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Browning susceptibility of new hybrids of yam (*Dioscorea alata*) as related to their total phenolic content and their phenolic profile determined using LC-UV-MS

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ABSTRACT

In the French West Indies, to cope with the yam disease anthracnose, new hybrids were selected for their resistance to this disease. However, some of them have quality flaws. The new hybrids of *Dioscorea alata* exhibited contrasted susceptibility to browning in relation to their total phenolic content ($r = 0.91$). The detailed polyphenol profiles of "INRA15", highly susceptible to browning, and of "Kabusah", with moderate susceptibility to this flaw, were achieved by HPLC coupled to UV–Visible and mass spectrometry. For the first time, total procyanidins of yam were finely characterized and quantified using HPLC after phloroglucinolysis, revealing that those compounds are by far the main polyphenols in the two cultivars. Differences in terms of browning susceptibilities of the two cultivars are clearly explained by their contrasted polyphenol profiles: (i) absence *versus* presence of catechin which is a substrate of polyphenol oxidase (PPO). - (ii) significant differences in procyanidin levels and in their average degree of polymerization potentially involved in PPO inhibition.

1. Introduction

Worldwide, 73 million tons of yam are produced each year, 97% of which is produced in West Africa (FAOSTAT, 2021). In this area, this staple food is therefore an essential source of energy for the 300 million people who consume it daily (Abiodun & Akinoso, 2015). In the French West Indies (FWI), namely Guadeloupe and Martinique islands, this tuber crop is highly appreciated by consumers but its production has declined by more than 90% since 1983 (Agreste, 2009, 2018). This decline is linked to fungal diseases, such as leaf anthracnose, environmental constraints in some production areas (soil pollution with chlordecone), economic issues (competition from imports) and shifts in consumers' habits.

Over the past twenty years, many changes in human diets have occurred due to the globalization of food and lifestyle (Martins, Barros, & Ferreira, 2016). These changes heavily affect tropical countries, especially their urban populations. They generally induce a higher consumption of foods, often imported, with high energy density but poor in micronutrients (Faller & Fialho, 2009). These imbalanced diets lead to an increase in metabolic disorders in humans (Martins al., 2016). Among the metabolic disorders, many people in some tropical and sub-tropical regions are overweight or suffer from obesity. In fact, 40% of the population is overweight in South America, North Africa and the Middle East (Rinaldo, 2020). Increasing the production and consumption of yams of good organoleptic and nutritional quality would increase food security and have beneficial effects on health (Chandrasekara & Kumar, 2016).

The organoleptic, nutritional and functional qualities of yam are scarcely documented. From a nutritional point of view, most studies have dealt with the characterization of starch of various varieties and species (Amani N'Guessan, Buléon, Kamenan, & Colonna, 2004; Tetchi, Rolland-Sabaté, Amani N'Guessan, & Colonna, 2007). Regarding phenolics, minerals and alkaloids, in most cases, only global assays measuring their total contents have been reported in yam. The total phenolic content of various cultivars was measured in relation to domestic cooking (Abiodun & Akinoso, 2015; Chen & Lin, 2007). Little interest has been drawn on the detailed phenolic profile of this tuber. In *Dioscorea (D.) alata*, sinapic and ferulic acids have been identified by HPLC-UV-MS (Akissoé, Mestres, Hounhouigan, & Nago, 2005; Fang et al., 2011) and chlorogenic and gallic acids were found using HPTLC (Lebot, Malapa, & Molisale, 2018). A few authors have studied the anthocyanin composition of colored (purple) cultivars and identified

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four of them as cyanidin or peonidin acylated glycosides (Fang et al., 2011). The presence of catechin and procyanidin dimers B1 and B3 was demonstrated in the pulp of this species (Lebot et al., 2018; Ozo, Caygill, & Coursey, 1984). With the exception of the aforementioned data, knowledge is still very scarce about yams regarding their composition in flavanols including catechins and their oligomer and polymer forms known as proanthocyanidins or condensed tannins, respectively. From an organoleptic and functional point of view, various varieties from West Africa were evaluated for their suitability to be processed while maintaining their quality. However, the varieties, the consumption patterns and the processing methods taken into account in these studies are specific to this region and do not correspond to those most frequently used in the Caribbean-Latin America zone.

To cope with anthracnose, new resistant hybrids have been selected at INRAE Guadeloupe since the 1980s. However, some of them have quality flaws, such as sensitivity to browning when cut. This sensitivity in yam is considered a major quality defect, that may result in rejection by consumers in West Africa, China and the FWI (Honfozo et al., 2021; Njoh Ellong, Billard, Pétro, Adenet, & Rochefort, 2015). Furthermore, little work has been devoted to the determining characteristics for organoleptic quality such as browning. Developing knowledge regarding the mechanisms of browning susceptibility in yam is necessary in order to be able to integrate objective quality criteria in the varietal selection programs. Our study aims to examine whether the susceptibility of some new hybrids of *D. alata* to browning when cut is linked to their total phenolic content and/or their phenolic profile.

2. Materials and methods

2.1. Materials

2.1.1. Description of plant material

The experiment was performed in Guadeloupe, an island of the French West Indies located at 16°15 of North Latitude and 61°35 of West Longitude. Six cultivars of yam (*D. alata*) were produced by the experimental farm of INRAE in Petit-Bourg in Guadeloupe. Five of them were new hybrids selected for their resistance to anthracnose (AL51, AL56, Caribinra (X17), Gwadinra (X142) and INRA15) and one, called Kabusah, was a commercial cultivar. This latter cultivar is amongst the most widely used by yam producers in Guadeloupe (Penet et al., 2016). These six cultivars were used to assess their browning susceptibility as related to the total phenolic content of their pulp.

2.1.2. Sample preparation

Yam tubers were harvested in Guadeloupe eight to nine months after planting, which corresponds to the commercial harvest period. After harvest, tubers were stored in an air-conditioned room at 23 °C for a maximum of one month.

For each cultivar, five tubers of weight close to the average tuber weight were chosen to assess their browning susceptibility. Within the month following harvest, three tubers of each cultivar were peeled and cut manually in small pieces that were frozen in liquid nitrogen and kept at −80 °C pending analysis. The other two tubers were cut longitudinally and immediately used to measure the color attributes and the browning index, within 10 min after cutting.

2.1.3. L* a*b* values and browning index

The color attributes (L*, a*, b*) were determined using a chromameter (Minolta, CR-300, Japan). They were measured at nine spots on each tuber. L* is the luminance, varying from 0 to 100. The browning of the pulp is positively correlated to the 100-L* parameter, as showed by Mestres, Dorthe, Akissoé, and Hounhouigan (2004).

Whiteness index (WI) was calculated according to Hsu, Chen, Weng, and Tseng (2003), as follows:

$$WI = 100 - ((100 - L^*)^2 + a^{*2} + b^{*2})^{0.5}$$

Eight trained panelists visually rated the Browning Index (BI), immediately after cutting, on a scale of 1–5. The scale was adapted from the one used by Nguyen, Ketsa, and Van Doorn (2003) to assess the intensity of surface browning due to chilling injury in banana. The scale we used was as follows:

1: No browning; 2: slight browning; 3: browning; 4: severe browning; 5: very severe browning. Taking into account our browning susceptibility data, two highly contrasted cultivars of yam, namely INRA 15 and Kabusah, were used to establish their phenolic profile. INRA15 is a new hybrid highly susceptible to browning when cut, whereas Kabusah exhibits a moderate susceptibility to browning (Fig. 1; Table 1).

2.1.4. Sample preparation for the determination of the phenolic profile

To determine their phenolic profile using HPLC-UV-MS, three extra tubers of the two contrasted cultivars were sent to the PRP Team (INRAE, UR 1268 BIA, Le Rheu, France) as fresh tubers. Sample preparation was performed as described, prior to HPLC analysis.

This fresh material was also used in two conditions either favoring or inhibiting enzymatic oxidation of polyphenols. About 40 g of sliced yam was ground in 40 mL of 50 mM acetate buffer (pH 4.3) containing 0.2 g/L sodium fluoride 3 (*unoxidized sample*, NOX) or in acetate buffer at 50 mM pH 4.3 (*oxidized sample, OX*). Oxidized and unoxidized liquid homogenates were then frozen and freeze-dried.

The powders obtained were then delipidated by homogenization in hexane. A centrifugation was performed to separate the hexane supernatant from the delipidated pellet (10000 g for 10 min on Thermo scientific Sorvall LYNX 6000 centrifuge). Delipidated powders were dried under nitrogen flow, frozen and freeze-dried. Freeze-dried samples were stored under argon at −20 °C until direct HPLC/MS analysis and after phloroglucinolysis.

2.2. Methods and analytical procedures

2.2.1. Determination of total phenolic content

Total phenolic content was assayed using the Folin-Ciocalteu method according to Singleton and Rossi (1965) and modified by Georgé, Brat, Alter, and Amiot (2005). Sodium carbonate and Folin-Ciocalteu reagent were purchased from Humeau Laboratories (La Chapelle-sur-Erdre, France).

Two grams of freezed pulp of yam was crushed in 20 mL of an acetone/water solvent (70:30). After stirring for 1 h, the mixture was centrifuged. The supernatant was filtered. 2.5 mL of Folin-Ciocalteu reagent $1/10^e$ and 2 mL of sodium carbonate 75 g/L was added to 0,5 mL of supernatant $1/5^e$. The interference with ascorbic acid and reducing sugars was taken into account by eluting these compounds on OASIS HLB 6 CC cartridges (Waters). The absorbance was measured at 760 nm using a UV–Visible spectrometer. The total phenolic content was expressed in milligrams per 100 g Fresh Weight using a calibration curve of gallic acid (mg GA/100 g FW).

2.2.2. Sensory analyses

Eighteen panelists were asked to rate the tubers for the Global Appreciation, using a hedonic test, as described by Sangketkit, Savage, Martin, Searle, and Mason (2000). The higher the Global Appreciation score, the more the cultivar fits with consumers' preferences. Global Appreciation was rated on a scale of 1–5 as follows:

1: Dislike extremely; 2: Dislike; 3: Neither dislike nor like; 4: Like; 5: Like extremely.

2.2.3. Determination of simple polyphenol compounds in yam tubers

Acetic acid, hexane, HPLC grade methanol and acetonitrile were obtained from Carlo Erba Reagents (Val de Reuil, France). Formic acid and sodium fluoride were purchased from VWR Prolabo (France). Ascorbic acid was sourced from Fisher Scientific (Loughborough, UK). Sodium acetate anhydrous, hydrochloric acid fuming 37% and phloroglucinol were obtained from Merck (Darmstadt, Germany). Standards

Fig. 1. Raw pulp of the varieties Kabusah (a) and INRA15 (b) within 10 min after the tuber was cut.

Table 1
Influence of the cultivar on the total phenolic content and the color attributes of the raw pulp of new hybrids of yam.

	Total phenolic content (mg GA/100 g FW)	100-L*	a*	b*	WI	Browning index
AL56	21 ± 7^{a}	15.8 ± 1.0^{ab}	-0.8 ± 0.1^{a}	13.6 ± 0.4^{a}	79.1 ± 1.1^{a}	2.0^{b}
X17 (Caribinra)	23 ± 10^{a}	14.8 ± 0.4^{a}	-1.8 ± 0.3^{a}	15.9 ± 1.7^{ab}	78.2 ± 1.5^{a}	1.0^{a}
AL51	32 ± 5^{ab}	18.5 ± 0.9^{b}	0.6 ± 0.1^{b}	19.1 ± 0.7^{b}	73.4 ± 0.1^{b}	2.0^{b}
Kabusah	46 ± 15^{b}	18.4 ± 2.0^{ab}	-1.5 ± 0.6^{a}	18.0 ± 4.0^{b}	74.1 ± 4.0^{ab}	1.5^{abcd}
INRA15	81 ± 13^{c}	24.1 ± 1.7^{c}	-0.04 ± 1.4^{ab}	30.5 ± 3.9^{c}	61.2 ± 4.1^{c}	3.0^{c}
X142 (Gwadinra)	146 ± 17^{d}	24.9 ± 4.2^{bc}	4.7 ± 1.5^{bc}	26.2 ± 0.6^{c}	63.5 ± 3.5^{c}	2.5^{d}
Cultivar	$P < 0.001$	$P < 0.005$	$P < 0.001$	$P < 0.002$	$P < 0.02$	$P < 0.005$

Mean ± Standard Deviation (n = 3 to assess the total phenolic content; n = 2 to measure the color attributes); GA: Gallic Acid.

of (+)-catechin and (−)-epicatechin, were obtained from Humeau Laboratories (La Chapelle-Sur-Erdre, France). Hyperoside (quercetin-3-*O*-galactoside), procyanidin B1, and procyanidin B2 and B3 standards were purchased from Extrasynthese (Genay, France).

Simple polyphenols (i.e., monomeric flavanols, flavonols, and anthocyanins) were extracted from freeze-dried delipidated yam powders using acidified methanol for 15 min in an ultrasonic bath containing ice (Brasson 2200, USA). Samples were then centrifugated at 10000 g for 3 min and the supernatant was recovered. A second extraction was performed on the pellet and both supernatants were pooled. Then, samples were evaporated under nitrogen flow and solubilized with 200 μL of methanol/water (50:50). After filtration, HPLC analyses were performed in duplicate. This procedure was performed on samples from each of the three tubers for the INRA 15 and Kabusah varieties and considering the two modalities (OX: oxidized and NOX: unoxidized).

2.2.4. Acidolysis of procyanidins oligomers and polymers in the presence of the phloroglucinol

The procedure was adapted from that of Kennedy and Jones (2001). Freeze-dried yam powder (200 mg) was dispersed in 0.8 mL of methanol containing phloroglucinol (75 g/L) and ascorbic acid (15 g/L). Then, the reaction started by adding 0.4 mL of HCl (0.3 N in methanol), and media were immediately incubated at 50 °C for 60 min. The reaction was stopped by using an ice bath and by adding 1.2 mL of 0.2 M aqueous sodium acetate. After filtration, samples were ready for HPLC analysis. All phloroglucinolyses were performed in duplicates. This procedure was performed on the three tubers for INRA 15 and Kabusah variety, also considering NOX and OX modalities.

2.2.5. HPLC-UV−Vis and mass spectrometry analysis

Analysis was carried out on an analytical HPLC-system composed of a thermostatted autosampler (model Surveyor, Thermo Finnigan, San Jose, CA, USA), a binary high-pressure pump (model 1100, Agilent Technologies, Palo Alto, CA, USA), a UV–Vis diode array detector (model UV6000 LP, Thermo Finnigan), and an ion trap mass spectrometer equipped with an electrospray source (model LCQ Deca, Thermo Finnigan). The column was a Purospher® STAR RP-18 end-capped (3 μm) Hibar® HR (Merck, 2.1 × 150 mm) thermostated at 30 °C. Solvents were (A) acidified pure water (0.1% formic acid) and (B) acidified acetonitrile (0.1% formic acid). The flow rate was 0.2 mL/min and the gradient was as follows: Initial, 3% B; 0–3 min, 7% B, linear; 3–21 min, 13% B, linear; 21–28 min, 13% B, linear; 28–32 min, 20% B, linear; 32–43 min, 30% B, linear; 43–51 min, 50% B, linear; followed by washing and reconditioning the column. The UV–Vis detection was

performed in the 240–600 nm range. The ESI source was used in the negative mode. The MS detection was carried out with the following parameters: MS spectra were acquired in full scan negative ionization mode in the m/z 50–2000 range to obtain the signals corresponding to the deprotonated $[M-H]^-$ molecular ions. The method also included the MS/MS dependent scan mode, which was used to obtain the product ion spectrum of the main molecular ions detected on the chromatogram in the full scan mode. The collision energy was optimized at 35% (arbitrary units) to clearly observe the production of both parent and main daughter ions. Data were collected and processed by XCalibur software (version 1.2, Thermo Finnigan).

By comparison with available standards, the retention times, UV–Vis spectra, full MS spectra, and MS/MS spectra were used for complete identification. When the standard was not available, those criteria were used for a partial identification only. Quantifications were carried out by integration of the peaks on UV–Vis chromatograms at 280 nm for flavanols and at 350 nm for flavonols (Fig. 2).

(+)-Catechin, (−)-epicatechin, procyanidin B1, procyanidin B2 and hyperoside were quantified according to their own calibration curves, whereas other compounds were quantified "as equivalent" according to a reference compound belonging to the same polyphenol class and showing a similar UV–Vis spectrum (Table 3).

2.2.6. Statistical analyses

Statistical analyses were performed using XLSTAT. Student's *t*-test was used for means comparisons. The Pearson test was used to calculate the correlations between the global appreciation and the total phenolic content, on one hand, and the color attributes on the other hand.

3. Results and discussion

3.1. Browning susceptibility of new hybrids of yam

3.1.1. Browning susceptibility of new hybrids of yam as related to their total phenolic content

In raw yams, our data show a significant effect of the cultivar on the total phenolic content ($P < 0.0001$), the browning index ($P < 0.005$) and the color attributes (L*, a*, b*) ($P < 0.005$) of the pulp (Table 1). Among the new hybrids, AL56 and X17 cultivars had the lowest total phenolic content, whereas INRA15 and X142 had the highest content. In comparison to Kabusah, AL56 and X17 contained half as many polyphenols whereas INRA 15 and X142 were two and three times more concentrated

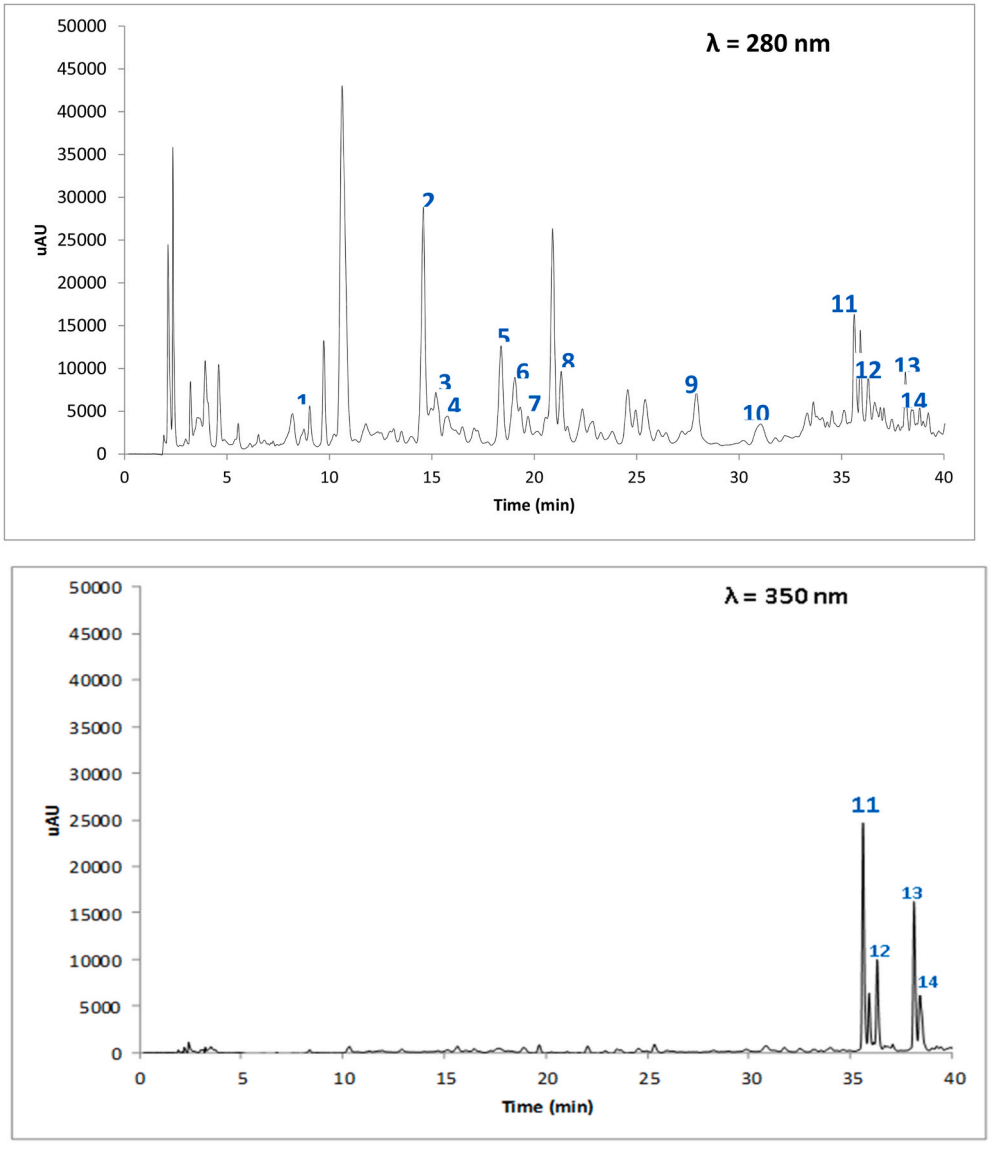

Fig. 2. Reversed phase UV–Visible HPLC chromatograms of the acidified methanol extract of Unoxidized Kabusah yam powder at (A) 280 nm, all phenolics are detected and (B) 350 nm, more specific for flavonols.

in polyphenols, respectively. The total phenolic content of the raw pulp of the new hybrids of *D. alata* studied ranged between 21 and 146 mg GA/100 g Fresh Weight (FW). Such a variability due to the variety has been previously shown in other hybrids of the same species (Njoh Ellong et al., 2015).

The Whiteness Index (WI) corresponds to the overall whiteness of the pulp and indicates the discoloration due to cutting the food product. 100-L* parameter, where L* is the luminance measured using a chromameter, is positively correlated to the browning of the pulp (Mestres et al., 2004). The new hybrids AL51, AL56 and X17 did not significantly differ from the control Kabusah neither for the Whiteness Index nor for Browning Index and 100-L* (Table 1). The 100-L* and the Whiteness Index values of AL51, AL56 and X17 cultivars ranged from 14.8 to 18.5 and from 73.4 to 79.1, respectively. On the contrary, INRA15 and X142 exhibited a significantly higher Browning Index and 100-L* and a lower Whiteness Index than Kabusah ($P < 0.02$). The 100-L* and the Whiteness Index values of the latter hybrids averaged 24.5 and 62.3, respectively. Whiteness Index is rarely calculated to characterize the color of the pulp of yam or other tropical roots and tubers. Only Hsu et al. (2003) have used this criterium to indicate the discoloration due to drying processes in *D. alata*. The correlation between 100-L* and Whiteness Index in the present study was found to be −0.96 ($P < 0.001$).

It is worth noting that the two varieties with the lowest values for the total phenolic content exhibited the lowest 100-L* values. Conversely, the two with the highest total phenolic content showed the highest 100-L* values. The correlations between the total phenolic content on one hand, and the Browning Index and the color attributes on the other hand, are presented in Table 2. The correlation between color attributes and total phenolic content is significant. It must be noted that the correlation between the Browning Index and the total phenolic content is not significant.

3.1.2. Browning after cutting as a major quality defect in yam

With regard to boiled yam, color and taste were found to be the main quality traits on which to rate *D. alata* for its sensory characteristics (Egesi, Asiedu, Egunjobi, & Bokanga, 2003). The Global Appreciation that we obtained by sensory analyses is significantly correlated to color attributes, except for a* values (Table 2). For example, the correlation between Global Appreciation and 100-L*, which reflects susceptibility to browning, is −0.94. This highly significant correlation confirms that in the French West Indies, as in West Africa (Honfozo et al., 2021), consumers prefer yams with white or cream pulp and that browning after cutting is considered a major quality flaw. Furthermore, as all the varieties have white or cream raw pulp, it must be noticed that the color of the pulp and the susceptibility to browning are two distinct parameters. The latter has to be studied as a specific quality trait.

Moreover, it is well known that color affects the acceptability of both fresh and processed plant derived foods (Rytel et al., 2019). Color interacts with other sensory attributes, mainly with taste (Francis, 1995; Zampini, Sanabria, Phillips, & Spence, 2007).

One of the hybrids we examined, INRA15, was previously evaluated for its texture, color and nutritional characteristics by Njoh Ellong et al. (2015). These authors found that INRA15 was the least popular variety due to its quality flaws, namely its large size and high susceptibility to browning when cut. Similar quality flaws have also been reported in new hybrids of dessert banana selected by Cirad for their resistance to Black Leaf Streak Disease (*M. fijiensis*). For example, Flhorban916 hybrid has been rejected by the export sector as it revealed not to be marketable in European markets due to its high susceptibility to bruising (Bugaud, Ocrisse, Salmon, & Rinaldo, 2014).

Browning is linked to the enzymatic oxidation of *o*-diphenols that occurs in tubers after cutting, as reported in raw yams and potatoes (Lebot, Malapa, Molisalé, & Marchand, 2006; Rytel et al., 2019). This oxidation takes place as soon as the cell structure is disrupted, which creates contact between the enzyme polyphenol oxidase (PPO) and its substrates (i.e. mainly *o*-diphenolic substrates and dioxygen) (Cheynier, 2005). The resulting *o*-quinones are involved in numerous chemical reactions that lead to the formation of phenolic oxidation products, some of them being brown pigments. These pigments are generally considered unfavorable except in some beverages such as tea or coffee, in which they are sought.

Table 2
Correlations between the total phenolic content, the global appreciation and the color attributes of the raw pulp of new hybrids of yam.

	Browning index	100-L*	a*	b*	WI
Total phenolic content	0.64 $^{(0.08)}$	0.91**	0.89*	0.78*	−0.85*
Global Appreciation	−0.83$^{(0.08)}$	−0.94*	−0.59$^{(NS)}$	−0.89*	0.92*

(n = 3 to assess the total phenolic content and the global appreciation score; n = 2 to measure the color attributes).
NS: Not Significant, *P < 0.05, **P < 0.01.

3.2. Determination of the detailed phenolic profiles of the unoxidized raw pulp of INRA15 and Kabusah yam tuber

3.2.1. Analysis of simple phenolics in methanol extracts by direct HPLC-UV/Visible-MS

There is paucity of information on the phenolic profile of yam. Pulp samples of raw INRA15 and Kabusah yam were analyzed by direct HPLC-UV/Visible-MS analysis of the corresponding methanol extracts. The UV 280 nm chromatogram of unoxidized Kabusah is shown in Fig. 2. It presented numerous peaks revealing the multiplicity of UV-absorbing constituents which are likely corresponding to phenolic compounds. Retention times, UV and MS data of the most important peaks are presented in Table 3. Some numbered peaks were fully identified according to their exact matching to commercial phenolic standards. Some others were partly characterized on the basis of their chromatographic, UV–Visible and MS parameters attesting to their belonging to a specific phenolic class (i.e. catechins, procyanidins oligomers and flavonols). Other more or less intense peaks on the UV 280 nm were referenced in Table 3 as "unidentified" since we were unable to formulate a hypothesis for their identification. Noticeably, no peak that could be attributed to the hydroxybenzoic or hydroxycinnamic acid class could be identified. This goes against the identification of ferulic, sinapic and chlorogenic acids in the pulp of *D. alata*, using either HPLC-DAD or HPTLC (Akissoé et al., 2005; Fang et al., 2011; Lebot et al., 2018). Our data mainly provide information on flavonols and flavanols. Catechins and procyanidins presented a maximum absorbance (λ max) at 280 nm, whereas flavonols were specifically characterized by a λ max at 350 nm. Regarding flavonols, hyperoside and isorhamnetin were observed in the pulp of both INRA15 and Kabusah, in contrast to the findings of Champagne, Hilbert, Legendre, and Lebot (2011) in *D. alata*.

(+)-Catechin, procyanidin dimers B1, B2, B3 and hyperoside were identified (Table 3) by comparison with commercial standards. A series of peaks identified as procyanidin trimers, tetramers and pentamers was characterized on the basis of their corresponding deprotonated molecule [M-H]- at m/z 865,1153 and 1441 observed on the corresponding MS spectrum, respectively.

The phenolic profile widely varied with the cultivar. In Kabusah, the major compounds were procyanidin B1 and one procyanidin trimer (peaks 2 and 5). It has been reported that the phenolic profile of yam varies with the species (Lebot et al., 2018). Moreover, our data show that there is intra-species variation among yam varieties in terms of their phenolic profile. Interestingly, (+)-catechin was not detected in the raw pulp of Kabusah whereas it is the predominant flavanol in INRA15 (Table 3). It amounted 4.0 mg/kg FW in INRA15, which is close to the 4.30–7.55 mg/kg FW recorded in *D. alata* by Ozo et al. (1984). (+)-Catechin is a good substrate for polyphenol oxidase in yam (Gnangui, Niamké, & Kouamé, 2009; Ozo & Cayill, 1986). A significant

Table 3
LC-UV−Visible/MS & MS/MS identification and quantification of the main simple phenolic compounds in the raw pulp of INRA15 and Kabusah yam.

peak	Rt (min)	λ max (nm)	$[M-H]^-$ (m/z)	MS/MS	Compound	INRA15 pulp (mg/kg FW)	Kabusah pulp (mg/kg FW)
1	8.0	279	865	695 (100); 575 (26); 577 (25)	Procyanidin trimer 1	0.96 ± 0.42	1.74 ± 0.33
	9.5	277	315	153 (100); 123 (7)	unidentified		
	10.4	278	594	321 (100); 272 (25)	unidentified		
	13.3	275	451	No MS^2 signal available	unidentified		
2	14.4	279	577	425 (100); 451 (45); 407 (29)	Procyanidin B1[a]	4.91 ± 2.7	11.74 ± 1.85
	15.5	277	577	Coelution; No MS^2 signal available	Procyanidin B3[a]	traces	traces
3	17.1	279	289	Coelution	(+)-Catechin[a]	4.04 ± 1.28	0.00
4	18.2	279	865	695 (100); 577 (51); 713 (38)	Procyanidin trimer 2	1.47 ± 0.71	4.90 ± 0.67
5	18.8	279	1153	1124 (100); 939 (46); 863 (43)	Procyanidin tetramer 1	0.00	3.96 ± 0.67
6	19.1	279	865	No MS^2 signal available	Procyanidin trimer 3	0.57 ± 0.04	1.37 ± 0.22
	20.7	278	465	303 (100); 327 (23)	unidentified		
7	21.1	279	577	425 (100); 407 (28); 451 (25)	Procyanidin B2[a]	0.00	3.89 ± 0.70
	22.1	278	1441	865 (100); 1271 (75); 863 (70)	Procyanidin pentamer		
	24.4	279	329	191 (100); 167 (42)	unidentified		
	24.7	279	465	303 (100); 345 (3)	unidentified		
	26.2	278	865	No MS^2 signal available	Procyanidin trimer 6		0.65 ± 0.11
	27	278	865	No MS^2 signal available	Procyanidin trimer 4	< LQ	0.89 ± 0.19
8	27.7	279	865	695 (100); 739 (57); 577 (36)	Procyanidin trimer 5		3.63 ± 0.48
9	30.8	279	1153	No MS^2 signal available	Procyanidin tetramer 3	< LQ	1.26 ± 0.16
10	35.4	355	609	300 (100); 301 (92); 343 (14)	Quercetin deoxyhexose-hexoside	0.18 ± 0.04	2.54 ± 0.53
11	36.1	355	463	301 (100); 300 (77); 343 (5)	Hyperoside (quercetin 3-galactoside)[a]	0.15 ± 0.01	0.82 ± 0.14
12	37.9	355	623	315 (100); 314 (32); 300 (18)	Isorhamnetin-deoxyhexose-hexoside 1	0.23 ± 0.02	1.69 ± 0.28
13	38.2	355	623	Coelution; No MS^2 signal available	Isorhamnetin-deoxyhexose-hexoside 2		

[a] Identified according to a commercial standard.

coefficient of correlation of 0.59 has been computed between the oxidation after cutting rated on a scale of 1–4 and the catechin content of the pulp (Lebot et al., 2018). Moreover, enzymatic oxidation of (+)-catechin leads to the formation of yellow products which are likely contributing to browning (Guyot, Vercauteren, & Cheynier, 1995). This is in line with our data indicating that the variety the richest in catechin has the highest 100-L* value.

Procyanidin B2, which is a dimer of (−)-epicatechin, was clearly detected in Kabusah. It should be noted that no free (−)-epicatechin was detected in any of the two varieties. Procyanidin B1 was found in the pulp of both Kabusah and INRA15, whereas traces of procyanidin B3 were shown in both varieties (Table 3). Only Ozo et al. (1984) have identified both procyanidin dimers B1 and B3 in several species of yam, including *D. alata*. As a whole, in our study, total procyanidin oligomers in the unoxidized pulp accounted for 34.6 mg/kg FW and 7.5 mg/kg FW in Kabusah and INRA15, respectively.

Regarding flavonols, quercetin deoxyhexose-hexoside was the predominant molecule of this class in Kabusah (rutin, peak 11) and amounted to 2.6 mg/kg FW. INRA15 contained about ten times fewer flavonols (peaks 11 to 13) than Kabusah. Among authors, the controversial results on the phenolic profile of *D. alata* might be linked to the variability due to the genotype (Fang et al., 2011; Lebot et al., 2018).

3.2.2. *Analysis of total flavanols (i.e. catechins + procyanidins oligomers and polymers) by HPLC after phloroglucinolysis reaction*

The present study focuses on the flavan-3-ols in yam, on which there is a lack of information. Catechin and epicatechin have been reported in the pulp of *D. alata* (Akissoé et al., 2005; Lebot et al., 2018; Ozo et al., 1984). As discussed in paragraph 3.2.1., our data revealed the presence of procyanidin dimers, trimers and tetramers in the pulp of INRA15 and/or Kabusah varieties. This suggests that procyanidins with higher degrees of polymerization are also present. For this reason, HPLC-UV-MS analysis after phloroglucinolysis was used to characterize and quantify the total flavanols including the non-extractable procyanidin polymers (Table 4).

Phloroglucinolysis reaction gives access to the average flavanol subunit composition of the total procyanidins (including oligomers and polymers). Acidolysis leads to the formation of flavanyl carbocations corresponding to the extension units of the procyanidin structure, whereas the terminal units are released in the medium as catechin molecules. Flavanyl carbocations are immediately trapped by nucleophilic phloroglucinol in excess, leading to flavanyl-phloroglucinol

Table 4
Composition of total flavanols (including catechins and total procyanidin oligomers) obtained after acidolysis in the presence of phloroglucinol in the raw pulp of two unoxidized cultivars of yam.

Variety	Free (+)-catechin (mg/kg FW)	Procyanidins (flavanol oligomers)				Total procyanidins (mg/kg FW)	Total flavanols (mg/kg FW)	DPn[d] of flavanols
		(+)-catechin t.su[ac] (%)	(−)-epicatechin t.su[ac] (%)	(+)-catechin e.su[bc] (%)	(−)-epicatechin e.su[bc] (%)			
INRA15	4.0 ± 1.3	21.4	0	14.9	63.7	238.2 ± 53.2	242.2 ± 53.8	5.0 ± 0.3
Kabusah	0.0	8.3	3.5	6.3	81.9	1123.0 ± 235.4	1123.0 ± 235.6	8.5 ± 0.3

[a] t.su, terminal subunits.
[b] e.su, extension subunits.
[c] Validation according to commercial standards.
[d] Average degree of polymerization.

adducts. Then, the HPLC analysis of the phloroglucinolysis media gives access to the concentration of each type of terminal and extension catechin unit that initially composed the procyanidin fraction. Therefore, it is possible to estimate the average distribution of the catechin units and the total concentration of procyanidins (Guyot, Marnet, Sanoner, & Drilleau, 2001). In addition, the molar ratio of all the subunits (extension + terminal subunits) on all the terminal subunits gives access to the average degree of polymerization (DPn). This parameter corresponds to the average number of subunits in a procyanidin molecule and is directly linked to the average molecular weight of the tannins in the sample. Noticeably, DPn is of great importance regarding procyanidins properties since it is closely related to their ability to associate proteins and polysaccharides (Le Bourvellec & Renard, 2012).

Regarding procyanidin extension subunits, two phloroglucinol adducts showing deprotonated molecules at m/z 413 and eluted at RT = 12.0 and 13.2 min were attributed to the two phloroglucinol adducts deriving from (+)-catechin extension units as previously observed in some grape seed procyanidins (Da Silva J.M.R., Rigaud J., Cheynier V., Cheminat A., & Moutounet M., 1991). (−)-Epicatechin extension units were identified according to their single phloroglucinol adduct (m/z 413) eluted at 13.8 min (Table 4). (−)-Epicatechin, expressed as a per cent of total procyanidins, is by far the main extension subunit of yam procyanidins in the two cultivars studied. Interestingly, (+)-catechin was more frequently found as terminal unit than (−)-epicatechin in both cultivars and no (−)-epicatechin was identified as terminal unit of the procyanidins in the pulp of INRA15.

The pulp of the new hybrid INRA15 contained 238 mg of flavanols/kg FW whereas the pulp of Kabusah amounted to 1123 mg/kg FW (Table 4). (−)-Epicatechin, as extension subunits, equaled 154 mg/kg FW in the pulp of INRA15 and 938 mg/kg FW in that of Kabusah. The data revealed that the average degree of polymerization (DPn) of procyanidins was very different from one cultivar to the other. Indeed, it was 5.0 for INRA15 and reached up to 8.5 for Kabusah ($P < 0.0001$) (Table 4). In apple, it has been demonstrated that the higher the average degree of polymerization of procyanidins, the stronger their inhibitory effect on polyphenol oxidase activity (Le Bourvellec, Le Quéré, Sanoner, Drilleau, & Guyot, 2004). This may be due to a tannin effect in which the binding of the polyphenols to the protein might reduce the catalytic properties of the enzyme. Our results suggest that the susceptibility to browning when cut depends on the total phenolic content and the catechin content of the pulp in yam, but also on the degree of polymerization of the flavanols.

To our knowledge, our data are the first to precisely describe qualitatively and quantitatively the composition of total procyanidins fraction in yam cultivars.

3.3. *Susceptibility of the two yam cultivars to "in vitro" enzymatic oxidation as measured by the changes of their detailed polyphenol profiles*

The influence of *in vitro* enzymatic oxidation on the contents in (+)-catechin, procyanidins oligomers and flavonols in the pulp of yam is presented in Fig. 3. The effect of the cultivar on these three groups of phenolic compounds is highly significant ($P < 0.01$). In INRA15, the induced oxidation led to a marked decline in the content of (+)-catechin. No more than 0.4 mg/kg FW of this compound was detected in the oxidized pulp of this new hybrid. Regarding procyanidins oligomers, the oxidation led to a massive 88% decline in INRA15 whereas it was a 36% decrease in Kabusah ($P < 0.01$). We noted that *in vitro* oxidation caused no significant change in the content in flavonols, whatever the cultivar.

Regarding total procyanidins assayed by HPLC after phloroglucinolyis, oxidation led to a marked decrease (66%) for INRA15, while it was not significant (21%) for Kabusah (Fig. 4).

In vitro enzymatic oxidation was performed to exacerbate the effect of slicing. Enzymatic oxidation had a tremendous effect on the reduction in the content in catechin, procyanidin oligomers and procyanidins of the raw pulp of INRA15. Conversely, only a moderate decline in the content in procyanidin oligomers was observed in Kabusah after enzymatic oxidation. These results clearly confirm that the susceptibility to oxidation leading to browning is highly dependent on the phenolic profile in *D. alata*. It is worth noting that, as compared to INRA15, Kabusah contained much more procyanidins with a higher average degree of polymerization (Table 4). The susceptibility to browning of INRA15 might also be due to a higher PPO activity (Akissoé et al., 2005; Peng et al., 2019). When comparing six different varieties including five resistant new hybrids, PPO activity in the raw pulp of INRA 15 was found to be amongst the highest (Njoh Ellong et al., 2015). Further

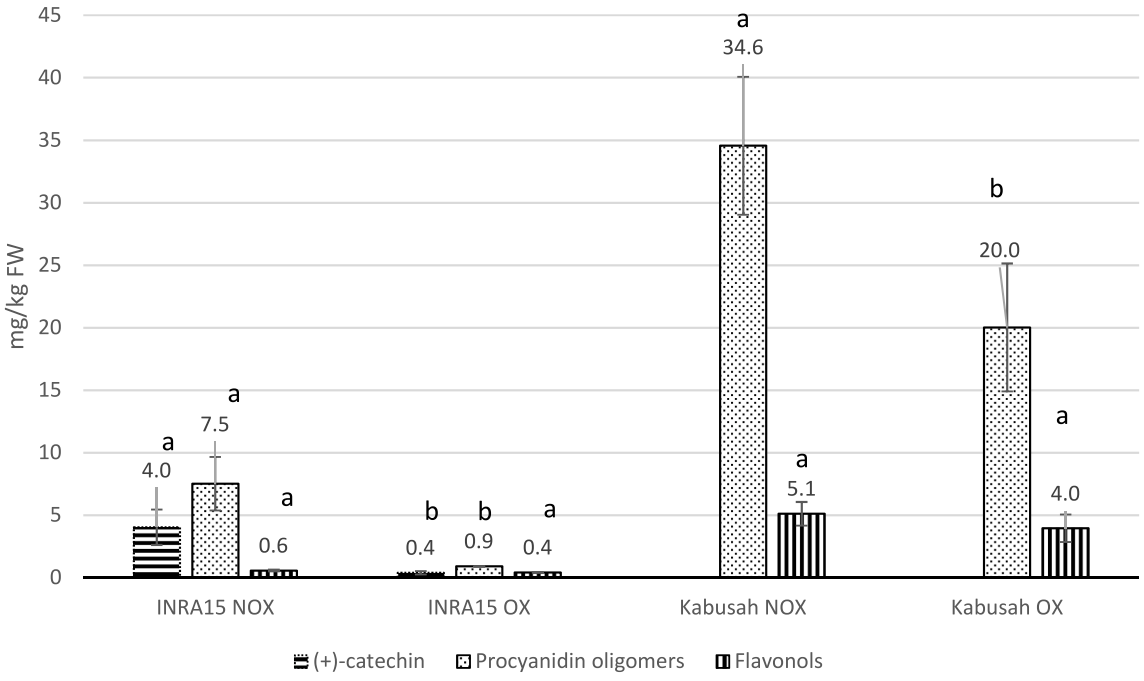

Fig. 3. Catechins, procyanidin oligomers and flavonols concentrations (mg/kg FW) in the raw pulp of INRA15 and Kabusah as measured directly by HPLC.

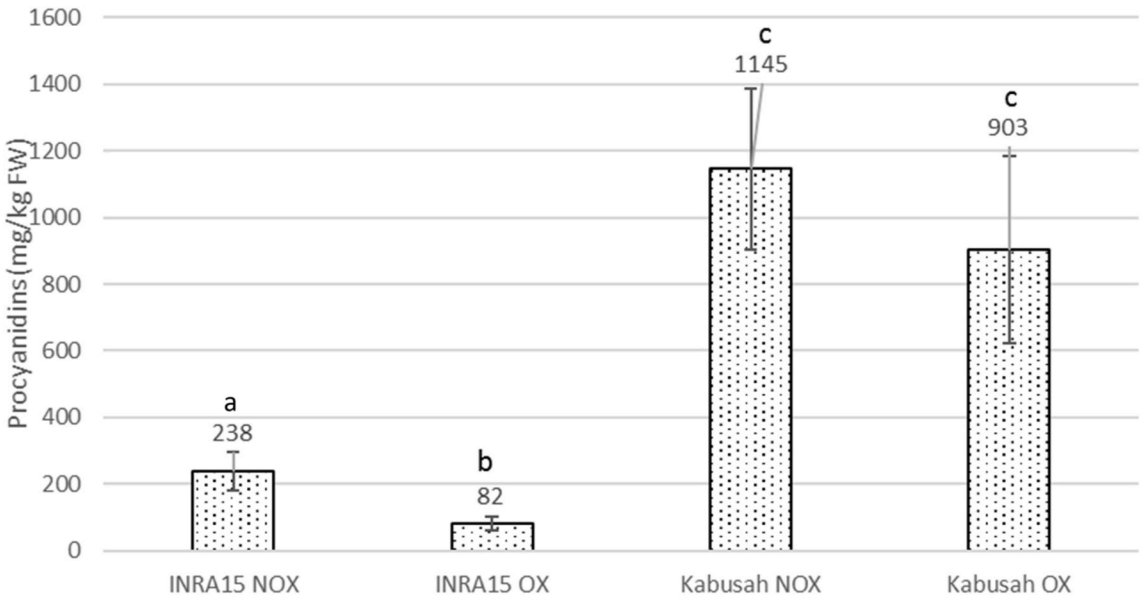

Fig. 4. Total procyanidin content (mg/kg FW) depending on the cultivar and the oxidation status as measured by HPLC after phloroglucinolysis in the raw pulp of INRA15 and Kabusah (n = 3; bars correspond to standard deviation).

knowledge is needed on polyphenol oxidase activity in the pulp of *D. alata* according to the cultivar.

The present study demonstrates that it is of the utmost importance to select new varieties not only for their resistance/tolerance to diseases but also, simultaneously, for their quality traits and flaws. This has also been pointed out in the selection of new hybrids of dessert bananas for their resistance to other fungal diseases. However, more knowledge is needed on the genetics of quality traits (starch content, textural properties) and also flaws (susceptibility to browning). In particular, their inheritance has to be calculated in order to be taken into account in breeding programs.

4. Conclusions

Our results on five new hybrids of yam selected for their resistance/tolerance to anthracnose clearly show that some of them are characterized by a high susceptibility to browning when cut. This susceptibility is considered a quality flaw by the consumers in the French West Indies as well as in West Africa. According to our results, this flaw is linked both to the total phenolic content and to the phenolic profile in *D. alata*. When comparing INRA15, which is highly susceptible to browning, to Kabusah, which exhibits moderate susceptibility to this defect, we obtained quite different qualitative and quantitative phenolic profile. Direct HPLC analysis and HPLC associated to phloroglucinolysis revealed that Kabusah was much more concentrated in polyphenols compared to INRA15. However, in both varieties, procyanidins were largely the main polyphenol class accounting for around 0.25 g/kg FW and 1.1 g/kg FW for INRA15 and Kabusah, respectively. The INRA15 variety contains about 4 mg/kg FW of catechin, which is known to be a good substrate of PPO. Conversely, the Kabusah variety contained a much higher level of procyanidins with a higher degree of polymerization compared to those compounds in INRA15. This is in accordance to its lower susceptibility to browning since procyanidins are likely contributing to inhibition of PPO activity and polymerized procyanidins are known to be more efficient inhibitors. Moreover, in *D. alata*, we demonstrated a high variability in the phenolic profile of the pulp owing to the variety. The present study focused on flavanols even though some flavonol glycosides were also detected and quantified. Further work is required to complete the phenolic profile of yam, taking into account the species and examining a larger panel of varieties. The data suggest that the presence of quality flaws should be taken into consideration at the very beginning when selecting new hybrids for their resistance/tolerance to diseases. A multi-criteria selection should be recommended, as far as the knowledge on genetics of quality traits and flaws can be extended.

Credit Authors'Statement

Dr. Dominique Rinaldo: Conceptualization, Data curation, Funding acquisition, Formal analysis, Investigation, Methodology, Software, Writing - original draft, Writing - review and editing, Hélène Sotin: Formal analysis, Investigation, Methodology, Software, Writing - original draft (Part of Materials and Methods), Dalila Pétro: Resources (Plant Material), Validation, Writing - original draft, Gildas Le-Bail: Investigation, Methodology. Dr Sylvain Guyot: Conceptualization, Methodology, Supervision, Writing - original draft, Writing – review.

Declaration of competing interest

The authors guarantee that there is no conflict of interest about this paper.

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