

Biochemical Origin of Browning during the Processing of Fresh Yam (*Dioscorea* spp.) into Dried Product

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This study was undertaken to follow the kinetics of polyphenoloxidase (PPO), peroxidase (POD), and phenolic compounds during yam blanching at different temperatures and after drying and to identify by high-performance liquid chromatography (HPLC) the main phenolic compounds present in yam products. PPO activity was 50% higher in nonprocessed freeze-dried Florido (*Dioscorea alata*) than in nonprocessed freeze-dried Deba (*Dioscorea rotundata*). It decreased progressively during blanching. Forty-five percent of PPO activity remained after 50 min of blanching at 60 or 65 °C, whereas the POD activity dropped sharply to less than 20% of the initial activity after 10 min of blanching, whatever the blanching temperature. No anthocyanidins could be detected by HPLC at 520 nm in nonprocessed freeze-dried yam. Flavanols with a maximum absorption wavelength (λ_{\max}) at 280 nm and cinnamic acid compounds with 320 nm λ_{\max} were detected. Catechin was identified as the major flavanol with concentrations ranging from 0.26 to 0.41 $\mu\text{M g}^{-1}$ depending on cultivar. One cinnamic compound, ferulic acid, was identified and assessed in both cultivars (0.03–0.04 $\mu\text{M g}^{-1}$). Total phenol, flavanol, and cinnamic contents decreased during blanching independently of temperature whereas some unresolved peaks were detected by HPLC in dried product. The latter was probably due to the consumption of coloring precursors and the appearance of polymerized or complex colored products.

KEYWORDS: Brown index; phenols; polyphenoloxidase; peroxidase; *Dioscorea rotundata*; *Dioscorea alata*

INTRODUCTION

Drying is a traditional process widely used in West Africa, mainly in Benin and Nigeria, to extend the shelf life of yam tubers. The flesh of the yam (*Dioscorea rotundata*) generally used is white or sometimes yellowish, but the flour made from the dried yam and the paste (amala) obtained from the flour turns brown or dark brown during processing (1–4). Browning is thus a serious problem in yam processing (5). It makes products obtained from dried yam less appealing visually and, if pronounced, detracts from the quality of the final product (1, 6–7).

The browning of fresh yam is usually attributed to phenolic compounds (8), while oxidation is catalyzed by polyphenoloxidase (PPO) (2, 9) and/or peroxidase (POD) (10, 11). In addition, nonenzymic reactions such as polymerization and complexation with metals, polysaccharides, or proteins (6, 12) are also suspected. Many polyphenols have been identified in various

yam species, such as (+)-catechin, which may be a substrate of O-diphenoloxidase (13), anthocyanins (cyanidin-3,5-diglucoside, cyanidin-3-monoglucoside, and cyanidin-3-rhamnoglucoside) (14), catecholamine, a leucoanthocyanidin (2), and cinnamic compounds (15).

Previous works have pointed out the relationship between the amala browning and the total phenol (TP) content of the flour (6, 16–17): The higher the phenol content is, the darker the product is. In West Africa, yams are traditionally blanched at intermediate temperature (60–75 °C) (18) before drying. In previous experiments, no significant variation in phenol content was observed during blanching whereas it increased dramatically during drying. At the same time, blanching reduces the activity of polyphenol acting enzymes. Two recent studies reported that POD activity almost disappeared after blanching at 65 °C for 20 min whereas about 50% of PPO activity was retained in the blanched yam tubers (17, 19). This is, however, in contradiction with the findings of Chilaka et al. (10), who reported that purified yam POD had a higher heat stability than purified yam PPO: Incubation at 80 °C for 2 h was required to inactivate the POD completely whereas 30 min at 70 °C was sufficient to inactivate the PPO. It was also reported that the residual PPO

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and POD activity remaining after blanching disappeared completely after drying at 40–50 °C for 5 days (17). There is thus a discrepancy between phenol content and phenol acting enzymes: The former increased when the latter exhibited low or no activity, i.e., during drying. On the other hand, some studies indicated that the browning of products obtained from dried yam may be linked to POD activity in the nonprocessed yam (16, 17, 20). The behavior of polyphenol acting enzymes during yam processing, particularly during blanching, and their role in the browning of dried yam thus still remain unclear.

This study was undertaken to identify the phenolic compounds responsible for the browning during the processing of fresh yam into dried yam, flour, and paste and to investigate the kinetic changes in phenolic compounds and PPO and POD activities associated with blanching.

MATERIAL AND METHODS

Yam Sampling for Blanching Kinetics. Yam tubers from cultivars of *D. rotundata* (Deba) and *Dioscorea alata* (Florido) were harvested at maturity on farms in the Northern region of Benin in December 2001. Only apparently sound tubers with no sign of insect attack were used. They were stored at ambient temperature (26–28 °C) until processed or analyzed.

The tubers were blanched at four temperatures (60, 65, 70, and 75 °C), with five blanching times (0, 7.5, 15, 30, and 50 min at 60 °C and 0, 5, 10, 20, and 50 min at 65, 70, and 75 °C). Each experiment (one cultivar, one blanching temperature, and five blanching times) was duplicated. For each experiment, 3–5 tubers were peeled in a water bath (28 °C) and then sliced into 1 cm × 10 cm parallelepiped using a manual rotative slicer (IITA, Ibadan, Nigeria). After homogenization, three samples of about 50 g were taken and immediately immersed in liquid nitrogen to serve as a blank (i.e., unblanched) sample. The remainder was blanched in a water bath thermostatically controlled at one of the required temperatures. Three samples were taken at each blanching time and treated as above. All samples were then stored at –20 °C, freeze-dried, and ground in a laboratory mill (Janke & Kunkel, IKA-Labortechnik, Germany). The flour was then stored at 4 °C until analysis. The three samples for each experimental point were analyzed separately, and mean values were calculated.

Yam Sampling for Cultivar and Drying Effect. Eight cultivars of *D. rotundata* (Baniouré, Deba, Gnidou, Kagourou, Porchebim, Tam-sam, Terlunto, and Yakarango) were harvested at maturity at the International Institute of Tropical Agriculture (IITA-Benin) experimental farm in December 2000. They were blanched at 65 °C for 20 min and dried at 40–50 °C for 5 days. For this experiment, the tubers were cut into slices (30 mm thick) and then frozen at –20 °C before freeze drying instead of being immersed in liquid nitrogen as above. As described in previous papers (17, 19), tubers were sampled onto nonprocessed freeze-dried, blanched freeze-dried, and blanched oven-dried. Samples were then stored at 4 °C until analysis.

PPO and POD Activity Determination. The PPO activity was measured using the oxygen consumption kinetic with 125 mM catechol as substrate in 0.2 M pH 6.8 phosphate buffer. The concentration of oxygen is registered as a function of time on 1 g of yam flour poured into the catechol solution. The maximum slope of the drop of oxygen concentration was used to calculate PPO activity (16). POD was extracted from 50 mg of yam flour with 1 mL of 0.2 M pH 7.0 phosphate buffer for 15 min at ambient temperature. The discoloration kinetic at 460 nm with 0.1 mL of POD extract and 2.9 mL of aqueous pyrogallol [1% (p/v) pyrogallol, 0.3% (v/v) H₂O₂] was registered, and the POD activity was calculated (16). PPO and POD relative activities were defined as the percentage of actual activity to initial enzyme activity measured for nonprocessed yam.

Phenolic Compounds Analysis. TP Content Determination. TPs were extracted from 350 mg of yam flour with 1 mL of methanol/water (85/15, v/v) for 1 h at ambient temperature. The optical density at 760 nm after reaction with Folin reagent was measured and compared with gallic acid standard solutions (16). Relative TPs were also

calculated as the percentage of the initial TP content in nonprocessed yam for each blanching experiment.

High-Performance Liquid Chromatography (HPLC Analysis). Phenol compounds were extracted by vigorously suspending 250 mg of yam flour in 1 mL of methanol/1.5N HCl (85/15, v/v) and stirring at ambient temperature (27–30 °C) for 30 min. The suspension was then centrifuged at 7000g for 5 min. The supernatant was filtered through 0.45 μm pore size filters, and 100 μL was injected onto the HPLC system. HPLC analyses were carried out on an Altima C18 5U column (250 mm × 4.6 mm). The solvent system used was derived from that of Guyot et al. (21) and consisted of two solvents: solvent A (acetonitrile/acetic acid/water; 3/2.5/95.5) and solvent B (acetonitrile/acetic acid/water; 95.5/2.5/3). The gradient applied was as follows: 0 min, 100% A, isocratic; 0–40 min, 70% A, linear; 40–43 min, 0% A, linear; 43–45 min, 0% A, isocratic; 45–48 min, 100% A, isocratic; 48–55 min, 100% A, isocratic. Absorption at 280, 320, and 520 nm was registered using Kroma System 2000 software (Bio-Tek Instruments). Commercial phenolic standards [*p*-coumaric acid, caffeic acid, ferulic acid, (+)-catechin, epicatechin, gallic acid, chlorogenic acid, catechol, 4-methyl catechol, and pyrogallol] from Sigma (St. Quentin, France) were injected separately and characterized by their retention times. Some standards tentatively identified in samples were coinjected with yam extracts to confirm the identity of the peaks.

Statistical Analysis. A multifactorial analysis of variance was performed with cultivar, blanching temperature, and blanching time as main factors (two cultivars × four blanching temperatures × five blanching times × two replicates) on PPO and POD activities and TP content. Correlation and regression analyses were also performed using Statistf software (ITCF, Boigneville, France).

RESULTS

PPO and POD Activities. The mean value of the PPO activity of the nonprocessed freeze-dried yam calculated from the eight blanching kinetic experiments (four temperatures × two replicates) (blank sample) was significantly higher for Florido than for Deba ($34.0 \pm 8.7 \mu\text{M} [\text{O}_2] \text{min}^{-1} \text{g}^{-1}$ and $21.4 \pm 4.3 \mu\text{M} [\text{O}_2] \text{min}^{-1} \text{g}^{-1}$, respectively). There was, however, great within cultivar variability, with a coefficient of variation of 22–23% for both cultivars. Even after correction in relative activity basis, the coefficient of variation calculated after analysis of variance (with cultivar, blanching temperature, and blanching time as the main factors) remained high (26.5%). No significant cultivar effect was observed ($P > 0.05$) for PPO relative activity during blanching, but both blanching temperature and blanching time significantly affected PPO activity. It decreased progressively during blanching (Figure 1): About 45% of the initial activity remained after blanching for 50 min at 60 or 65 °C whereas only low activity (<20%) remained after 50 min at 70 or 75 °C. The heat of inactivation (ΔH) of PPO was calculated from Arrhenius plotting of these data. It was similar for both cultivars: $189 \pm 11 \text{ kJ mol}^{-1}$ for Deba and $194 \pm 6 \text{ kJ mol}^{-1}$ for Florido.

The evolution of POD was followed only for Deba. It was of $158 \pm 32 \text{ mDO min}^{-1} \text{g}^{-1}$ in nonprocessed freeze-dried tuber and decreased abruptly within less than 10 min whatever the blanching temperature (Figure 2). About 20% of the initial activity remained after 7.5 min at 60 or 65 °C, whereas no activity was observed after 10 min at 70 °C or 5 min at 75 °C.

Phenolic Compounds. As with PPO activity, there was great within cultivar variability in the TP content of the nonprocessed samples. The coefficient of variation was 17% for Deba and 33% for Florido. The mean value calculated from eight experiments was nevertheless significantly higher for Florido than for Deba (5.8 ± 1.9 and $2.0 \pm 0.3 \mu\text{M g}^{-1}$ gallic acid db, respectively). Relative TP content decreased progressively to about 40% of the initial TP content in nonprocessed yam (Figure 3) by the end of blanching for Deba (a) and Florido

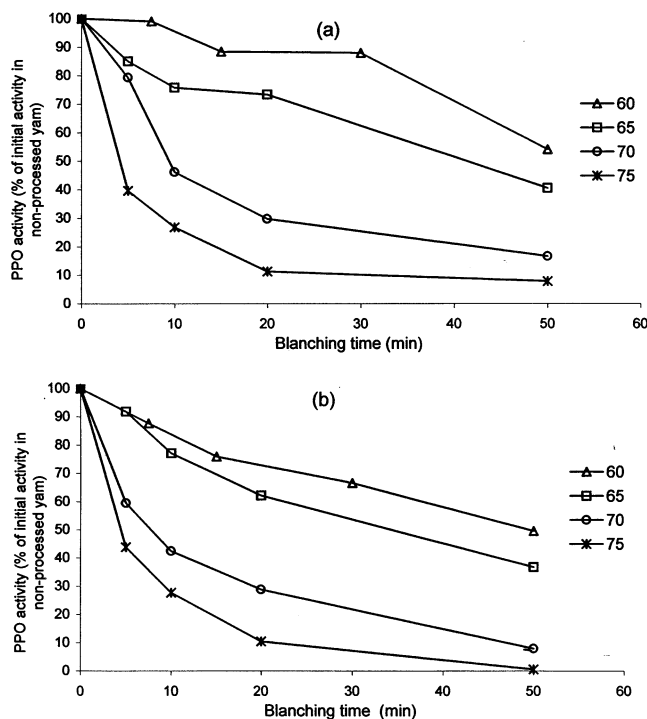


Figure 1. Evolution of PPO activity at different temperatures for Deba (a) and Florido (b).

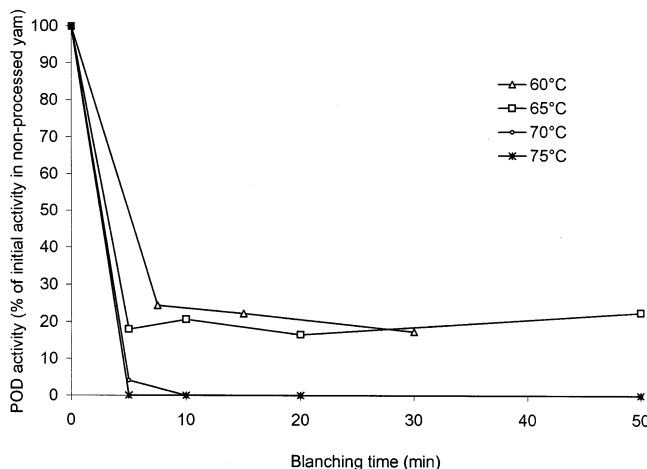


Figure 2. Thermal inactivation curve of POD for the Deba cultivar.

(b), whatever the temperature. No general trend could be identified for the temperature effect. The remaining TPs content were significantly higher ($P < 0.05$) at the start of blanching at 60 °C for Florido (Figure 3b) but not for Deba (Figure 3a).

No anthocyanidins could be detected by HPLC at 520 nm. Only flavanols with maximum absorption wavelength (λ_{\max}) at 280 nm and cinnamic acid compounds with 320 nm λ_{\max} were detected. Figure 4 shows chromatograms recorded at 280 and 320 nm for Deba (a) and Florido (b). Seventeen peaks were detected at 280 nm in Deba and Florido extracts. They were numbered based on their retention times. Among them, a chromatogram from nonprocessed freeze-dried Deba extract showed 15 peaks with four major peaks (6, 12, 13, and 16) accounting for 50% of the total flavanol peak areas. Eleven peaks were detected for Florido including five major peaks (1, 4, 5, 6, and 12), each representing more than 10% of the total flavanol peak area. Thirteen peaks were detected at 320 nm in the extracts of nonprocessed freeze-dried yam and numbered with "b" letter. They included three major peaks (8b, 10b, and

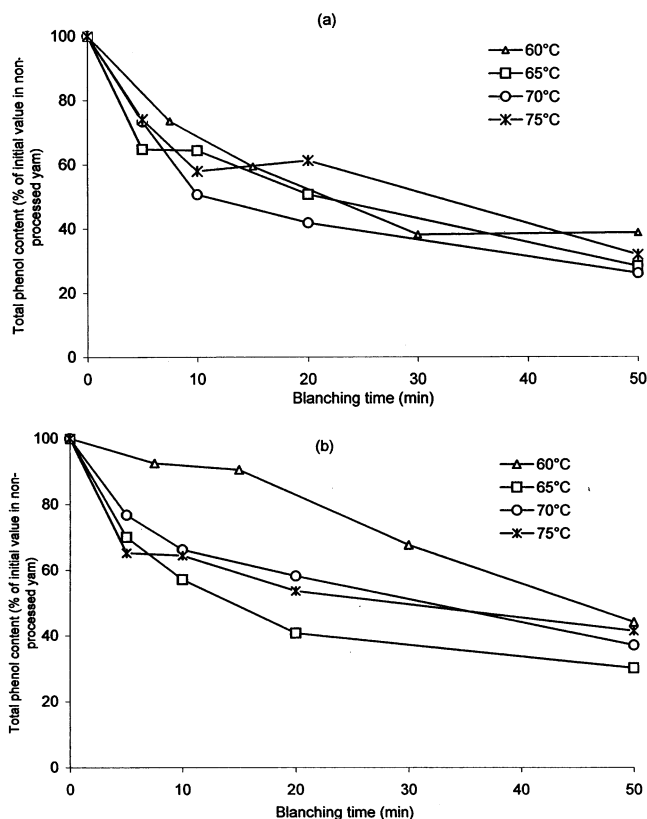


Figure 3. Evolution of TP content of Deba (a) and Florido (b).

11b) for Deba and four for Florido (3b, 4b, 5b, and 8b), representing more than 70% of the total chromatogram area. Except for peak 8, which eluted at 25.3 min, the other major peaks were cultivar specific.

Numerous phenolic standards were injected to try to identify them in yam extracts based on their retention time. Those suspected of being present in yam extracts were coinjected with yam extracts. A single but greater peak observed at the same retention time with the mixed injection confirmed the identity of the component. Among these standards, the only identified flavanol was catechin (peak 6). It was the major peak, with a retention time of 13.1 min, and represented about 16% of the total flavanol peak areas for Deba (*D. rotundata*) and 10% for Florido (*D. alata*). Nonprocessed freeze-dried Deba thus contained $0.26 \pm 0.08 \mu\text{M g}^{-1}$ db of (+)-catechin (14.6% of TP content) vs $0.42 \pm 0.08 \mu\text{M g}^{-1}$ db for Florido (11.7% of TP content). The only identified cinnamic compound was ferulic acid (8b), with a retention time of 25.3 min. It represented about 34% ($0.028 \pm 0.005 \mu\text{M g}^{-1}$ db) of the peak cinnamic areas for Deba and 17% for Florido ($0.037 \pm 0.011 \mu\text{M g}^{-1}$ db).

Peak areas on both detection wavelengths decreased during blanching, and some disappeared completely. Only three of the cinnamic compounds detected in the nonprocessed freeze-dried yam remained assessable after blanching for 50 min. No clear effect of blanching temperature on either flavanols or cinnamic compounds was observed. For Deba, cinnamic compounds decreased markedly during the first 20 min of blanching, followed by a plateau at the end of blanching (Figure 5a,b). For Florido, the ferulic acid (8b) that eluted at 25.3 min disappeared more slowly when blanching was performed at 60 °C than at the other temperatures (65, 70, and 75 °C) whereas (5b) peak was not affected by blanching temperature.

Phenolic compounds were also extracted and assessed by HPLC for the eight cultivars traditionally blanched. Only the

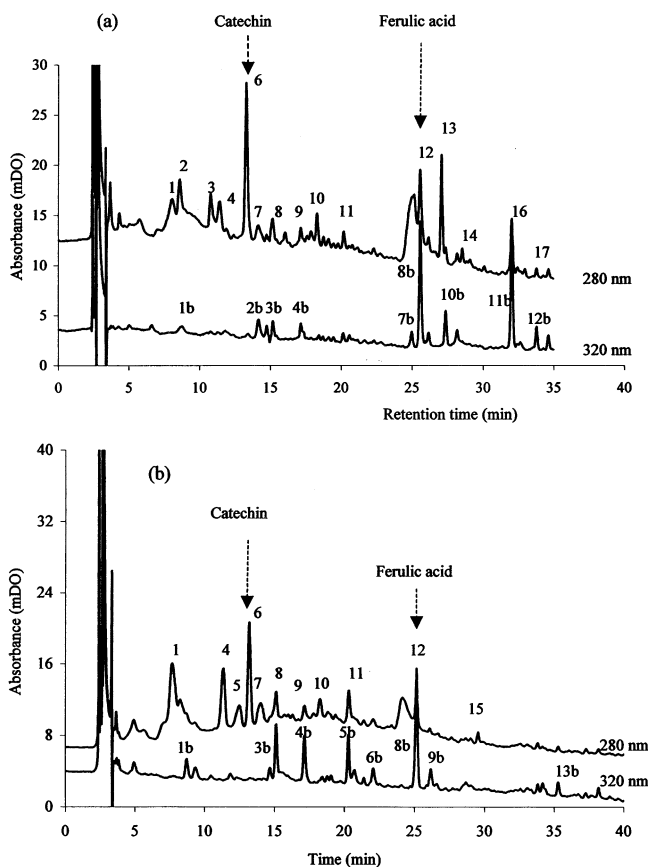


Figure 4. Chromatograms at 280 (upper) and 320 nm (lower) for phenolic compounds extracted from nonprocessed freeze-dried Deba (a) and Florido (b).

results for cinnamic acid compounds (detected at 320 nm) are presented (Table 1). The eight nonprocessed freeze-dried *D. rotundata* cultivars contained the cinnamic compound eluted at 32 min (peak 11b). Ferulic acid (8b) was detected in all cultivars except Gnidou. In most cases, the area of these peaks was less after drying. However, two complex unresolved peaks appeared at 9 and 11.3 min for five of the eight cultivars.

DISCUSSION

The PPO activity of the nonprocessed freeze-dried yams (blank sample) was within the range previously reported (16, 17, 22). No general conclusions can be drawn on cultivar effect as PPO activity varies considerably with season. Ozo and Caygill (22) noted a 50% variation in the PPO activity of *D. alata* and *D. rotundata* cultivars between seasons. In addition, there was great within cultivar variability in PPO activity, despite the precautions taken to obtain a representative sample of each cultivar: 3–5 tubers used for each experiment, randomized by fine slicing and homogenization before subsampling. The variability was, however, within the range previously reported by Isamah et al. (23).

Previous studies realized on purified enzymes reported that yam PPO was largely inactivated after 2–30 min at 60–65 °C, with the activity reduced to less than 20% of the initial enzyme activity in nonprocessed yam (22, 24). In our experiments, PPO activity was measured in situ and around 50% of PPO activity remained after 50 min at 60–65 °C. It can be hypothesized that the yam structure and/or components stabilized the PPO during blanching, preventing its inactivation. Yam PPO was, however, less heat stable than PPO from other food plants such

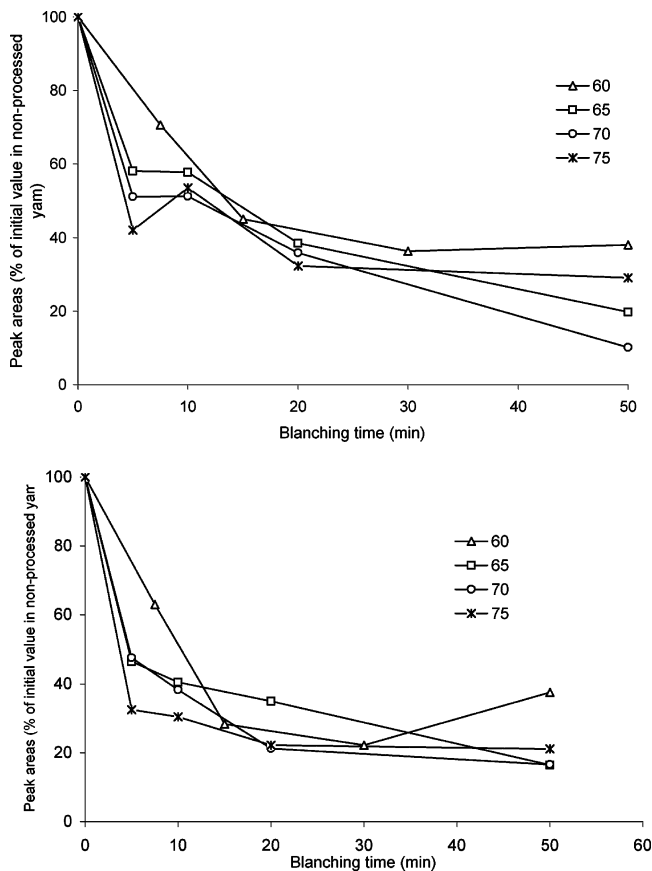


Figure 5. Effect of blanching time on cinnamic compounds extracted from Deba: 8b (ferulic acid, a) and 11b (b) peaks.

Table 1. Peak Areas of Cinnamic Compounds in Nonprocessed Freeze-Dried and Blanched Oven-Dried Flours

yam cultivar	sum of peak area at 9 and 11.3 min (cm ²)		peak area (cm ²)					
			4b		8b		11b	
	NFD ^a	OD ^b	NFD ^a	OD ^b	NFD ^a	OD ^b	NFD ^a	OD ^b
Banioure	0.0	107.1	6.5	0.0	6.2	0.0	31.9	12.3
Deba	0.0	0.0	4.6	11.7	2.3	8.7	16.5	23.8
Gnidou	0.0	0.0	0.0	0.0	0.0	0.0	20.0	8.3
Kagourou	0.0	0.0	0.0	0.0	3.4	0.0	23.6	10.5
Porchebim	0.0	30.5	7.3	16.2	15.0	0.0	22.8	11.1
Tamsam	5.3	90.7	6.2	5.0	16.7	0.0	20.2	11.9
Terlounto	0.0	12.8	0.0	0.0	7.4	0.0	18.3	8.9
Yakarango	0.0	9.3	0.0	0.0	2.5	0.0	12.5	4.9

^a Nonprocessed freeze-dried. ^b Oven-dried.

as banana, the PPO of which was stable at 70 °C for 30 min (25). As the PPO is not completely inactivated, it might nevertheless contribute to browning during the drying stage.

The abrupt decrease in POD activity after 10 min at 60–65 °C reveals that yam POD is less stable than yam PPO. This finding is in contradiction with Chilaka et al. (10), who reported that purified yam PPO was completely inactivated after 30 min at 70 °C whereas 2 h at 80 °C was necessary to inactivate purified yam POD. The same study also reported that purified inactivated yam POD could partially recover its activity after storage at 25 °C for 48 h, whereas this was never observed in our experiments. These discrepancies may be explained by the differences in experimental conditions: Our inactivation experiments were performed in situ, i.e., in a water bath, in the presence of the natural biochemical and structural environment of the enzymes, whereas those of Chilaka et al. (10) were

performed *in vitro*, using purified enzymes incubated in 0.1 M sodium phosphate buffer (pH 7.0) for PPO and in 0.05 M sodium acetate buffer (pH 5.4) for POD (10). It can be inferred that interactions with components of the yam tissue favored irreversible POD degradation whereas the yam tissue offered some protection against PPO degradation. It should be noted in particular that low pH (4.0) prevents yam PPO from the heat denaturation (26) that would normally occur in the cytoplasm where the PPO is located (9). This incidentally implies that the addition of plant leaves (*Piliostigma thonningii*, *Sorghum vulgare bicolor*, *Lophira lanceolata*, and *Tectona grandis*) in the blanching bath in the traditional process (18) might also modify the kinetic of enzymic thermoinactivation even if they are thought to limit heat damage during blanching and to preserve for insect attack during storage.

PPO inactivation energy was at least twice as high as the activation energy measured for *D. bulbifera* (26). Although there were significant differences in their PPO activities, the heat of inactivation of *D. alata* (Florida) and *D. rotundata* (Deba) was very similar. A set temperature can therefore be used for blanching yam, whatever the cultivar. Ige and Akintunde (27) recommended a blanching temperature of 60 °C. If blanched at a lower temperature, the yam pieces darkened within a short time because of residual PPO activity, which we measured at 45%. When blanched at 65 °C or above, the yam pieces were almost cooked and did not present the desired flabby texture, because of starch gelatinization. In fact, traditional Benin yam flours, normally blanched at between 60 and 65 °C, contain a partly gelatinized starch (28). However, the gelatinization onset temperature varies between 70 and 75 °C for *D. cayenensis-rotundata* and *D. alata* cultivars (29). A blanching temperature of between 65 and 70 °C (at or just below the gelatinization onset temperature) might therefore be a good compromise between complete PPO and POD inactivation and starch gelatinization.

TP content decrease during blanching should have been related to phenolic oxidation catalyzed by PPO or POD. As PPO and POD inactivation increased with blanching temperature, the decrease ought to have been less intense and slower at high temperature, but this was not observed. Another explanation for the decrease in TPs may be their solubilization in the blanching bath, as already noted in steeping experiments (19). The high decrease in TP measured during this experiment (60% loss) may therefore be linked to the fineness of the sample size (parallelepipeds with a 1 cm² cross-section) as compared to the 3 cm thick slices used in previous experiments (17), which would favor phenol leaching. It can therefore be hypothesized that two antiparallel phenomena are acting during blanching: enzymic oxidative degradation of the phenols, which is greater at lower temperature before PPO and POD inactivation, and diffusion solubilization, which increases with blanching temperature.

The absence of anthocyanidins was unexpected, as previous studies (2, 14, 30, 31) reported their presence. It is likely that they are in fact present but in a complexed form. Anthocyanidins can present substitutions (such as methyl, hydroxyl, glycosyl, and acyl) on the aromatic skeleton, which move their absorption wavelength from 520 nm (32, 33). We found a higher (+)-catechin content for the *D. rotundata* cultivar (Deba) than reported by Ozo et al. (31) but a lower one for *D. alata* (Florida). In any case, this component was the main polyphenol in nonprocessed yam. It is a good substrate for PPO and can undergo oxidative polymerization to form tannins, so it is a good candidate for explaining yam browning (22, 31).

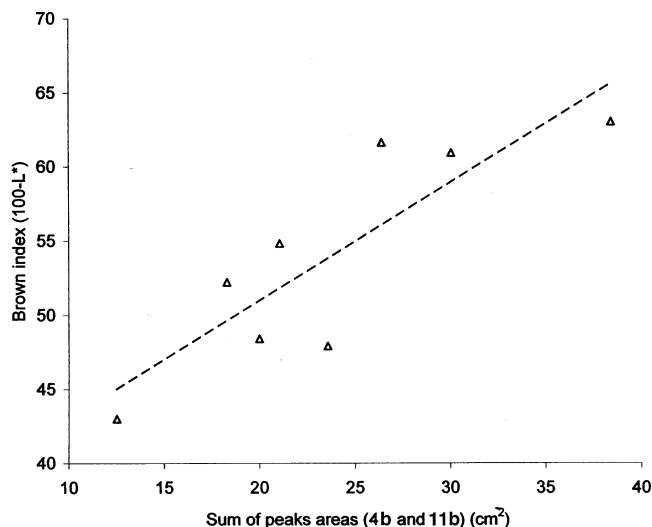


Figure 6. Relation between amala brown index and sum of 4b and 11b peak areas extracted from nonprocessed freeze-dried yams.

Various cinnamic compounds were evidenced in nonprocessed *D. rotundata* and *D. alata* cultivars. Most of them seemed specific for a particular species, but ferulic acid was one of the most widely distributed and important. This confirmed the large distribution and variability of cinnamic compounds in yam species (14). Fewer cinnamic peaks were evidenced after drying although TP content increased (17). This discrepancy may be due to the polymerization and/or complexation of oxidized polyphenols (34, 35), leading to peaks that are unresolved even though they are present. This is consistent with the results of Guyot et al. (21) and Ozo et al. (31), who observed chromatograms showing alterations of peaks (particularly in the case of catechin and chlorogenic acid), which were partly converted into secondary peaks detectable after thiolysis. We did in fact observe the appearance of complex unresolved peaks (at 9 and 11.3 min) in most cultivars after drying. The sum of areas of these peaks could be positively correlated ($r = 0.8$) with amala brown index determined in a previous study (17). A positive correlation was also evidenced between the amala brown index and the areas of some peaks detected at 320 nm in nonprocessed freeze-dried yam. Particularly the sum of peaks 4b and 11b areas could explain 73% of the amala brown index (Figure 6). Those peaks might therefore be precursors of the brown compounds formed during processing and particularly after drying.

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