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Changes in Some Biochemical Ripening Parameters as Related to the Formation of Dark-colored Pigments in the Peel of Banana Fruits

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ABSTRACT

The study was undertaken to determine the effects of palm kernel or coconut oil fractions on the ripening of banana fruits. Palm kernel oil and coconut oil were fractionated using column chromatography. Green mature banana fruits were anointed with 100 μL of oil fractions. Three oil fractions in each case induced a blackening of the peel of bananas. The blackened fruits still contained considerable amounts of chlorophylls while control bananas had negligible levels of green pigments in the peel. The content of total carotenoid was higher in the peel of most of dark-colored fruits than in the peel of controls. Qualitative analysis of carotenoid revealed the exceptional presence of reddish pigments in the peel of blackened bananas. A transient accumulation of proteins was observed in the pulp of banana fruits treated with oil fractions that induced a blackening of fruits. The pH slightly decreased in the peel and pulp extracts from dark-colored fruits. Treatments with oil fractions that did not induce a blackening of the peel of bananas did not remarkably influence the parameters measured. These results indicated that some constituents of palm kernel oil and coconut oil could serve as intermediates in the formation in aqueous environment of the peel of dark-colored pigments which might induce alterations in metabolic processes in ripening banana fruits.

Key words: Banana fruits, blackening, metabolic disturbance, pigments, ripening

INTRODUCTION

Fruit ripening evolved a complex network of metabolic processes that include pigment, carbohydrate and aroma metabolism. Although ethylene is generally considered as the main triggering factor, an interaction between different hormones is necessary for the control and regulation of the fruit ripening process. Like in other natural biological processes, the mechanisms of hormone action on fruit ripening are based on the regulation of gene expression through a cascade of signal transduction from membrane receptors (Luettge *et al*., 2002). Thus, any alteration in the chemical nature of membranes may lead to modifications in the response of tissues or organs to the hormonal signals. In this context, it has been reported that short chain saturated fatty acids might affect ethylene action in flower abscission of the ethylene-overproducer Arabidopsis mutant (Friedman *et al*., 2003). Furthermore, coating of banana fruits with crude Palm Kernel Oil (PKO)

or coconut oil (CNO) induced a prolongation of their shelf life (Aghofack-Nguemezi *et al*., 2006). When coating with these oils was simultaneously associated with treatments by dipping in solutions of calcium chloride or magnesium sulfate at different concentrations, there was a synergistic retarding effect of oil and divalent cations on the ripening of mature green banana fruits (Aghofack-Nguemezi and Dassie, 2007). Moreover, although singly treatments with CNO or PKO had retarding effects on the ripening, simultaneous treatments with auxins at concentrations $\geq 10^{-4}$ M and any of the two oils rather accelerated this process in banana fruits (Aghofack-Nguemezi *et al*., 2008).

Palm kernel nut and coconut enclose phenolic compounds and carbohydrates (Atasie and Akinhanmi, 2009; Marina *et al*., 2009). They also contain many complex lipids, free fatty acids and volatile n-alkanes (Akpanabiatu *et al*., 2001; Pantzaris and Ahmad, 2001). Thus, crude PKO and CNO are likely to include various compounds characterized by different chemical and physical properties. The present study was undertaken to examine into the influence of treatments with CNO and PKO fractions obtained using column chromatography on some characteristic parameters of the ripening process in banana fruits.

MATERIALS AND METHODS

Extraction and fractionation of oils using column chromatography: Oils were obtained from the endosperm of palm kernel and coconut after grinding and squeezing. 45 g of each oil was dissolved in 300 mL hexane. Thereafter, 50 g of silica gel (ø = 0.063-0.2 mm) were added to the oil solution. Hexane was then removed using a rotary evaporator (Büchi R200) at 50% under reduced pressure. This preparative gel on which oil has been fixed was sequentially spread on the slab and introduced in an oven at 70°C for complete evaporation of hexane. The separation gel was prepared by introducing 230 g of silica gel into a beaker containing 400 mL of hexane. The separation gel was then introduced into a glass column with enough hexane followed by the addition of the preparative gel. Cotton wet with hexane was finally put at the top of the preparative gel to stabilize the column. The elution of the loaded column was done using sequentially hexane, ethyl acetate and methanol. The two oils and their fractions were diluted using hexane to give different stock solutions of 100 μg mL^{-1}.

Thin layer chromatography oils and their fractions: Ten microliter of oil solutions and their fractions were deposited on chromatographic aluminum sheet precoated with silica gel 60 F_{254} (Merck, Germany). This thin layer chromatographic plate was then eluted using a solvent system comprising hexane and ethyl acetate (95/5, v/v). After completion of the chromatography, the plate was dried under concentrated hot air. The different spots were sequentially revealed using UV light at 254 and 365 nm and spray with sulfuric acid.

Treatment of banana fruits and assessment of ripening stages: *Musa acuminata* Colla var. William fruits were donated by the High Penja Plantation Company in Jombe, Cameroon. Banana fruits of second hands of freshly harvested bunches were collected in order to reduce variations and obtain consistent data according to Dadzie and Orchard (1997). They were either anointed or not with 100 μL of coconut or palm kernel oil. Untreated fruits were used as controls. Control and treated bananas were packed in transparent polythene bags. The different stages of ripening were visually accessed according to the banana color stage chart proposed by Dadzie and Orchard (1997).

Banana fruits at stage 1 (green, unripe), 2 (start of ripening and visual color change), 4 (yellowier than green) and 7 (yellow, slightly flecked) of ripening of control fruits were used for the determination of the contents in water, total proteins, pigments (chlorophyll a, chlorophyll b and total carotenoid) and of the pH.

Determination of water, pigments, total proteins contents and the pH: Fresh peels and pulps of banana fruits were weighed and dried in an oven at 105°C for 24 h. they were then weighed and the water content was calculated using fresh and dry matter weight according to Chapman (1976).

For the determination of pigment content two grams of banana peels were ground and extracted with 7.2 mL acetone. The homogenate was allowed to stand for 10 min and filtrated. The filtrate was centrifuged for 10 min at 1000 rpm. Optical densities of each supernatant were measured at 470, 647 and 663 nm. The concentrations of chlorophyll a, chlorophyll b and total carotenoids were calculated using the formulae described by Lichtenthaler (1987).

The quantification of total proteins was done according to Bradford (1976). The pH was determined in aqueous extracts from banana peels and pulps.

Statistical analysis: Group comparisons were made using One-Way-Analysis of Variance (ANOVA) to see if variations among the means were significantly different than expected by chance. The Student-Newman-Keuls Test (Garcao and Mattioli, 2009) was used to compare means differences, whereby a p-value of <0.05 was considered as statically significant.

RESULTS

Five fractions were obtained from the column chromatography of each oil. The Ratio of Front (RF) of spots on the thin layer chromatogram showed that the two first fractions of CCO might have similar chemical nature. The fraction 3 of PKO (FP3) should have almost the same chemical composition as the fraction 3 of CCO (FC3). Some fractions from both oils had the same number of spots, but the RF values of these spots were different. Some spot belonging to different fractions had similar RF values (Table 1).

The green color of the peel of bananas turned quickly into black after treatment with the oil fractions FC3, FC4, FC5, FP3, FP4 and FP5. Thin Layer Chromatograms (TLC) of extracts from the peel of control green-unripe banana fruits revealed the presence of chlorophyll and different carotenoids. The extracts from peel of control fully ripe yellow banana fruits contained only

Table 1: Number and Ratio of Front (RF) of oil fractions on thin layer chromatogram

	Coconut oil		Palm kernel oil	
Fraction No.	No. of spots	RF values	No. of spots	RF values
1	1	0.63	1	0.68
2	1	0.64	1	0.87
3	2	0.5; 0.77	2	0.53; 0.80
4	2	0.41; 0.66	3	0.38; 0.51; 0.8
5	2	0.38; 0.75	1	0.8

RF: Ratio of front

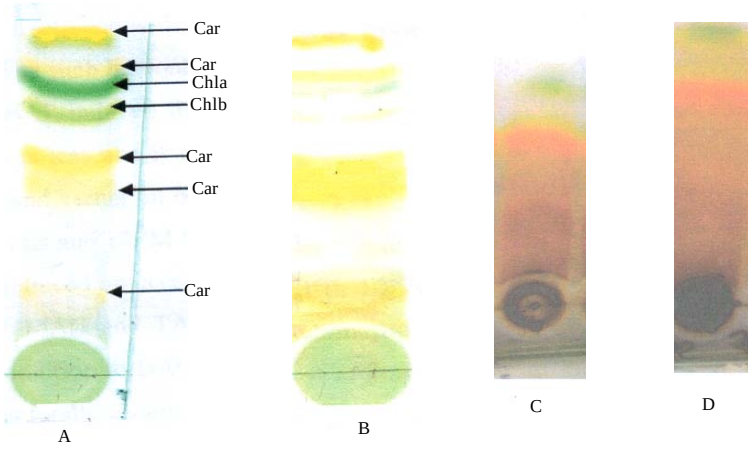

Fig. 1: Thin layer chromatogram of extracts from the peel of control green-unripe banana fruits (A), control fully ripe fruits (B) and blackened fruits treated with the fraction number 3 of palm kernel oil (C) or coconut oil (D). Car, carotenoid; chl a, chlorophyll a; chl b, chlorophyll b

carotenoids and no more chlorophyll. At this stage 7 of ripening however, extracts of the peel of banana fruits treated with oils fractions that were responsible for the blackening were still containing chlorophyll. Furthermore, extracts from the blackened peel also contained some unknown reddish colored pigments. No dark-colored pigment could be found on TLC of the acetonic extract from the peel of blackened banana fruits (Fig. 1A-D).

The contents of chlorophyll a (chla) in the peel of both control and treated banana fruits dropped significantly throughout the ripening. The level of chla in the peel of bananas treated with FC3 was higher at ripening stages 2 and 4 than control fruits and those found in the peel of fruits that received other treatments. At stage 7 of ripening of control fruits, the contents of chla in the peel of bananas treated with FP3, FP4 and FC5 were higher than those observed in the peel of controls and fruits that were anointed with other oil fractions. There was a significant linear decrease in the chlb content in the peel of banana fruits treated with almost all oil fractions with increasing ripening stage excepted in the peel of fruits that were anointed with FP3 and FP4. In the latter cases, the chl b content remained nearly constant from stage 2 to stage 7 of ripening (Table 2).

Total carotenoid (car) contents increased gradually from stage 1 to stage 7 of ripening in the peel of banana fruits treated with FP3 and FP4, this increase being more pronounced in the peel of fruits treated with FP3. No significant increase in the levels of car with ripening stages could be observed in the peel of control as well as other treated banana fruits. At stage 4 of ripening, the level of *car* was higher in the peel of fruits treated with FP3 than in the peel of control and fruits that were anointed with other oil fractions. At stage 7 of ripening, the peel of banana fruits treated with FP3 or FP4 contained more *car* than the peel of control fruits and the peel of bananas that have been anointed with other oil fractions (Table 2).

The protein content in the pulp of banana fruits treated with FC3 decreased strongly from stage 1 to stage 2 of ripening remained constant between stage 2 and stage 4 and then increased toward stage 7 of ripening. An opposite trend to these changes could be observed in the protein contents

Table 2: Effects of treatments with coconut oil (FC) or palm kernel oil (FP) fractions on the levels of chlorophyll a (chla), chlorophyll b (chlb) and total carotenoid (car) in the peel and proteins in the pulp of banana fruits at different ripening stages (1, 2, 4 and 7). Values are means of at least three replications

	Treatments							
Parameter and ripening stage	Control	FC1	FC2	FC3	FC5	FP1	FP3	FP4
Chla [µg g^{-1}]								
1	135.7^{a}	135.7^{a}	135.7^{a}	135.7^{a}	135.7^{a}	135.7^{a}	135.7^{a}	135.7^{a}
2	39.7^{a}	46.8^{a}	47.4^{a}	99.8^{d}	76.7^{bcd}	53.3^{abc}	51.0^{ab}	77.9^{cd}
4	11.9^{a}	29.4^{ab}	36.6^{bc}	78.8^{d}	44.3^{bc}	46.9^{bc}	41.0^{bc}	42.8^{c}
7	1.8^{a}	9.0^{ab}	7.7^{ab}	4.0^{a}	27.2^{bc}	13.0^{ab}	42.7^{c}	39.0^{c}
Chlb [µg g^{-1}]								
1	84.1^{a}	84.1^{a}	84.1^{a}	84.1^{a}	84.1^{a}	84.1^{a}	84.1^{a}	84.1^{a}
2	22.1^{a}	23.4^{a}	23.0^{a}	56.3^{b}	40.1^{ab}	22.4^{a}	46.9^{ab}	42.9^{ab}
4	6.4^{a}	33.2^{bc}	18.6^{ab}	67.6^{bc}	22.7^{ab}	23.9^{ab}	50.3^{cd}	43.5^{d}
7	2.3^{a}	4.1^{a}	3.9^{a}	8.6^{a}	13.0^{a}	5.6^{a}	55.4^{b}	44.1^{b}
Car [µg g^{-1}]								
1	23.4^{a}	23.4^{a}	23.4^{a}	23.4^{a}	23.4^{a}	23.4^{a}	23.4^{a}	23.4^{a}
2	18.1^{a}	18.8^{a}	16.2^{a}	47.6^{b}	14.6^{a}	21.2^{a}	93.7^{c}	56.3^{c}
4	21.5^{ab}	18.4^{a}	32.6^{ab}	42.9^{bc}	18.1^{a}	20.9^{ab}	190.7^{d}	61.6^{c}
7	21.2^{a}	21.2^{a}	20.3^{a}	21.6^{a}	25.2^{a}	20.1^{a}	216.7^{b}	280.9^{c}
Protein [µg g^{-1}]								
1	47.5^{a}	47.5^{a}	47.5^{a}	47.5^{a}	47.5^{a}	47.5^{a}	47.5^{a}	47.5^{a}
2	48.0^{a}	46.8^{a}	48.6^{a}	13.0^{d}	56.1^{ab}	64.4^{abc}	68.6^{bc}	75.3^{c}
4	41.1^{a}	46.1^{a}	67.8a^{b}	13.5^{c}	65.5^{ab}	78.9^{b}	68.9^{ab}	65.0^{ab}
7	45.0^{a}	46.6^{ab}	46.1^{ab}	62.8^{b}	33.9^{ab}	41.1^{ab}	46.1^{ab}	41.1^{ab}

Values bearing different superscript letters within the same row are statistically significant at $p \leq 0.05$

in the pulp of fruits anointed with FP1, FP3, FP4 and FC5. At ripening stages 2 and 4, the protein content in the pulp of fruits treated with FC3 was significantly lower than that found in the pulp of controls and fruits treated with other oil fractions. At these stages, the protein content was higher in the pulp of bananas treated with FC5, FP1, FP3 and FP4 than controls and other treated fruits. At stage 7 of ripening, the pulp of bananas anointed with FC3 had a higher level of protein than that observed in the pulp of controls and other treated banana fruits (Table 2).

Treatments of banana fruits with fractions of CNO or PKO did not generally have a great influence on the water content and pH in both the peel and pulp. Nevertheless, some minor changes could be observed. At the ripening stage 2, the water content was higher in the peel of controls than treated fruits, except bananas anointed with FC1 and FC2. The water content in the peel of banana fruits treated with FC5 was significantly lower than that found in the peel of control and other treated fruits at stage 4 of ripening. There were decreases in water contents in the pulp of fruits anointed with FC5, FP1, FP2, FP3, FP4 and FP5 at stage 4 of ripening as compared to control fruits. At ripening stage 7, the pH values of the peel extracts of bananas treated with FC5, FP4 and FP5 were lower than those measured in the peel extracts of controls and fruits anointed with other oil fractions. At this ripening stage, pH values of the pulp extracts of bananas anointed with FC5, FP3, Fp4 and FP5 were lower than those measured in the extracts of the pulp of other fruits. PH values of both peel and pulp extracts of banana fruits treated with FC2 were higher than those measured in other fruits at the ripening stage 7 (Table 3).

Table 3: Effects of treatments with coconut oil (FC) or palm kernel oil (FP) fractions on the Water Content (WC) and pH in the peel and pulp of banana fruits at different ripening stages (1, 2, 4 and 7). Values are means of at least three replications

Parameter and ripening stage	Treatments										
	Con-trol	FC1	FC2	FC3	FC4	FC5	FP1	FP2	FP3	FP4	FP5
WC [%]											
Peel											
1	90.1^{a}	90.1^{a}	90.1^{a}	90.1^{a}	90.1^{a}	90.1^{a}	90.1^{a}	90.1^{a}	90.1^{a}	90.1^{a}	90.1^{a}
2	91.8^{a}	90.4^{ab}	91.5^{a}	88.9^{b}	89.4^{b}	89.6^{b}	89.8^{b}	89.5^{b}	88.6^{b}	88.7^{b}	88.6^{b}
4	92.1^{a}	90.0^{a}	90.2^{a}	89.9^{a}	88.7^{a}	81.2^{b}	89.6^{a}	89.4^{a}	88.9^{a}	87.4^{a}	89.9^{a}
7	89.6^{ab}	89.5^{ab}	87.0^{a}	91.9^{b}	88.5^{b}	89.1^{ab}	89.2^{ab}	89.8^{ab}	88.9^{ab}	89.7^{ab}	90.1^{ab}
WC [%]											
Pulp											
1	66.1^{a}	66.1^{a}	66.1^{a}	66.1^{a}	66.1$_{a}$	66.1^{a}	66.1^{a}	66.1^{a}	66.1^{a}	66.1^{a}	66.1^{a}
2	64.7^{a}	65.1^{ab}	65.7^{ab}	65.8^{ab}	73.4^{b}	72.1^{ab}	71.6^{ab}	71.3^{ab}	71.2^{ab}	68.6^{ab}	68.7^{ab}
4	74.2^{a}	75.9^{b}	75.4^{b}	74.2^{a}	75.0^{ab}	72.7^{c}	72.1^{cd}	72.5^{c}	71.3^{d}	71.1^{d}	71.1^{d}
7	76.2^{a}	79.9^{a}	75.8^{a}	76.2^{a}	76.4^{a}	77.8^{a}	72.8^{a}	72.3^{a}	72.0^{a}	72.0^{a}	73.2^{a}
pH											
Peel											
1	5.3^{a}	5.3^{a}	5.3^{a}	5.3^{a}	5.3^{a}	5.3^{a}	5.3^{a}	5.3^{a}	5.3^{a}	5.3^{a}	5.3^{a}
2	5.1^{a}	5.2^{a}	5.0^{a}	5.2^{a}	5.2^{a}	5.2^{a}	5.1^{a}	5.2^{a}	5.3^{a}	5.3^{a}	5.3^{a}
4	5.8^{a}	5.7^{a}	5.6^{ab}	5.5^{ab}	5.5^{ab}	5.5^{ab}	5.8^{a}	6.0^{ab}	5.7^{a}	5.9^{a}	5.6^{ab}
7	5.2^{a}	5.2^{a}	5.6^{b}	5.2^{a}	5.2^{a}	4.2^{d}	5.2^{a}	5.1^{a}	5.0^{a}	4.9^{c}	4.9^{c}
pH											
Pulp											
1	5.6^{a}	5.6^{a}	5.6^{a}	5.6^{a}	5.6^{a}	5.6^{a}	5.6^{a}	5.6^{a}	5.6^{a}	5.6^{a}	5.6^{a}
2	4.2^{a}	4.3^{a}	4.7^{b}	4.3^{a}	4.4^{ab}	5.0^{c}	4.5^{ab}	4.3^{a}	5.0^{c}	4.9^{c}	5.1^{c}
4	5.6^{a}	5.5^{a}	5.3^{ab}	5.1^{ab}	5.2^{ab}	5.2^{ab}	5.4^{ab}	5.5^{ab}	5.3^{ab}	5.3^{ab}	4.2^{c}
7	5.4^{a}	5.2^{a}	5.6^{b}	5.1^{ab}	5.3^{ab}	4.6^{c}	5.0^{ab}	5.1^{ab}	4.5^{c}	4.5^{c}	4.9^{c}

Values bearing different superscript letters within the same row are statistically significant at $p \leq 0.05$

DISCUSSION

The anointment of green mature banana fruits with some fractions of CNO and PKO induced a rapid and intense blackening of the peel. The blackening reported for the first time here is quite different from browning that occurs in mature-green banana fruits after wounding or removal of the peel and which is a result of the oxidation some phenolic compounds, principally dopamine (John and Marchal, 1995). It is probably rather similar to the blackening of banana peel during storage at ≤4°C. In support to this results, Promyou *et al.* (2008) reported that the early peel blackening at low temperature of banana cultivar Hom Thong was correlated with increased lipoxygenase activity, which may be the cause of blackening and increased cathecol oxidase activity, which is apparently involved in the blackening reaction; peel blackening in the banana cultivar Namwa, in contrast, was not correlated with any of both parameters measured.

No black spot could be found on TLC of extracts from the peel of the blackened fruits (Fig. 1). The dark-colored pigments could then not be extracted together with chlorophylls and carotenoids using acetone. This is an indication that those pigments involved in the darkening of the peel of bananas after treatment with some oil fractions should strongly be either hydrophilic or non-polar. This corroborated with the finding that palm kernels have a thin testa that contains phenolic compounds (Sreedhara *et al.*, 1992; Ramaswamy and Rege, 1976). It is well established that the oxidation of certain phenolic compounds in an aqueous medium generate complexes derivatives

which take on dark color at low concentrations (Mijangos *et al*., 2006; Slattery *et al*., 1982). Besides phenolic compounds, anthraquinones can also serve as intermediates in formation of dark-colored pigments (Saiz-Jimenez *et al*., 1975). Furthermore, it has been shown that the amount of even numbered n-alkanes (C_{22}-C_{32}) was significantly higher in darker colored beeswax as compared to light colored beeswax (Namdar *et al*., 2007). N-alkanes were among volatile compounds found in heated samples of coconut oil (Pai *et al*., 1979). Contrarily to beeswax, the presence of anthraquinones has not yet been reported neither in palm kernel oil nor in coconut oil. Thus, PKO and CNO fractions responsible for the blackening of banana peels might contain phenolic substances and volatile *n*-alkanes that could impart the black color to the peel of treated banana fruits. Oil fractions that induced the blackening of the peel of fruits subsequently induced a delay in the breakdown of chlorophylls and a stimulation of the accumulation of the total carotenoid in the peel (Table 2). In support to these results, it is well known that phenolic compounds, vitamin C and vitamin E have strong antioxidative properties (Halliwell, 2007; Kountouri *et al*., 2007; Torres *et al*., 1999; Postaire *et al*.,1997). They can scavenge free radicals that are potentially aggressive and that accelerate the degradation of a great number of biological molecules including chlorophylls a and b, proteins and lipids. Chlorophyll breakdown occurs both enzymatically and by oxidative degradation (Matile and Hörtensteiner, 1999). Phenols present in some of the oil fractions could protect chlorophyll molecules in the peel of banana fruits from non-enzymatic oxidative catabolism. It is not exactly known how phenol compound could influence the enzyme-catalyzed pathway of chlorophyll degradation. Furthermore, the mechanisms whereby phenols could stimulate the biosynthesis of carotenoids during the ripening of banana fruits are not known. Nevertheless, phenolic compounds might play a regulatory role in the action of ethylene and auxin. These two phytohormones play an important role in the control of the ripening of climacteric fruits such as bananas and peaches (Cheng *et al*., 2009; Trainotti *et al*., 2007). Phenolic compounds have also been classified as natural growth inhibitors (Kifeli and Kalevitch, 2003; Kifeli and Kadyrov, 1971). In addition to their individual inhibitory effects, they have been shown to antagonize different plant hormones including abscissic acid and ethylene (Leslie and Romani, 1988; Apte and Lalorava, 1982). Furthermore, phenolic compounds are suggested to inhibit auxin catabolism and regulate its transport (Galis *et al*., 2002; Rubery, 1990). Besides inducing changes in the quality and quantity of pigments in the peel, oil fractions that induced more intensively the blackening of banana peel also induced alterations in protein content and pH in the peel or pulp (Table 3). Thus, most of the metabolic processes in the blackened bananas were seemingly disturbed. Obviously, changes in the biosynthesis and activity of ethylene and auxin as a result of treatments of banana fruits with these oil fractions led to a general metabolic disorder. However, the almost overall altering effect of treatments with these oil fractions could not be attributed only to the presence of phenolic compounds and volatile even-numbered n-alkanes therein (Gopalakrishnan *et al*., 1987; Pai *et al*., 1979). These oils fractions content also non-polar complexes lipids and free fatty acids as reported elsewhere (Wiberg and Bafor, 1995). In order to elucidate the specific role of each constituent of peel blackening inducing oil fractions on the ripening process in banana fruits, further study should be undertaken to purify these constituents and test their individual effect. Treatment with oil fractions that did not induce a blackening of the peel of bananas did not influence remarkably the contents of chla, chlb, car and protein. These oil fractions might thus not contain organic substances that triggered changes in the ripening process when bananas were treated with crude oils. Alterations in the ripening process of banana fruits

after treatments with crude CNO or PKO as previously reported (Aghofack-Nguemezi *et al*., 2006) could also rather be a result of synergistic effects of several different chemical constituents.

CONCLUSION

Palm kernel oil and coconut oil might contain phenolic compounds, even numbered n-alkanes or other intermediates in the formation of dark-colored pigments. Treatments of banana fruits with oil fractions containing these substances induced a rapid intense blackening of the peel and subsequently alterations especially in chlorophyll breakdown and carotenoid biosynthesis, thereby delaying the ripening. This raised the open question as whether there is any determinant and main triggering reaction pathway among several metabolic processes (catabolism of chlorophylls, synthesis of carotenoids, transformation of starch into sugar, cell wall loosening, softening of cell membranes, etc.) that usually accompany ripening in bananas.

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