± 0.09 b	4.46 ± 0.02 b
	14	4.41 ± 0.04 b	5.51 ± 0.03 c
10	4	3.51 ± 0.08 ab	3.43 ± 0.10 ab
	7	3.95 ± 0.05 ab	3.86 ± 0.03 ab
	14	2.04 ± 0.01 a	2.11 ± 0.09 a
16	4	4.50 ± 0.03 bc	4.52 ± 0.30 b
	7	6.10 ± 0.07 d	6.16 ± 0.09 d
	14	6.13 ± 0.03 d	6.23 ± 0.05 d
22	4	6.56 ± 0.07 d	5.56 ± 0.07 c
	7	7.57 ± 0.05 e	7.46 ± 0.10 e
	14	7.62 ± 0.10 e	7.56 ± 0.06 e
25	0	2.31 ± 0.03 a	2.65 ± 0.05 ab
	4	5.30 ± 0.05 c	5.51 ± 0.10 c
	7	6.10 ± 0.08 d	6.14 ± 0.08 d
	14	7.76 ± 0.15 e	7.82 ± 0.20 e

In a column and on a line, the values followed by the same letter are not significantly different (Newman-Keuls test at 5%). Values are the mean of triplicate; ±SD: standard deviation; PAL: phenylalanine ammmonia lyase; dw: dry weight.

TAL activity evolves similarly as those of PAL. In contrast, it was significantly lower regardless of temperature and storage time (Table 3). This result suggests that biosynthesis of phenolic compounds during cold stress would be oriented towards PAL pathway in pineapple [24].

Table 3. Effect of temperature and storage time on TAL activity in pineapple fruit

Storage temperature (°C)	Storage time (days)	Activity of TAL (µkat/min/g dw)	
		Smooth Cayenne	MD2
4	4	1.01 ± 0.01 a	1.10 ± 0.02 ab
	7	1.14 ± 0.01 b	1.17 ± 0.02 b
	14	1.16 ± 0.02 b	1.11 ± 0.01 ab
10	4	1.19 ± 0.01 bc	1.21 ± 0.02 b
	7	1.16 ± 0.03 b	1.13 ± 0.02 ab
	14	1.10 ± 0.02 b	1.05 ± 0.03 a
16	4	1.22 ± 0.01 c	1.14 ± 0.01 ab
	7	1.17 ± 0.02 b	1.11 ± 0.01 ab
	14	1.10 ± 0.02 b	1.07 ± 0.02 a
22	4	1.15 ± 0.02 b	1.06 ± 0.02 a
	7	1.12 ± 0.0 4 b	1.18 ± 0.02 b
	14	1.06 ± 0.04 ab	1.10 ± 0.01 ab
25	0	1.06 ± 0.03 ab	1.15 ± 0.01 ab
	4	1.30 ± 0.02 b	1.21 ± 0.01 b
	7	1.26 ± 0.03 c	1.22 ± 0.01 b
	14	1.18 ± 0.01 b	1.14 ± 0.03 ab

In a column and on a line, the values followed by the same letter are not significantly different (Newman-Keuls test at 5%). Values are the mean of triplicate; ±SD: standard deviation; TAL: tyrosine ammmonia lyase; dw: dry weight.

At 22°C, PPO activity remained statistically equal to that of day 0 irrespective of fruit time storage. For MD2 variety, PPO activity was increased with time storage at temperatures of 4, 16 and 25°C. For both varieties, preservation at 10°C for 14 days gave identical activities to those obtained at 22°C, temperature at which no browning was observed on pineapple fruit. Therefore, the temperature of 10°C would be e best indicated for the conservation of pineapples. This seems to justify the low activity of PAL observed at 10°C (Table 4). Indeed, there would be fewer phenolic substrates available for PPO. These results are consistent with those of Coseteng and Lee [20]. Indeed, they reported the existence of a correlation between TPC and PPO activity in pineapple internal browning. For all storage times at 22°C, PPO activity remained substantially equal to that of control in the two pineapple varieties. This result shows that the increase in PPO activity was due to variation in storage temperature. Furthermore, when pineapple was removed from refrigerated containers, they would have physiological injuries due to the transition from lower to higher temperatures [25]. This causes browning of pineapple flesh [26]. The very low TPC observed at 8°C after 14 days of storage would indicate that this storage time impairs the quality of the pineapple less. So, knowing that maritime transport of pineapples to Europe lasts about two weeks, fruit storage at 10°C would allow the arrival to have pineapples less brown and therefore of good commercial quality.

Catalase, like PPO, was more active in the smooth Cayenne variety than in MD2 variety. In Smooth Cayenne, CAT activity increased with duration for preservation at 4, 10, 16 and 25°C. Regardless to storage at 10°C for 14 days, CAT activity was statistically equal to that of control (day 0) in each variety (Table 5). At 22°C, enzyme activity remains statistically equal to that of control, whatever the storage time. For MD2, CAT activity increased with duration for preservation at 4, 16 and 25°C. In both pineapple varieties, storage at 10°C for 14 days was given identical activities to those obtained at 22°C. This enzyme activity evolution could be justified by its role of detoxification in fruits [27]. Thus, PPO activity seems to be at toxins origin found in the fruits (browning of fruits).

Everything seems to be as if the browning caused by PPOs was automatically cleaned up by CAT's action. Indeed, several factors lead to the generation of reactive oxygen species (ROS) that causes oxidative stress, such as internal browning [28, 29]. Furthermore, hydrogen peroxide (H_2O_2) is a highly involved ROS in plant response to stress, and CAT capable of breaking down this substance is a key enzyme in the ROS-scavenging in plant in plants [30]. Therefore, CAT appears to detoxify plant cells by catalyzing a dismutation reaction where one molecule of H_2O_2 is degraded to H_2O and then another is oxidized to O_2 [31]. The activity of ascorbate peroxidase (AsPerox) increases in both varieties for all of these preservatives at different temperatures.

Table 4. Effect of temperature and storage time on PPO activity in pineapple fruit

Temperature (°C)	Storage time (days)	Activity of PPO (µkat/min/g dw)	
		Smooth Cayenne	MD2
4	4	1.16 ± 0.00 b	1.23 ± 0.02 c
	7	1.44 ± 0.02 d	1.36 ± 0.01 cd
	14	1.54 ± 0.02 e	1.65 ± 0.02 de
8	4	0.93 ± 0.08 a	1.00 ± 0.01 a
	7	1.05 ± 0.02 ab	1.02 ± 0.01 a
	14	1.21 ± 0.02 bc	1.09 ± 0.05 ab
16	4	1.44 ± 0.08 d	1.15 ± 0.01 b
	7	1.55 ± 0.05 e	1.50 ± 0.06 d
	14	1.82 ± 0.01f	1.55 ± 0.05 d
22	4	1.17 ± 0.02 b	1.06 ± 0.07 a
	7	1.20 ± 0.01 bc	1.10 ± 0.05 ab
	14	1.22 ± 0.02 bc	1.05 ± 0.03 a
27	0	1.20 ± 0.03 bc	1.04 ± 0.02 a
	4	1.26 ± 0.02 c	1.25 ± 0.01 c
	7	1.28 ± 0.03 c	1.22 ± 0.01 c
	14	1.97 ± 0.01 f	1.94 ± 0.03 e

In a column and on a line, the values followed by the same letter are not significantly different (Newman-Keuls test at 5%). Values are the mean of triplicate; ±SD: standard deviation; PPO: polyphenol oxidase; dw: dry weight.

Table 5. Effect of temperature and storage time on CAT activity in pineapple fruit

Temperature (°C)	Storage time (days)	Activity of CAT (µkat/min/g dw)	
		Smooth Cayenne	MD2
4	4	0,96 ± 0,02 bc	1,57 ± 0,06 c
	7	0,63 ± 0,01 b	1,61 ± 0,02 c
	14	1,01 ± 0,02 c	2,10 ± 0,01 cd
10	4	0,65 ± 0,01 b	0,67 ± 0,01 ab
	7	0,64 ± 0,01 b	0,50 ± 0,03 ab
	14	0,26 ± 0,01 a	0,31 ± 0,01 a
16	4	0,98 ± 0,01 bc	0,84 ± 0,01 b
	7	0,74 ± 0,01 bc	2,05 ± 0,01 cd
	14	1,72 ± 0,01 c	2,41 ± 0,01 d
22	4	0,30 ± 0,01 a	0,29 ± 0,02 a
	7	1,38 ± 0,01 d	1,38 ± 0,02 c
	14	1,05 ± 0,02 c	1,00 ± 0,03 c
25	0	0,20 ± 0,01 a	0,28 ± 0,02 a
	4	0,90 ± 0,02 bc	0,95 ± 0,01 b
	7	1,46 ± 0,03 d	1,52 ± 0,01 c
	14	1,08 ± 0,01 c	1,24 ± 0,03 c

Data are expressed as mean of three replicates; ±SD: standard deviation; on a line and in a column, means followed by a different letter are significantly different (Newman Keuls at 5%); CAT: catalase; dw: dry weight.

The highest activities are obtained at 4°C after 7 days of storage. Beyond this storage period, AsPerox activity decreases in all temperatures (Table 6). However, AsPerox is more active in MD2 than in Smooth Cayenne. The activity of this enzyme is related to the ascorbic acid content of pineapple fruit [32].

Table 6. Effect of temperature and storage time on AsPerox activity in pineapple fruit

Temperature (°C)	Storage time (days)	Activity of AsPerox (µkat/min/g dw)	
		Smooth Cayenne	MD2
4	4	0.67 ± 0.10 ab	0.72 ± 0.02 cb
	7	1.54 ± 0.11 cd	1.69 ± 0.03 d
	14	0.99 ± 0.03 b	1.55 ± 0.02 c
10	4	1.56 ± 0.03 cd	1.50 ± 0.01 c
	7	1.66 ± 0.08 d	1.52 ± 0.03 c
	14	1.14 ± 0.06 bc	1.07 ± 0.00 bc
16	4	1.14 ± 0.01 dc	0.43 ± 0.00 a
	7	1.83 ± 0.02 d	1.58 ± 0.00 c
	14	1.35 ± 0.03 c	1.49 ± 0.03 c
22	4	0.82 ± 0.00 ab	0.90 ± 0.03 b
	7	1.48 ± 0.00 c	1.22 ± 0.02 bc
	14	1.16 ± 0.01 bc	1.12 ± 0.00 bc
25	0	0.18 ± 0.03 a	0.63 ± 0.04 ab
	4	0.90 ± 0.02 b	0.95 ± 0.01 b
	7	1.46 ± 0.03 c	1.52 ± 0.01 c
	14	1.08 ± 0.01 bc	1.24 ± 0.03 bc

Data are expressed as mean of three replicates; ±SD: standard deviation; on a line and in a column, means followed by a different letter are significantly different (Newman Keuls at 5%); AsPerox: ascorbate peroxidase; dw: dry weight.

Indeed, Teisson *et al.* [6] showed that the ascorbic acid, which is substrate of AsPerox prevents enzymatic browning by inhibiting PPO activity. These results reveal that MD2 would be richer in ascorbic acid than the Smooth Cayenne [26, 33]. There would therefore be a greater inhibition of browning in MD2 than that in Smooth Cayenne. Asada [34] reported that in the case of plant cells, there is an alternative and more effective detoxification mechanism against H_2O_2, which would complement the action of CAT. This detoxification mechanism is the reduction of H_2O_2 to H_2O by AsPerox [35]. The combined action of these two antioxidant enzymes seems to be necessary to avoid the browning of pineapple after cold stress during transport to Europe. ROS-scavenging enzymes in plants have been extensively studied and the results have shown that AsPerox's activity in response to environmental stress increases with other enzymes, such as CAT [36]. These results highlight the importance to study these enzymes in order to better understand the biological processes that may be related to oxidative stress reactions, such as internal browning in fruits. The main findings showed that storage time and temperature preservation influence strongly the internal browning in pineapple. MD2 seems to resist at cold stress than the Smooth Cayenne. TPC has a relationship to phenolic biosynthesis enzyme such as PAL and TAL. However, phenolic biosynthesis borrows PAL pathway. The internal browning of pineapple observed after cold storage of fruits would have an enzymatic origin. Indeed, it seems to be caused by PPO oxidative action on phenolic compounds. However, detoxification enzymes such as CAT and Asperox appear to have a positive effect on the detoxification of browning cells by decomposing H_2O_2 from stress. They would also inhibit PPOs thereby reducing their oxidizing effect of phenolic compounds. Results obtained to let foresee that the technical itineraries which modulate the activity of the antioxydant enzymes upwards must be taken into account in management of pineapple conditioning towards export. Indeed, these enzymes are strongly implied in internal browning reduction of post-harvest fruit. Such solutions seem to be oriented towards the use of potassium during pineapple crops [37, 38].

CONCLUSION

During postharvest storage, pineapple browns easily and consequently loses much of its commodity value. Such knowledge is of great importance in developing storage strategies designed to maintain pineapple quality in postharvest storage and thus extend its shelf life and pineapple commercial value. Thus, studies of antioxidant enzymes have been carried out in relation to the oxidation enzymes and biosynthesis of phenolic compounds. PPO (the key enzyme of browning) is more active in Smooth Cayenne than the MD2 during storage. This enzyme activity is related to diphenol produced under PAL activity. The results showed that MD2 is more resistant to internal browning than the Smooth Cayenne. Thus, activation of antioxidant enzymes or detoxification enzyme are necessary to reduce or prevent fruit browning. The preservation of pineapples at 10°C for 14 days is better. However, many pineapples from Côte d'Ivoire are refused because of the browning observed after their arrival in Europe. It is possible that cultural techniques, in particular the use of pesticides may be responsible of the increased sensitivity to browning on internal browning. It is therefore essential to seek to reduce the internal browning of the Smooth Cayenne in order to preserve its physicochemical properties after export and thus increase its quality so that it regains its market share on the international plane. This would revive this speculation which has long been the wealth of countries like Côte d'Ivoire and would fight strongly poverty in production areas.

CONFLICT OF INTEREST

The authors state the absence of conflict of interest that could potentially have a relationship with the conduct of this research.

REFERENCES

1. Shoda, M., S., Sakiyama, S., Terakami, F., Hosaka, N., Shigeta, C., Nishitani and T., Yamamoto, 2012. DNA profiling of pineapple cultivars in Japan discriminated by DDR markers. Breed. Sci., 62 (4): 352-359.

2. FAOSTAT, 2016. Pineapple. http://faostat3.fao.org. Accessed July 8, 2017.

3. Mangara, A., A.A. N'Da, K., Traoré, K., M., Khéhé, K., Soro, M., Touré, 2010. Étude phytoécologique des adventices en cultures d'ananas [*Ananas comosus* (L.) Merrill.] dans les localités de Bonoua et N'douci en basse Côte d'Ivoire. J. Applied Biosci., 36: 2367-2382.

4. Workman, D., 2017. Pineapples exports by country. www.worldstopexports.com/pineapples-exports-by-country. Accessed July 8, 2017.

5. FIRCA, 2014. Fonds interprofessionnel pour la recherche et le conseil Agricole, Rapport Annuel, Abj. CI.

6. Teisson C., J.C., Combres, P.P., Martin and J., Marchal, 1979. Internal browning of pineapples fruits. Fruits 34(4): 245-26.

7. Hepton, A., 2003. Cultural System. In: The pineapple: Botany, production and uses (eds D.P. Bartholomew, R.E. Paul and K.G. Rohrbach) pp 109-142. CAB International, New York.

8. Ramon, G. .L and K., Delanie, 2012.Characterization of MD-2 pineapple planting density and fertilization using a grower survey. HortTechnology, 22(5): 644-650.

9. Sanchez, M.L.A., 2005. MD2 pineapple variety production guide. Pesticide Initiative Programme, Europe-Africa-Caribbean-Pacific Liasson Committee. Brussels, Belgium.

10. Bartholomew, D.P., 2009. MD-2 pineapple transforms the world's pineapple fresh fruit export industry. Pineapple News 16: 2-5.

11. Cheriot, S., 2007. Rôle des produits de la réaction de Maillard dans l'inhibition de l'oxydation enzymatique des phénols et des lipides. Thesis, Inst. Sci. Indust. Vivant Env., Agro Paris Tech., France.

12. Schoner, S., H., Krause, 1990. Protective systems against active oxygen species in spinach: response to cold acclimation in excess light. Planta, 180: 383-389.

13. Baker, C., E., Orlandi, 1995. Active oxygen in plant pathogenesis, Annu. Rev. Phytopathol., 33: 299-321.

14. Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance, Trends Plant Sci., 7: 405-410.

15. Antonio, G.S., C.T. Luiz, B., Neide and F.D.S.S., Luis, 2004. Reduction of internal browning of pineapple fruit (*Ananas comusus* L.) by preharvest soil application of potassium. Posth. Biol. Technol. 35: 201-207.

16. Kouakou, T.H., E.A. Due, N.E.J.P. Kouadio, S. Niamke, Y.J. Kouadio, T.P. Waffo, A. Decendit and J.M. Merillon, 2009. Purification and characterization of cell suspensions peroxidase from cotton *(Gossypium hirsutum* L.). Applied Biochem. Biotechnol., 157 : 575-592.

17. Regnier, T., 1994. Composés phénoliques du blé dur (*Triticum turgidum* L. var durum) ; Variation au cours du développement et de la maturation du grain relation avec l'apparition de la moucheture. Thesis, Univ. Montpellier II, France.

18. Cheng, G.W. and P.J., Breen, 1991. Activity of phenylalanine ammonia-lyase and concentrations of anthocyanins and phenolics in developing strawberry fruit. J. Amer. Soc. Hort., 116(5): 865-869.

19. Berner, M., D., Krug, C., Bihlmaier, A.,Vente, R.Muller and A., Bechthold, 2006. Genes and enzymes involved in caffeic acid biosynthesis in Actinomycete *Saccharothrix espanaensis*. J. Bacteriol., 188: 2666-2673.

20. Coseteng, M. and C.Y., Lee, 1987. Changes in apple polyphenol oxidase and polyphenol concentrations in relations to degree of browning. J. Food Sci., 52: 985-989.

21. Aebi, H., 1984. Catalase *in vitro*. Methods Enzymol., 105: 121- 126.

22. Dorival, M.A. and M., Ann, 2014. Catalase activity is stimulated by H_2O_2 in rich culture medium and is required for H_2O_2 resistance and adaptation in yeast. Redox Biol., 2: 308-313.

23. Nakano, Y. and K., Asada, 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in Spinach chloroplasts. Plant Cell Physiol., 22: 867-880.

24. Yeoh, W.K. and A., Ali, 2017. Ultrasound treatment on phenolic metabolism and antioxidant capacity of fresh-cut pineapple during cold storage. Food Chem., 216:247-253.

25. Cantwell, M. and T. Suslow, 2007. Cabbage (round and Chinese types). In Technology Research and Information Center, Department of Plant Sciences, University of California, Davis, CA, USA.

26. Meheriuk, M., R.K., Prange, P.D., Lidster and S.W., Porritt, 1994. Affections après récolte de la pomme et de la poire. Agriculture et Agroalimentaire Canada, publication n° 1737F, pp. 27-31.

27. Kirkman, H. N. Rolfo, M. Ferraris, A. M. Gaetami, G.F., 1999. Mechanisms of protection of catalase by NADPH. Kinetics and stochiometry. J. Biol. Chem., 274: 13908-13914.

28. Hameed, A., M.A., Sheikh, 2007. Changes in catalase, peroxidase activities and soluble proteins in wheat le8aves on thiourea and H2O2 treatments. Biosci. Res., 4: 21-27.

29. Mhamdi, A. G., Queval, S., Chaouch, S., Vanderauwera, F., Van Breusegem and G., Noctor, 2010. Catalase function in plants: A focus on Arabidopsis mutants as stress-mimic models. J. Exp. Bot., 61: 4197-4220.

30. Demidchik, V., 2015. Mechanisms of oxidative stress in plants: From classical chemistry to cell biology. Environ. Exp. Bot., 109: 212-228.

31. Mhamdi, A., G., Noctor and A., Baker, 2012. Plant catalases: Peroxisomal redox guardians. Arch. Biochem. Biophys., 525: 181-194.

32. Raven, E.L., L., Lad, K.H. Sharp, M. Mewies and P.C., Moody, 2004. Defining substrate specificity and catalytic mechanism in ascorbate peroxidase. Biochem. Soc. Symp., 71: 27-38.

33. Wisdom, W., K.S., Firibu, S., Matilda, S.B., Agnes and S., Samuel, 2009. A comparison of some physical, chemical and sensory attributes of three pineapple (*Ananas comosus)* varieties grown in Ghana. Afr. J. Food Sci., 3(1): 22-25.

34. Asada, K., 1992. Ascorbate peroxidase - a hydrogen peroxide-scavenging enzyme in plants. Physiol. Plant., 85 (2) : 235-241.

35. Anjum, N.A., P.,Sharma, S., Gill, M., Hasanuzzaman, E.A., Khan, K., Kachhap, A., Mohamed, P., Thangavel, G., Devi, P., Vasudhevan, A., N., Khan, A., Misra, A., Lukatkin, H., Singh, E., Pereira, N., Tuteja, 2016. Catalase and ascorbate peroxidase-representative H2O2-detoxifying heme enzymes in plants. Environ. Sci. Pollut. Res. Int., 23(19): 19002-19029.

36. Shigeoka, S,. T., Ishikawa, M., Tamoi, Y., Miyagawa, T., Takeda, Y., Yabuta Y and K., Yoshimura, 2002. Regulation and function of ascorbate peroxidase isoenzymes. J. Exp. Bot., 53: 1305-1319.

37. Nanayakkara K.P.G.A., H.M.W. Herath, Y.D.A. Senanayake, 2002. Effects of pre-harvest treatments of potassium, post-harvest treatments of calcium, potassium, abscisic acid and light on reducing internal browning in pineapple (*Ananas comosus* (L.) Merr. cv mauritius) under cold-storage. Acta Horticult., 666(666): 321-327.

38. Soares, G.A., L.C., Trugo, N., Botrel, L.F.S., Souza, 2005. Reduction of internal browning of pineapple fruit (*Ananas comusus* L.) by preharvest soil application of potassium. Posth. Biol. Technol., 35(2): 201-207.