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Comparison of Content in Phenolic Compounds, Polyphenol Oxidase, and Peroxidase in Grains of Fifty Sorghum Varieties from Burkina Faso

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Analysis of fifty sorghum [*Sorghum bicolor* (L.) Moench] varieties used in Burkina Faso showed that they have different contents of phenolic compounds, peroxidase (POX), and polyphenol oxidase (PPO). Most of the varieties (82%) had a tannin content less than 0.25% (w/w). POX specific activity was higher than the monophenolase and *o*-diphenolase specific activities of PPO. For POX, there was a diversity of isoforms among varieties. No clear correlation could be made between the quantitative composition of the grain in phenolics, PPO, and POX, and resistance of plant to pathogens. In general, varieties good for a thick porridge preparation ("tô") had low phenolic compounds content and a medium POX activity. From the red varieties, those used for local beer ("dolo") had a high content in phenolic compounds and PPO, and a low POX activity. The variety considered good for couscous had a low POX content. The characteristics might be useful as selection markers for breeding for specific applications.

KEYWORDS: Phenolic compounds; tannins; polyphenol oxidase; peroxidase, sorghum

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is the staple cereal in sub-Saharan Africa and India where 300 million people rely on its grains (*1*). Burkina Faso is the world leader in sorghum consumption per inhabitant (*2*). In Burkina Faso sorghum represents 48−57% of the total cereal production (*2*, *3*). The grain is mainly used for human nutrition, with an average consumption of 200 kg per person per year (*3*). White grains are used for local foods such as "tô" or other porridges, and "couscous", etc. Tô is a thick porridge prepared by cooking a slurry of cereal flour in boiling water. Couscous is a steamed and granulated food with a different type of texture, also prepared from cereal flour. Malt from red sorghum grains is widely used for the preparation of a cloudy local beer ("dolo") and for nonfermented beverages. The knowledge about the content of phenolic compounds (PC), polyphenol oxidase (PPO), and peroxidase (POX) within cereal grains is scarce. However, these biochemical constituents have been shown to be determinants in food quality (*4*, *5*).

PPOs [monophenol, 3,4-*L*-dihydroxyphenylalanine: oxygen oxidoreductase, EC.1.14.18.1] are copper-containing oxidases that catalyze the O_2-dependent oxidation of catechols to the corresponding quinones (*o*-diphenolase or catecholase activity). They may also catalyze the regioselective (*ortho*) aerobic hydroxylation of monophenols to *o*-diphenols and their subsequent oxidation to *o*-quinones (monophenolase or cresolase activity). In most plants POXs [donor: H_2O_2 oxidoreductase, EC. 1.11.1.7] are heme-containing enzymes that catalyze the conversion of H_2O_2 to water using PC as a hydrogen donor. POX and PPO activities have been detected in the leaves (*6*−*9*) and grains (*10*−*12*) of sorghum.

POX and PPO may act synergistically in enzymatic browning, because PPO may promote POX activity by generating H_2O_2 from the oxidation of PC (*13*). They play an important role in plant defense by the oxidation of endogenous PC into quinones, which are toxic to the invading pathogens and pests (*8*, *14*). The resulting quinones may undergo nonenzymatic autopolymerization or covalent hetero-condensation with proteins and carbohydrates to produce colored compounds (*4*, *15*). These compounds may also constitute a physical barrier against biotic and abiotic stresses (*14*, *16*). In food, the reaction products of these enzymes may not only affect taste, bitterness, astringency,

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and color, but when interacting with proteins, these products may hinder digestibility and palatability, thereby reducing the nutritional value of foods (*4*, *17*). In barley, POXs and PPOs have been found to be involved in oxygen scavenging during the mashing process (*18*). Interestingly, POX could modify the functional properties of food macromolecules, notably hydroxycinnamic acids containing polysaccharides (pectins, arabinoxylans, etc.) and tyrosine-containing proteins, via the mediation of their homo and hetero-cross-linking (*19*). This property may be desired in bakery, as it results in a better quality of both the dough and the baked product (*20*).

Sorghum and barley are the two important food grains reported to contain significant quantities of PC (*15*, *21*). PCs are plant secondary metabolites biosynthesized through the shikimic acid pathway, in which phenylalanine ammonia-lyase is the key enzyme (*4*, *5*). PCs are believed to be involved in plant growth and reproduction, protection against UV radiation, and resistance to pathogens and predators (*5*). Some PCs present in food may have dietary and therapeutic effects (*4*, *5*). However, tannins are often considered as antinutritional factors, because they inhibit hydrolytic enzymes and link with macronutrients to form indigestible complexes (*21*). Further, vicinal hydroxy groups of PCs (caffeic acid, chlorogenic acid, quercetin, etc.) may chelate metal ions and reduce their bioavailability (*5*, *22*). Possible carcinogenic effect (*23*) and pro-oxidant activity (*24*) of PCs are also reported. Nevertheless, the enzymatic oxidation of PCs considerably enhances their enzyme inhibitory effect and toxicity, and also reduces their health promoting properties (*24*, *25*).

The objective of this study was first to determine the content of PCs and their oxidative enzymes in fifty sorghum varieties that are produced in Burkina Faso and used for food processing, and, second, to relate the determined biochemical properties to food use as these are reported to be determinants of food quality.

MATERIALS AND METHODS

Chemicals and Reagents. Electrophoresis gels (IEF, pH 3−9) were purchased from Amersham Pharmacia Biotech. 4-Hydroxyanisole (4HA) and gallic acid (3,4,5-trihydroxybenzoic acid) were from Aldrich. Catechin and 3,4-dihydroxyphenylpropionic acid (DHPPA) were from Across Organics. Folin−Ciocalteau's reagent, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS), insoluble poly(vinylpyrrolidone) (PVP), 3,3′-diaminobenzidine (DAB), bovine serum albumin (BSA), and 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) were from Sigma Chemicals Co. Hydrogen peroxide and ferric ammonium citrate (FAC) were from Merck. All other chemicals were of analytical grade.

Sorghum Grains. Grains of 50 sorghum [*Sorghum bicolor* (L.) Moench] varieties were kindly provided by the Centre de Coopération International en Recherche Agronomique pour le Développement (CIRAD) at Ouagadougou (Burkina Faso) and Montpellier (France), and the Centre National de Recherche Scientifique et Technique (CNRST) of Burkina Faso. The selection included local varieties as well as internationally improved varieties in order to represent the most widely cultivated sorghums in Burkina Faso (**Table 1**). Varieties were grown (1996−1998) in the natural season, in the semi-arid environment of West Africa (temperature 30−42 °C; precipitation 600−800 mm/year), at the Breeding Station in the village of Saria (Burkina Faso). Of the varieties 90% were white and 10% were red, which reflects the actual figure of sorghum production in Burkina Faso. The varieties were chosen according to their resistance or susceptibility to pests (anthracnose, sorghum midge, sooty stripe and the parasitic plant striga), sensitivity or insensitivity to photoperiod, drought tolerance or susceptibility, grain hardness or softness, and suitability or not for traditional processing into local foods (tô and couscous) or beer (dolo). All the outlined known properties of the plant and grain processing were obtained from either the literature (*26*−*31*) or from CIRAD and CNRST. For convenience, the sorghum varieties were designated with Arabic numbers from 1 to 50 preceded by C. The order was used as it occurred in the collection of breeders. The mature grains (ungerminated) were surface-sterilized by washing with 5% (v/v) aqueous sodium hypochlorite for 5 min with stirring. The grains were dried by ventilation at room temperature (20−25 °C) to a moisture content of 12−14% (w/w), and ground into flour in a microanalytical mill (Fritsch, Marius Instruments, The Netherlands) to pass a screen of 0.5 mm. Milling was done at 4 °C, and was performed carefully to avoid overheating. The flours were stored at −80 °C prior to analysis to prevent endogenous enzymatic or nonenzymatic reactions.

Determination of Total Phenolic Compounds Content. Total PC was extracted from 50 mg of sorghum flour by continuous stirring with 1.5 mL of 1% (v/v) HCl in methanol at 25 °C, for 20 min (*32*). The suspension was centrifuged (5000*g*, 10 min, 25 °C) and the supernatant was collected. The residue was re-extracted with HCl/methanol as described above, and the two supernatants were pooled. Total PC content was determined using Folin−Ciocalteau's method (*33*) adapted to a 96-well plate assay. To 10 μL of extract was added 25 μL of Folin−Ciocalteau's reagent (50%, v/v). After 5 min of incubation, 25 μL of 20% (w/v) sodium carbonate solution and water were added to the mixture to have a final volume of 200 μL. Blanks were prepared for each sorghum sample by replacing Folin−Ciocalteau's reagent with water. Gallic acid was used as a standard and results were expressed as gallic acid equivalent per gram of flour (w/w). The standard was always freshly prepared. The absorbances (after 30 min) were measured at 760 nm using a multiwell plate reader (EAR 400, Labinstruments, Australia).

Determination of Tannin Content. Tannins were extracted from 50 mg of sorghum flour with 75% DMF and quantified by the FAC method as described by Beta et al. (*34*) using catechin (flavan-3-ol monomer) as a standard. The assay was monitored at 525 nm. The standard was always freshly prepared. Tannin content was expressed as catechin equivalent per gram of flour (w/w).

Extraction of Enzymes. Enzyme extracts were prepared by mixing 250 mg of sorghum flour with 1.2 mL of 50 mM Tris−HCl buffer pH 7.3 containing 0.5 M $CaCl_2$ and 2% (w/v) PVP, at 4 °C for 1 h. The homogenate was centrifuged (14000*g*, 4 °C, 45 min) and the resulting supernatant was used as crude extract of both PPOs and POXs. Total protein was quantified by the linearized method of Bradford (*35*) using the ratio of A_{620}/A_{450} versus protein concentration. BSA was used as standard.

Enzyme Assays. The spectrophotometric assay for PPO was performed as described by Espín et al. (*36*, *37*). 4HA and DHPPA were used as phenolic substrates to determine the monophenolase and *o*-diphenolase activities of PPO, respectively. The enzyme extract (10 μL) was incubated with 150 μL of 50 mM sodium acetate buffer pH 5.5, 10 μL of 40% (v/v) DMF, and 10 μL of 50 mM MBTH, at 25 °C, for 5 min. The reaction was started by addition of 20 μL of 100 mM of the phenolic substrate (prepared in 0.15 mM phosphoric acid). The reaction was monitored at 500 nm. Control assays, in which the enzyme extract or substrates were replaced by buffer were performed. One unit of PPO activity (U) is defined as the amount of enzyme producing 1 μmol of MBTH-quinone-adducts per min resulting from the oxidation of 4HA or DHPPA. POX activity was measured spectrophotometrically by monitoring the H_2O_2-dependent oxidation of ABTS at 25 °C. The reaction mixture consisted of 10 μL of 200-fold diluted crude enzyme extract, 20 μL of 100 mM ABTS, 10 μL of 100 mM H_2O_2, and 160 μL of 50 mM sodium acetate buffer pH 5.0. Control assays in which the enzyme extract or substrates were replaced by buffer were performed. The reaction was monitored at 405 nm. One unit of POX activity (U) is defined as the amount of enzyme releasing 1 μmol of ABTS radical/min under the assay conditions.

All enzyme assays were monitored with a multiwell plate reader SLT340 ATTC (Labinstruments, Australia) on-line interfaced to a computer (Macintosh Performa 450). Kinetic data were determined in the linear phase of reaction traces using DeltaSoftII version 4.1S (Biometallic, Inc.). The reactions were monitored over 3 min. The initial slopes of the reaction traces caused by enzyme activities were corrected with the slopes of the blanks.

Table 1. List of Local and Improved Sorghum Varieties Used in Burkina Faso[a]

variety number	name	genetic type	country of origin	race	grain testa	color of grain/plant	known particular properties	
							plant/grain	food[b]
1	CEF 322/53-1-1	IL	Burkina Faso	C	-	W/R	post-flowering drought resistant	good for tô
2	Sariaso 10	IL	Burkina Faso	C	-	W/tan	post-flowering drought resistant	poor for dolo
3	IRAT 204	IL	Senegal	C	-	W/tan	sooty stripe and anthracnose susceptible	/
4	BF 89-18/139-1-1	IL	Burkina Faso	C	-	W/tan	post-flowering drought susceptible	good for tô
5	BF 88-2/31-3	IL	Burkina Faso	C	-	W/tan	pre-flowering drought susceptible	/
6	SRN 39	IL	Sudan	C	-	Y/tan	striga resistant	/
7	Framida	IL	South Africa	KC	+	R/R	striga resistant	good for dolo
8	IS 15401	LR	Cameroon	GC	-	W/R	striga resistant	/
9	S 29	LR	Burkina Faso	G	-	W/R	striga susceptible	good for tô
10	F2-20	IL	Burkina Faso	C	-	W/tan	leaf anthracnose resistant	/
11	CE 180-33	IL	Senegal	C	+	W/tan	leaf anthracnose susceptible	/
12	ICSV 1049	IL	Burkina Faso	C	-	W/tan	sooty stripe resistant	good for tô
13	ICSV 745	IL	India	C	-	W/tan	sooty stripe susceptible; sorghum midge resistant	/
14	IRAT 174	IL	Burkina Faso	C	-	W/R	photoperiod sensitive	/
15	Cauga 22-20	IL	Burkina Faso	GC	+	W/R	photoperiod sensitive	/
16	G 1414	LR	Burkina Faso	G		W/R	photoperiod sensitive	/
17	Cauga 108-15	IL	Burkina Faso	GC	-	W/R	photoperiod insensitive	/
18	Magadji 1-509	LR	Burkina Faso	GC	-	R/R	photoperiod insensitive	/
19	ICSV 1002	IL	Burkina Faso	C	-	W/tan	leaf anthracnose resistant	good for tô
20	BC1 S29/2-2	IL	Burkina Faso	G	-	W/R	sorghum midge susceptible	good for tô
21	Kaapelga	LR	Burkina Faso	G	-	W/tan	hard grains (PSI< 10)	good for tô
22	IRAT 277	IL	Burkina Faso	C	-	W/tan	soft grains (PSI >16)	poor for tô
23	BF 88-2/31-1	IL	Burkina Faso	C	-	W/tan	-	poor for tô
24	CEM 326/11-5-1-1	IL	Mali	GC	-	W/tan	hard grains (PSI< 10)	good for tô
25	CEF 396/12-3-1	IL	Burkina Faso	GC	-	W/R	hard grains (PSI< 10)	good for tô
26	G 1636	LR	Burkina Faso	G	-	W/tan	soft grains (PSI >16)	/
27	Nazongala tan	IL	Burkina Faso	G	-	W/tan	soft grains (PSI >16)	good for tô
28	Nongomsoba	LR	Burkina Faso	G	-	W/tan	soft grains (PSI >16)	good for tô
29	CGM 19/9-1-2	IL	Mali	G	-	W/R	-	good for dolo
30	Kokologho	LR	Burkina Faso	C	+	W/R	post-flowering drought resistant	/
31	IRAT 202	IL	Senegal	C	+	W/tan	pre-flowering drought resistant	good for couscous
32	Tiamassie 289	LR	Burkina Faso	G	+	W/R	-	poor for tô
33	Kapla-57	LR	Burkina Faso	G	+	R/R	sorghum midge susceptible	good for dolo
34	IRAT 9	IL	Cameroon	C	+	R/R	grain mold resistant	good for dolo
35	Sariaso 9	LR	Burkina Faso	G	-	W/R	sooty stripe resistant	good for tô
36	IRAT 10	IL	Niger	C	-	W/R	grain mold susceptible	/
37	CEF 395/9-2-3	IL	Burkina Faso	GC	-	W/tan	hard grains (PSI < 10)	good for tô,
38	G 1296	LR	Burkina Faso	GC	-	R/R	good for dyeing	/
39	Nafo-Natogué 775	LR	Burkina Faso	G	-	R/R	-	good for dolo
40	Farkakofsi 781	LR	Burkina Faso	G	+	R/R	-	good for dolo
41	Sariaso 808	LR	Burkina Faso	G	+	R/R	-	good for dolo
42	Zugilga	LR	Burkina Faso	G	+	R/R	-	good for dolo
43	90L1235	IL	USA	GC	-	W/R	sorghum midge resistant	/
44	CCGM 1/19-1-1	IL	Mali	G	-	W/R	sorghum midge susceptible	/
45	CK 60	IL	USA	K	-	W/R	striga susceptible	/
46	CGM 19/9-1-1	IL	Mali	G	-	W/R	striga susceptible	/
47	B 35	IL	USA	D	-	W/R	post-flowering drought resistant	/
48	Tx 7000	IL	USA	C	-	W/R	post-flowering drought susceptible	/
49	Segaolane	IL	Bostwana	C	-	W/R	pre-flowering drought resistant	/
50	Ajabsido	LR	Sudan	C	+	W/R	pre-flowering drought resistant	/

[a] Abbreviations: C, *Caudatum*; G, *Guinea*; CG, *Guinea-Caudatum*; D, *Durra*; K, *Kafir*; KC, *Kafir-Caudatum*; R, red; W, white; Y, yellow; IL, Inbred line; LR, Landrace; PSI, particle size index. Grain with (+) or without (–) pigmented testa layer. /, not known. [b] Note that all white varieties are generally used for tô and porridge preparation and the red ones are used for brewing dolo.

Zymography of POX and PPO. For enzyme zymography, IEF was carried out with a PhastSystem unit (Amersham Pharmacia Biotech) using pH 3−9 gradient gels, according to the manufacturers instructions. The crude extract (2 μL) from each variety was loaded onto the gel. After IEF the gels were stained separately for POX and PPO activities. For the zymography of POX the gel was incubated in 50 mM sodium

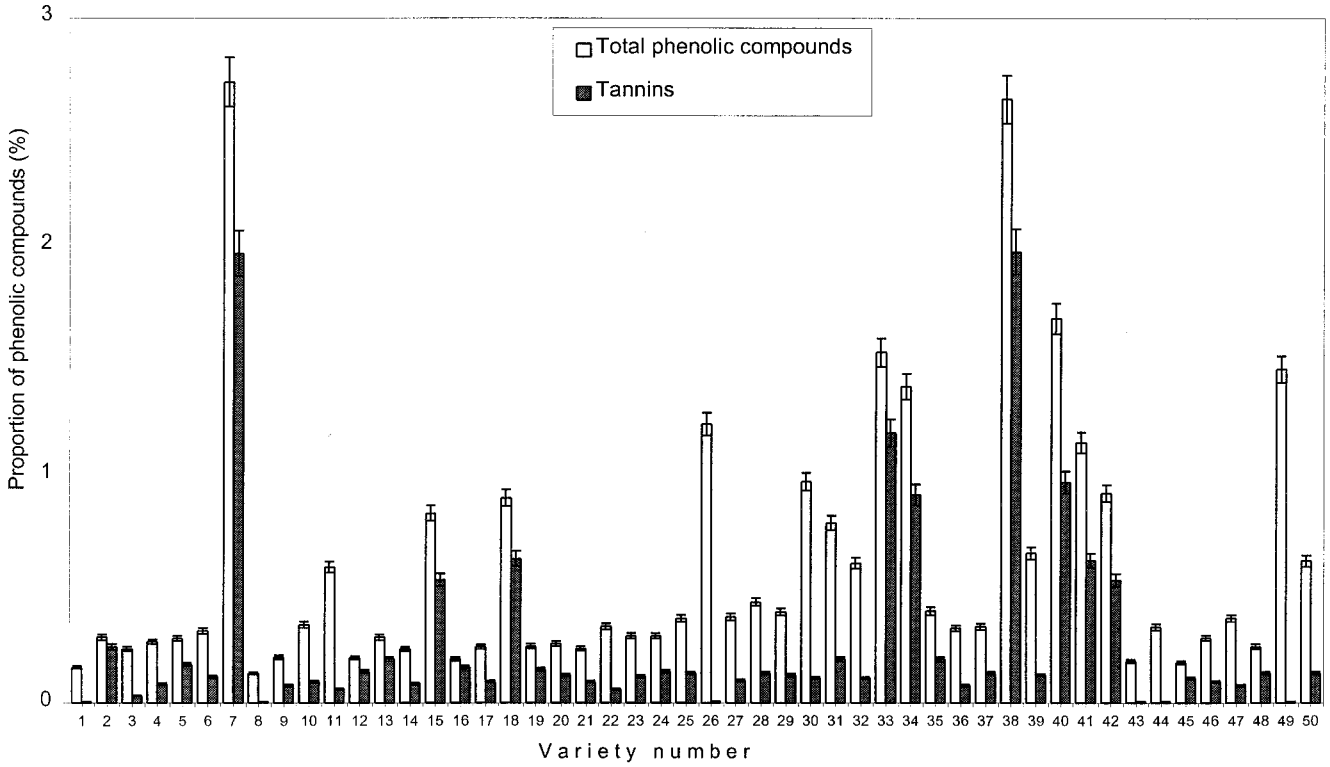

Figure 1. Total phenolic compounds and tannin contents of sorghum varieties. The standard error bars represent the average standard error for each experiment.

Table 2. Pearson Correlation (*r* Values) Matrix between Phenolic Compounds, Oxidative Enzymes, and Proteins of Sorghum Grains

	total phenolic compounds	tannins	monophenolase	*o*-diphenolase	peroxidase
tannins	0.88[a]				
monophenolase	0.12	0.17			
o-diphenolase	–0.04	–0.19	0.35		
peroxidase	–0.24	–0.19	–0.01	0.07	
proteins	0.29	0.39	0.05	–0.23	–0.26

[a] Significant ($p < 0.001$), $n=50$.

citrate buffer pH 5.0, containing 1 mM DAB and 10 mM H_2O_2, for 30 min. The zymography of *o*-diphenolase activity of PPO was performed as described by Dicko et al. (*38*).

Statistical Analysis. All assays were carried out in triplicate, and the means and standard deviations are reported. Differences in mean performance for each composition among sorghum varieties were tested by the Student's *t*-test. Pearson linear correlation coefficients were used to assess relationships among total PC, tannin, PPO, POX, and protein contents.

RESULTS

Phenolic Compounds in Sorghum Varieties. The 50 sorghum varieties varied significantly ($p < 0.01$) in their total PC and tannin contents (**Figure 1**). Total PC and tannin contents were highly positively correlated among varieties (**Table 2**). The mean values of total PC and tannin in the varieties were 0.60 and 0.27%, respectively. The average content of PC was higher in the red grains (1.39 and 0.90% of total PC and tannin, respectively) than in the white grains (0.40 and 0.11% of total PC and tannin, respectively). The average content of total PC is lower in the hard grains (0.31%) than in the soft grains (0.59%). The highest concentration of total PC and tannin was found in variety C7 (2.72 and 1.97%, respectively) and variety C38 (2.64 and 1.97%, respectively). All the varieties contained PC, but some of them (C1, C8, C26, C43, C44, and C49) contained almost no tannin (**Figure 1**). Tannin content-based classification of varieties (**Table 3**) shows that the majority (82%) have low tannin content (≤ 0.25%). Varieties with relatively high tannin content (≥ 0.75%) accounted for 10%. Among tannin containing varieties, the variation was 6-fold. The inter-varietal difference in total PC content was a factor 20.

Table 3. Classification of Burkina Faso Sorghum Grain Varieties Based on Tannin Content

tannin content (%)	group	percentage of varieties
≤ 0.25	low	82
0.26–0.5	medium	4
0.51–0.75	high	4
≥ 0.75	very high	10

Oxidative Enzymes in Sorghum Varieties. The mean values of the monophenolase and *o*-diphenolase specific activities of PPO in sorghum varieties were 0.9 mU/mg and 45.3 mU/mg, respectively. The *o*-diphenolase activity of PPO was 50−100 times higher than the monophenolase activity among varieties (**Figure 2**). Inter-varietal difference in monophenolase activity was a factor of 3, but the varieties exhibited almost similar *o*-diphenolase activity (**Figure 2**). There was no significant ($p < 0.001$) difference between the monophenolase and *o*-diphenolase activities of PPO in the group of red and white grains. The mean value of the POX specific activity (**Figure 3**) in the varieties was 1.90 U/mg. Average POX activity was higher in the group of white grains (2.0 U/mg) than in the red grains (1.6 U/mg). Inter-varietal difference of POX specific activity was at most a factor of 5. Variety C17 (Cauga 108-15) displayed the highest POX specific activity (4.23 U/mg). Lowest POX specific activity (0.81 U/mg) was found in the variety C31

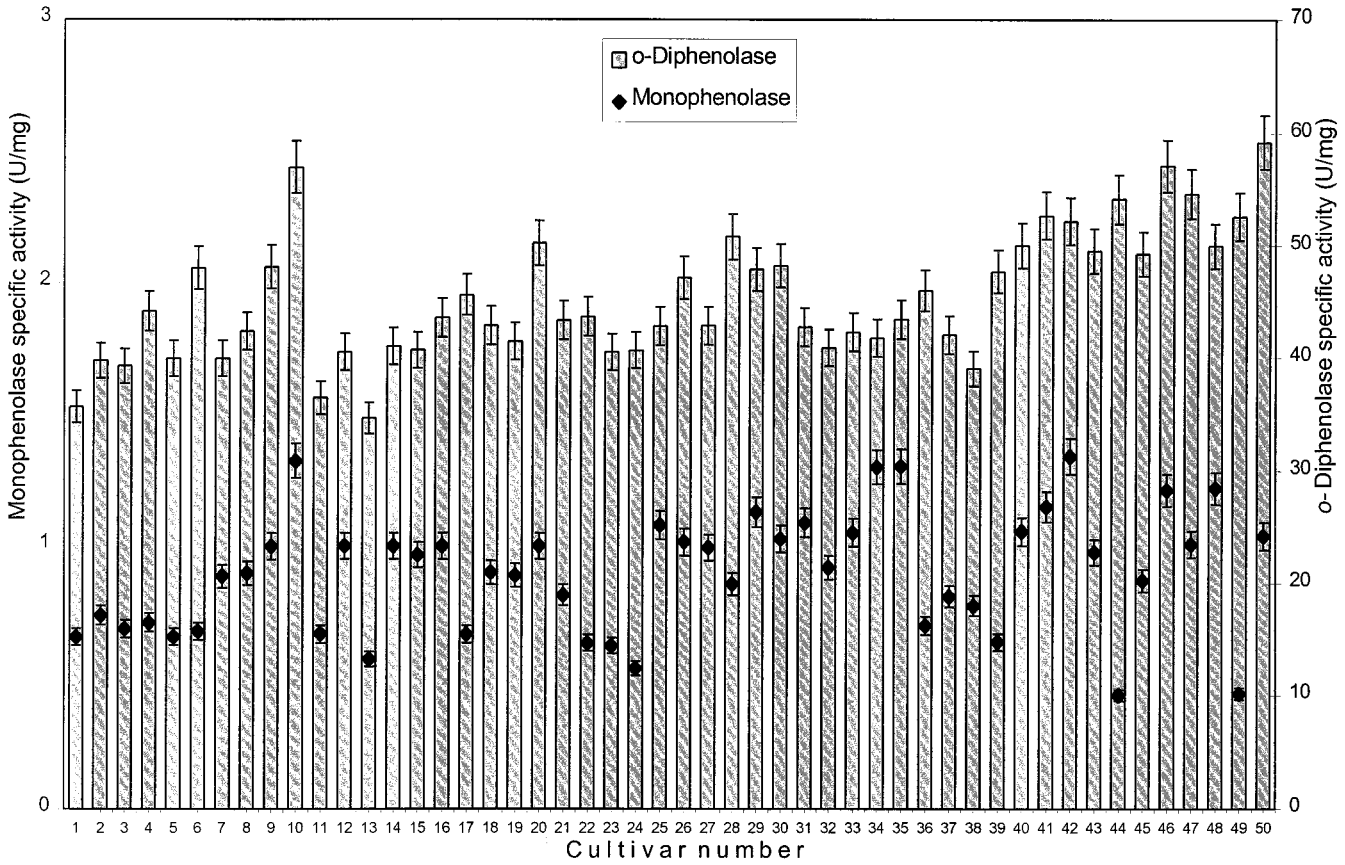

Figure 2. Monophenolase and *o*-diphenolase specific activities of PPO in sorghum varieties. The standard error bars represent the average standard error for each experiment.

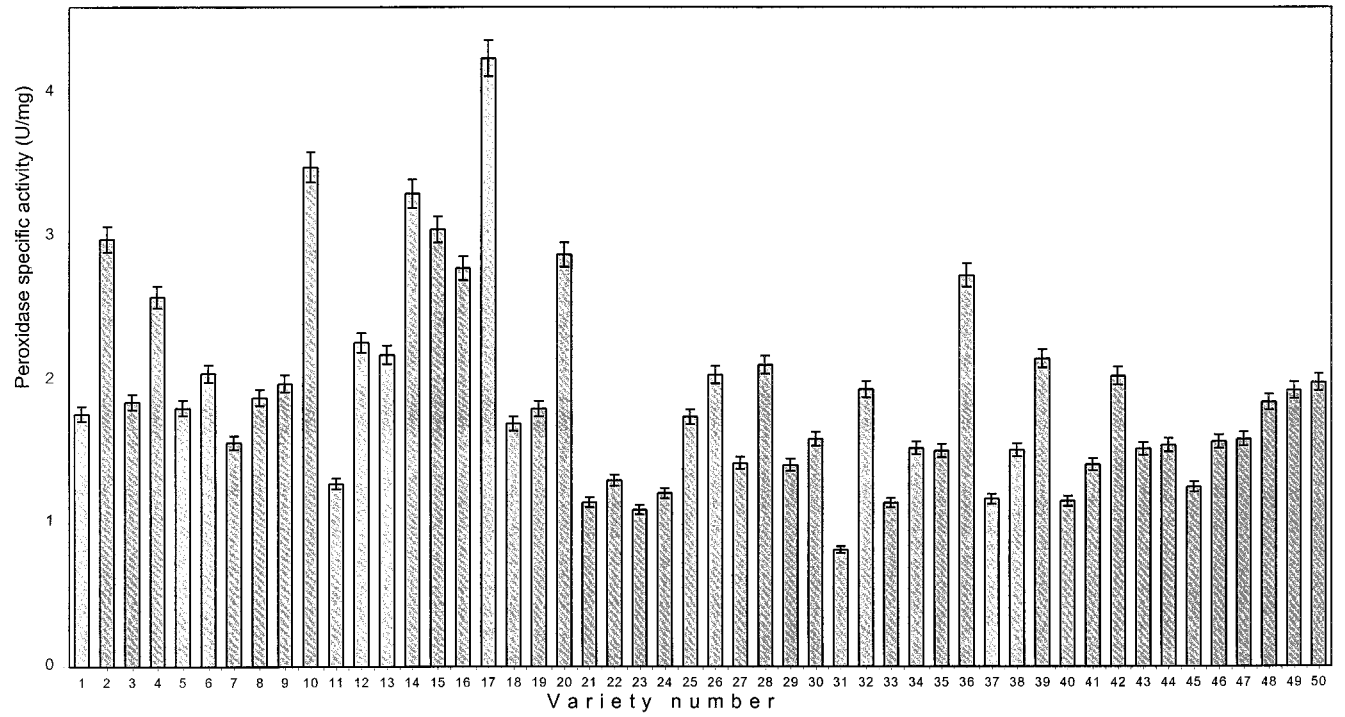

Figure 3. POX specific activities in sorghum varieties. The standard error bars represent the average standard error for each experiment.

(IRAT 202). Specific activity of POX was twenty (C31) to ninety (C17) times higher than the PPO *o*-diphenolase activity. No significant correlation was found between phenolics, monophenolase, and *o*-diphenolase activities of PPO, and POX activity of all varieties (**Table 2**).

Zymography of PPO and POX in the grain extracts allows the detection of isoenzymes present in the varieties. A band of PPO was found at p$I \geq 9$ for all varieties. As an illustration (**Figure 4**), PPO is shown for eight varieties. Cationic (p$I \geq 9$) POX isoenzymes were ubiquitous in all varieties (**Figure 5**).

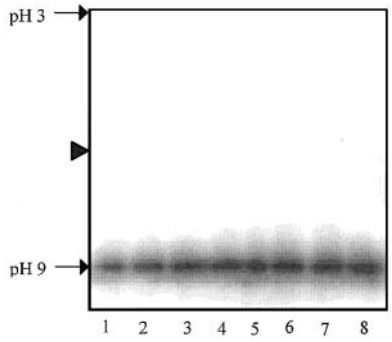

Figure 4. Zymograms of *o*-diphenolase activity of PPO in sorghum varieties. Because all varieties presented the same PPO pattern, eight of them were arbitrarily selected as examples. Lanes 1–8 are crude extracts from grains of sorghum varieties C1, C5, C10, C15, C20, C25, C30, and C35, respectively. The samples were applied in the middle of the gels (at right arrow).

However, in some varieties neutral and anionic POX isoenzymes could be detected in the pH range of 4 to 8 **(Figure 5**).

Comparison of the Average Content of PC, PPO, and POX in Groups of Varieties According to their Food Properties. The average content of PC, PPO, and POX in varieties grouped according to their known properties and food processing is illustrated in **Table 4**. The group of varieties that is less suitable for tô has a low average content of POX (1.2 U/mg) compared to the group of varieties good for tô (1.8 U/mg). Tô varieties have, interestingly, less total PC (0.32%) and tannin (0.11%) than the other groups good for "dolo" and "couscous". Varieties that are good for dolo have noticeably high total PC (1.18%) and tannin (0.71%) contents. Those that are poor for dolo differ in all aspects (PC, PPO, and POX) from those that are good for dolo. The variety used for couscous had the lowest POX activity (0.8 U/mg) of all the varieties investigated **(T**able 4).

DISCUSSION

Phenolic Compounds and their Oxidative Enzymes in Sorghum Grain. Although total contents of PC, tannin, and oxidative enzyme may change according to age/harvesting-time and environmental conditions, it was shown for wheat, barley, and other plants that mainly genetic factors are responsible for differences among varieties (*4*, *5*, *15*, *39*). Because the grains were not dehulled prior to surface-sterilization, and the incubation time was kept short (5 min), sodium hypochlorite is not likely to react with polyphenols. Best extraction results for total phenol and tannins were obtained with HCl-methanol (*32*, *40*) and DMF (*41*), respectively. Whereas some polyphenol assays (FAC, vanillin-HCl, butanol-HCl, Prussian blue, etc.) are supposedly specific for different types of polyphenols (*40*), Folin−Ciocaltau's assay for total phenol has been recommended because of its wide applicability for biological materials (*33*). Although the FAC method for tannins quantitation may include nontannins phenolic compounds, it has been chosen as standard assay for sorghum tannins (*41*). It may allow distinction among sorghum varieties containing free and condensed polyphenols (tannin). In agreement with earlier studies (*32*, *34*), several sorghum varieties were found to contain no tannin. A high tannin content may be a positive agronomic trait but less suitable for food usage due to its alleged anti-nutritional properties (*21*−*23*). In line with the general observation, sorghum grains with high phenolic content were found to be soft (*42*). To prevent the reaction of oxidative enzymes with endogenous polyphenols, insoluble PVP was used in the enzyme's extraction buffer. The occurrence of POX and PPO in mature sorghum grains found in the present study disagrees with those of Glennie (*12*) who found that for *Sorghum bicolor* var. NK-300, PPO and POX activities could not be detected in mature grain. Because cereals do not contain laccase (EC. 1.10.3.2) (*15*), and knowing that 4HA and DHPPA are not oxidized by POX in the absence of hydrogen peroxide (*38*), it may be inferred that the O_2-dependent oxidase activity is essentially due to PPO. The higher *o*-diphenolase of PPO (as compared to the monophenolase activity) is due to the lower turnover rate of the monophenolase activity of plant PPOs (*43*). The band at p$I \geq 9$ may be due to several cationic PPO isoenzymes having high p*I* values. Thus, the difference in monophenolase activity among the varieties might originate from the heterogeneity of cationic PPO isoenzymes (*6*). The varieties are highly polymorph in their POX composition, in agreement with the results of Ollitrault et al. (*11*) who also found varietal differences of POX isoenzymes in germinated sorghum grains. Cationic POXs were also the only detectable isoenzymes in the grain of *Sorghum* var. Frontier 400 (*10*) and the major isoenzymes in the first internodes of *Sorghum vulgare* var. Wheatland milo (*9*). Similar results were reported for barley and wheat kernels, where 93% and 98% of the POX activities, respectively, are cationic isoenzymes (*44*). The average specific activity of POX in the fifty sorghum varieties (1.90 U/mg) is higher than the POX specific activity in both barley (1.35 U/mg) and wheat (1.35 U/mg) kernels (*44*). The inter-varietal difference in POX activity (5-fold) among Burkina Faso sorghums is higher than that found (1.2-fold) between Nigeria sorghums (*45*). The enzyme assays and zymogram studies showed that POXs in sorghum grain have more isoforms and a higher activity than PPOs. Luthra et al. (*8*) analyzed sorghum leaves and also showed that POX activity was several times higher than PPO activity. From this, it may be tentatively concluded that POX is more involved in the in vivo oxidation of PC than PPO. Although the average POX specific activity was higher in the group of white grains than that in the red, the color of the grain could not be significantly linked to its content in POX and PPO. Therefore, the appearance of the grain may be controlled by physiological parameters other than their content in oxidative enzymes. Indeed, several interacting factors (pericarp color and thickness, presence or absence of testa, endosperm texture, etc.) are known to influence the appearance of the grain.

The phenolic, POX, and PPO contents of the grains could not be simply related to the susceptibility or resistance of individual sorghum plant to striga, anthracnosis, sorghum midge, or sooty stripe. This also holds true for the abiotic factors such as drought and photoperiod.

Relationship between PC, PPO, and POX Contents in Grain and Food Application. It is known in industrial brewing that high POX activity may not be desirable, because POX may catalyze the oxidative polymerization of endogenous PC of malt, notably the anthocyanidins, leading to flavor deterioration and the occurrence of haze (*15*, *18*). Dolo sorghum varieties have a higher content in total PC ($p < 0.05$) and tannin ($p < 0.1$), and higher monophenolase activity ($p < 0.05$) than varieties that are poor for dolo. Their content in PC is also higher than that in varieties used for tô and couscous, as well as the mean value. PPO may be involved in the oxidation of endogenous PC to yield the desired opaque color of dolo and influence its organoleptic properties. Thus, red varieties with high PC and PPO contents and a low POX activity may be the best for dolo preparation. Among dolo varieties, C40 (Zouobdo) and C39

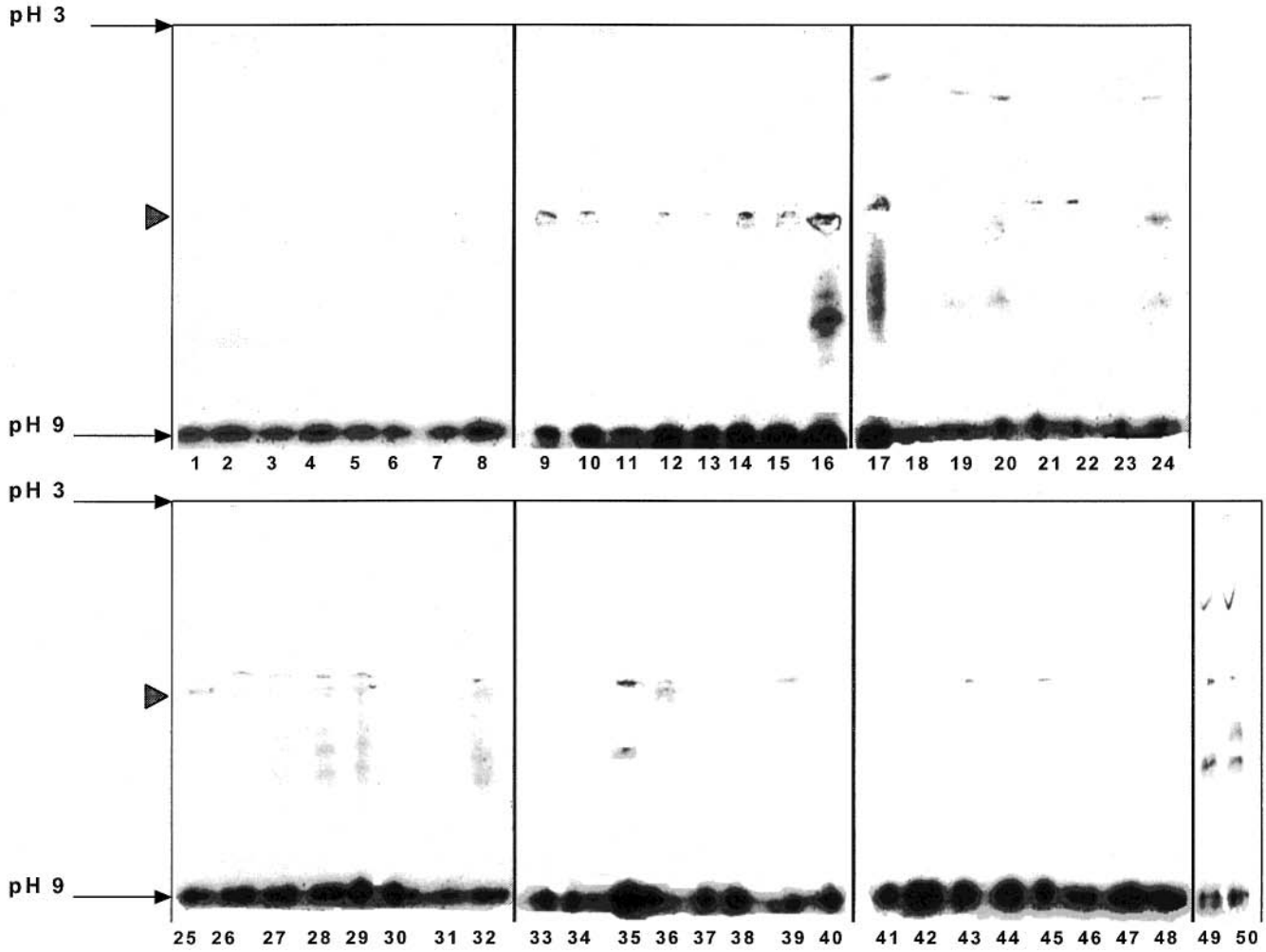

Figure 5. Zymograms of POX activity in sorghum varieties. Each Arabic number represents a sorghum variety. Samples were applied in the middle of the gel (at right arrow).

Table 4. Comparison of the Average Content (Value ± Standard Error) of Phenolic Compounds and Their Oxidative Enzymes in Groups of Sorghum Varieties of Known Food Properties

	group properties	total phenolics (%)	tannins (%)	monophenolase (mU/mg)	*o*-diphenolase (mU/mg)	peroxidase (U/mg)
food	good for tô ($n = 12$)	0.32 ± 0.03	0.11 ± 0.01	0.8 ± 0.1	43.4 ± 1.3	1.8 ±0.1
	poor for tô ($n = 2$)	0.31 ± 0.02	0.09 ± 0.01	0.6 ± 0.1	42.1 ± 3.4	1.2 ± 0.1
	good for dolo ($n = 8$)	1.18 ± 0.05	0.71 ± 0.03	1.1 ± 0.1	47.0 ± 3.8	1.5 ± 0.1
	poor for dolo ($n = 2$)	0.29 ± 0.01	0.25 ± 0.01	0.7 ± 0.1	39.7 ± 3.2	3.0 ± 0.2
	good for couscous ($n = 1$)	0.79 ± 0.04	0.19 ± 0.01	1.1 ± 0.1	42.7 ± 3.4	0.8 ± 0.1
grain	hard grain ($n = 4$)	0.31 ± 0.02	0.12 ± 0.01	0.8 ± 0.1	42.2 ± 3.4	1.3 ± 0.1
	soft grain ($n = 4$)	0.59 ± 0.03	0.08 ± 0.01	0.9 ± 0.1	46.1 ± 3.7	1.7 ± 0.1
	white color ($n = 40$)	0.40 ± 0.02	0.11 ± 0.01	0.9 ± 0.1	45.2 ± 3.5	2.0 ± 0.1
	red color ($n = 10$)	1.39 ± 0.07	0.90 ± 0.01	1.0 ± 0.1	45.7 ± 3.6	1.6 ± 0.1
	mean value ($n = 50$)	0.60 ± 0.03	0.27 ± 0.01	0.9 ± 0.1	45.3 ± 2.3	1.9 ± 0.1

(Nafo-Natogué) have high (0.97%) and low (0.12%) tannin contents, respectively. The later also has a medium POX activity (2.15 U/mg), therefore, it may be the most suitable for industrial brewing purposes. The group of varieties poor for tô differs from the group good for tô, exclusively with respect to its low POX content. In tô preparation, the formation of a thick paste is necessary. Thus, in analogy with what was found in wheat dough (*20*) POX may mediate the gelatinization of sorghum flour during tô preparation. Among tô varieties, C1 (CEF 322/53-1-1) and C21 (Kaapelga) have white flour and low PC and PPO contents, and a suitable POX activity. Hence, these varieties may be the most suitable for this local food. The variety C31 (IRAT 202), reputed to be the best sorghum variety for "couscous" preparation in West Africa, notably in Senegal, deviates from the other varieties in its low content of POX (0.8 U/mg). That may be justified by the fact that for couscous preparation the formation of a gel mediated by POX via the cross-linkage of macromolecules is not desired. Therefore, "Couscous" varieties might have a low POX content. It is known that the suitability of sorghum varieties for food and beverages is a function of the chemical and physical properties of kernels and process conditions. In addition to these parameters, the composition of the grain in oxidative enzymes may also play an important role in grain quality.

Although PCs and their oxidative enzymes are thought to be involved in plant responses to physical stress and to pathogens,

the present study shows that these constituents of the grains could not be used as a marker for the described agronomic properties of the fifty varieties. It remains to be investigated whether these constituents in the whole plant are useful indicators, or whether specific polyphenols and their oxidative enzymes are induced upon challenging the plant with a pathogen. The present work has given some indications for the use of biochemical properties as criteria to select for varieties most suited for a specific food application.

ABBREVIATIONS USED

ABTS, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); BSA, bovine serum albumin; DAB, 3,3′-diaminobenzidine; DHPPA, 3,4-dihydroxyphenylpropionic acid; DMF, N,N′-dimethylformamide; FAC, ferric ammonium citrate; IEF, isoelectrofocusing; 4HA, 4-hydroxyanisole; MBTH, 3-methyl-2-benzothiazolinone hydrazone hydrochloride; PC, phenolic compounds; POX, peroxidase; PPO, polyphenol oxidase; PVP, poly(vinylpyrrolidone).

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