International Journal of Engineering Research and Science & Technology

www.ijerst.org

ISSN : 2319-5991

Vol. 20 No. 4 (2024)



ijerst.editor@gmail.com

editor@ijerst.com

International Journal of Engineering Research and Science & Technology

PHENOLIC COMPOUNDS AS QUALITY-GRADE MARKERS FOR THE PREPARATION OF HUMAN FOODS

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Abstract: Phenolic compounds and related enzymes such as phenol biosynthesizing enzym es (phenylalanine ammonia lyase) and phenol catabolizing enzymes (polyphenol oxidase and peroxidase) are determinants for sorghum utilization as human food because they influence product properties during and after sorghum processing. Phenolic compounds are quality-grade markers for the preparation of several foods because of enzyme inhibitory activities, color, or antioxidant activities. Large inter-varietal differences in contents of phenolic compounds and their antioxidant activities among sorghum varieties exist. Moreover, some red sorghum varieties have higher antioxidant activities than the most important sources of natural antioxidants. Oxidation products of peroxidase and polyphenol oxidase (benzoquinones and polymeric compounds) affect food quality. This paper reviews the current advances in phenolic compounds and phenolic enzymes in sorghum as human food, with emphasis on nutritional and health aspects. This may provide some guidance for researchers in further Investigations and for industries in developing practical health agents and functional foods.

Key words: sorghum, phenolic compounds, antioxidants, 3-deoxyanthocyanidins, proanthocyanidins, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase.

INTRODUCTION

Sorghum bicolor (L.) Moench is the fifth most important cereal crop after wheat, rice, maize, and barley in terms of production (FAO, 2005). The total world annual sorghum production is over 60 million tons from a cultivated area of 46 millions ha. Sorghum is particularly adapted to drought prone areas: hot, semi- arid tropical environments with 400-600 mm rainfall-areas that are too dry for other cereals. The sorghum genome is currently sequenced (Paterson et al., 2003; http://fungen.org/Sorg-hum.htm).

Phenolic sorghum phytochemicals are important for human nutrition (Awika et al., 2004a). Phenolic compounds are the most widely distributed secondary metabolites, ubiquitously present in the plant kingdom. Among cereals, sorghum has the highest content of phenolic compounds reaching up to 6% (w/w) in some varieties (Deshpande et al., 1986; Beta et al., 1999, Doka et al., 2004; Awika and Rooney, 2004a, Dicko et al., 2005a). Almost all classes or phenolic compounds arefound in sorghum (Chung et al., 1998; Krueger et al., 2003, Awika and Rooney, 2004a).

Proanthocyanidins (condensed tannins), originally classified as antinutritional factors, may have health benefits for humans (Waniska, 2000, Parr and Bolwell, 2000; Clifford, 2000; Awika, 2004a). Some flavanols, e.g. flavan-4-ols have particular therapeutic interest because of their antitumor activity (Ferreira and Slade, 2002). Procyanidins may inhibit the growth of several viruses including the human immunodeficiency virus 1 (HIV-1) (Chan and Kim, 1998; Okuda et al., 1991, Lu et al., 2004). Phenolic compounds, together with other natural

compounds scavenge free radicals (antioxidant activity). Interestingly, independent of germination, sorghum grains display high antioxidant activities related to their phenolic content (Dicko et al., 2005a, Dykes et al., 2005).

Moreover, some red sorghum varieties have higher antioxidant activities than the most important sources of natural antioxidants such as Vaccinium species, e.g. blackberries (Awika et al., 2004a; Dicko et al., 2005a). Since sorghum is a staple food in many African countries, it may be the main potential source of natural antioxidants. Epidemiological studies suggest that the consumption of whole cereal grains including sorghum lowers the mortality from cardiovascular disease, which is probably linked to their antioxidant properties (Kushi et al., 1999; Awika and Rooney, 2004a).

The biosynthesis of phenolic compounds in plants proceeds through the production of phenylalanine, which is subsequently deaminated by the enzyme phenylalanine ammonia lyase (PAL) . PAL is indirectly associated with the synthesis of several phenolic constituents, including cell wall polymers (Figure 1) (Parr and Bolwell, 2000). PAL activity has been detected in half of the sorghum varieties before germination and in all screened varieties after germination (Dicko et al., 2006). Polyphenol oxidases and peroxidases are the main enzymes involved in cereal browning. In sorghum, PPO is present in the leaves (Stafford and Dresler, 1973; Vaughn and Duke, 1981) and in the grain (Glennie, 1981; Dicko et al., 2002a, 2006a). Peroxidases are ubiquitously present and relatively abundant in sorghum grain both before and after germination (Dicko et al., 2002a, 2006a). Polyphenol oxidase and peroxidases being involved in the oxidative cross-linking of phenolic compounds are determinants of food quality by influencing product properties during and after processing (Tomas-Barberan and Espín, 2001).

SORGHUM PHENOLIC COMPOUNDS BIOCHEMISTRY

Classes of phenolic compounds in sorghum

Phenolic compounds, of which more than 8000 are known, embrace a wide range of plants secondary metabolites (Harborne, 1994; Pietta, 2000). Phenolic compounds are the most widely distributed secondary metabolites, ubiquitously present in the plant kingdom. Located in the vacuole, they are found in free form or linked to carbohydrates (glucose, galactose, rhamnose, mannose, rutinose etc.). Among cereals, sorghum has the highest content of phenolic compounds reaching up to 6% (w/w) in some varieties (Deshpande et al., 1986; Beta et al., 1999, Awika and Rooney, 2004a) (Tables 1 and 2). While all sorghums contain phenolic compounds, its genotype and the environment in which it is grown influence the amount present in any particular cultivar. The main classes of phenolic compounds are simple phenols, hydroxybenzoic acids, hydroxycinnamic acids, flavonoids (flavanols, flavones, flavanones, isoflavones and anthocyanins), chalcones, aurones (hispidol), hydroxycoumarins, lignans, hydroxystilbenes and polyflavans (proanthocyanidins and pro-deoxyanthocyanidins) (Chung et al., 1998; Krueger et al., 2003).

These compounds are soluble in water or organic solvents (methanol, HCl-methanol, acetone, dimethylformamide, etc.). Sorghum does not contain tannic acid and hydrolysable tannins (Waniska, 2000, Awika et al., 2004a). Sorghums with a pigmented testa and spreader genes (B1B2S) or with purple/red plants and thick pericarp genes have the highest levels of phenolic compounds (Dykes et al., 2005). Sorghums with a black pericarp have higher levels of flavan-4-ols and anthocyanins than the other varieties. This suggests that genes for plant color, pericarp thickness, presence of a pigmented testa, and spreader genes increase phenolic levels (Dykes et al., 2005, Dicko et al., 2005a).

Lignans and hydroxystilbenes have not been detected in sorghum grain (Awika et al., 2004a). Tables 1 and 2 give the approximate range and intervarietal contents of phenolic

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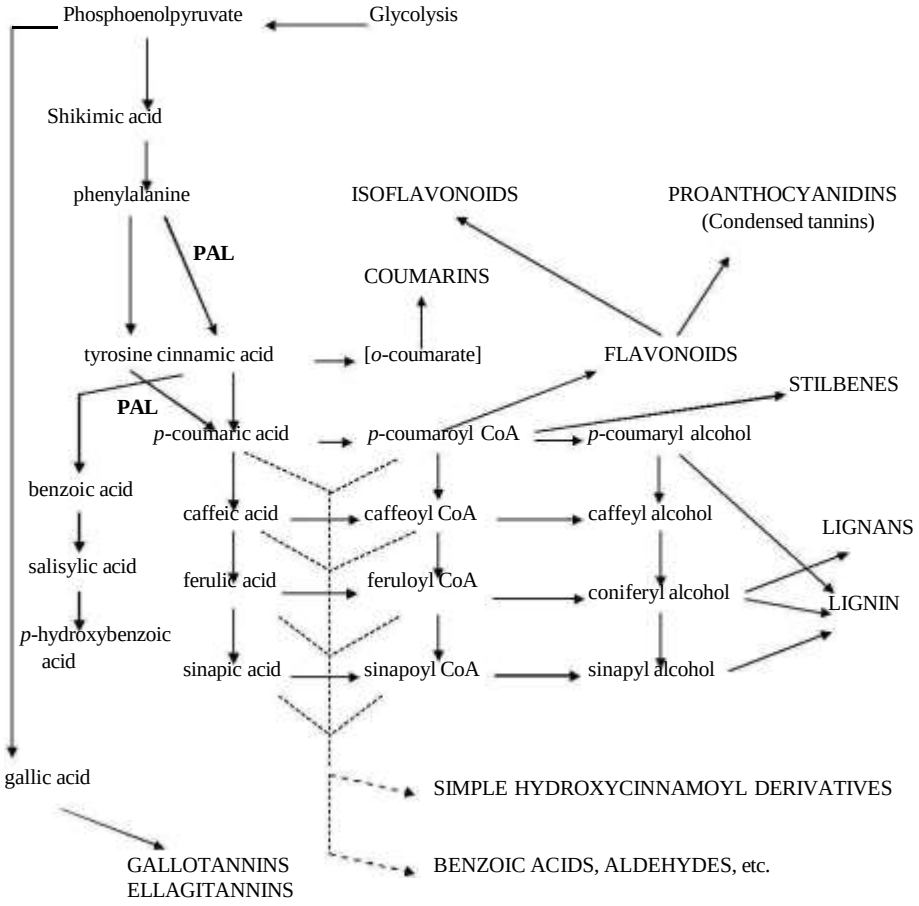

Figure 1. Schematic illustration of the biosynthesis of different phenolics from shikimic acid (Parr and Bolwell, 2000; Ryan and Robards, 2002). PAL= phenylalanine ammonia lyase.

Table 1. Phenolic contents in sorghum grain

Phenolic compounds	Content mg/g dry weight	References
Hydroxybenzoic acids		
p-Hydroxybenzoic	15-36	Hahn et al. (1983)
Gallic	26-46	Hahn et al. (1983)
Protocatechuic	24-141	Hahn et al. (1983)
Vanillic	8-50	Hahn et al. (1983)
Hydroxycinnamic acids		
p-Coumaric	100-200	Verbruggen et al. (1993)
Caffeic	25-52	Hahn et al. (1983)
Ferulic	300-500	Verbruggen et al. (1993)
Sinapic (sinapinic)	50-140	Hahn et al. (1983)
Flavonoids		
Anthocyanins	0-2800	Sérémé et al. (1992); Awika et al. (2003, 2004b)
3-deoxyanthocyanidins	0-4000	Dicko et al. (2005a)
Flavan-4-ols	0-1300	Bate-Smith (1969); Audilakshmi et al. (1999); Dicko et al. (2005a)
Proanthocyanidins	0-68000	Beta et al. (1999); Awika and Rooney (2004a); Dicko et al. (2005a)

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Table 2. Comparison of total phenolic compounds and phenolic enzymes in sorghum varieties

Variety code	Total phenolics[a] (%)		Antioxidant activities[b]		PAL ($mU\ mg^{-1}$)		POX ($U\ mg^{-1}$)		mono-PPO[e] ($mU\ mg^{-1}$)		di-PPO ($mU\ mg^{-1}$)	
	g-	g+	g-	g+	g-	g+	g-	g+	g-	g+	g-	g+
V1	0.58	0.96	43	54	nd	10.3	68.8	81.2	0.7	1.0	35.6	16.3
V2	0.85	0.61	59	21	3.9	1.7	11	49.9	0.7	0.2	39.7	18.7
V3	0.72	0.69	42	42	2.3	9.9	17.4	79.9	0.7	0.8	39.2	24.4
V4	0.66	0.41	45	12	4.1	1.8	15.1	106.7	0.7	0.9	44.2	14.6
V5	0.72	0.63	44	19	nd	5.8	93.3	175.9	0.7	0.9	39.9	11.3
V6	1.38	1.22	52	64	1.1	11.1	31.7	100.7	0.7	0.7	48.0	14.1
V7	0.59	0.87	33	50	nd	15.5	81.8	124.7	0.9	0.7	39.9	23.0
V8	0.71	0.67	40	35	2.4	8.7	59.9	76.3	0.9	0.5	42.4	15.7
V9	0.87	0.91	42	42	nd	2.5	56.5	75.8	1.0	0.1	48.1	14.0
V10	0.68	0.88	48	46	nd	10.1	60.8	71.4	1.3	0.2	57.0	14.5
V11	0.69	0.73	44	40	nd	7.1	50.9	81.6	0.7	0.2	36.4	20.7
V12	0.64	0.81	34	39	nd	3.2	73.2	99.9	1.0	0.5	40.5	17.3
V13	0.61	0.82	29	42	nd	8.8	44.2	95.4	0.6	0.5	34.6	15.1
V14	0.55	0.81	29	36	13.1	6.2	41.3	54.5	1.0	0.8	41.0	9.5
V15	0.76	1.22	37	51	9.0	11.2	59.7	144.3	1.0	0.8	40.7	0.9
V16	0.55	0.86	35	38	9.2	1.2	42.8	184.0	1.0	0.3	43.6	15.1
V17	1.28	1.47	66	53	3.1	2.8	20.9	89.6	0.7	0.4	45.6	14.7
V18	1.74	1.85	55	43	6.2	6.0	8.8	89.2	0.9	0.7	42.9	18.1
V19	3.01	2.95	80	70	2.9	6.6	15.2	109.7	0.9	1.0	41.4	20.3
V20	0.71	0.84	49	32	nd	0.9	22.6	105.3	1.0	1.4	50.3	9.7
V21	0.76	0.74	36	51	nd	16.5	62.3	75.8	0.8	1.9	43.3	19.8
V22	0.82	0.51	40	17	4.7	2.0	24.4	42.5	0.6	0.5	43.7	18.3
V23	0.64	0.78	37	19	5.1	0.6	23.2	83.0	0.6	1.2	40.5	20.5
V24	0.66	0.46	35	14	nd	1.4	25.7	56.5	0.5	0.8	40.6	27.0
V25	0.82	0.72	34	31	5.6	2.3	21	98.5	1.1	0.8	42.8	17.2
V26	0.92	0.75	42	25	3.9	1.2	24.6	75.3	1.0	0.3	47.1	21.0
V27	1.20	1.22	62	49	nd	3.8	12	15.6	1.0	0.6	42.8	15.3
V28	0.96	0.61	44	19	2.2	9.2	18.9	16.4	0.9	0.8	50.8	16.9
V29	0.87	0.79	48	22	4.1	1.9	21.4	49.1	1.1	0.6	47.9	13.0
V30	1.50	1.18	67	42	4.4	8.1	31.5	82.4	1.0	2.3	48.3	18.5
V31	0.65	0.63	35	37	nd	0.1	86.6	107.5	1.1	1.9	42.7	24.4
V32	0.73	0.83	36	48	nd	13.7	40.2	76.5	0.9	0.9	40.9	39.5
V33	0.60	0.97	30	43	2.7	13.6	37	92.6	1.1	1.4	42.3	40.7
V34	0.81	1.02	43	41	5.1	16.9	18.4	26.1	1.3	1.3	41.8	62.5
V35	1.28	0.97	52	44	3.6	19.7	36.3	45.7	1.3	1.9	43.4	54.6
V36	0.70	0.99	34	46	nd	30.1	71.7	86.6	0.7	1.6	46.0	43.5
V37	1.47	1.75	71	58	2.0	6.0	35.9	88.3	0.8	1.1	42.0	42.1
V38	0.66	1.16	40	45	nd	7.4	75.8	180.3	0.8	1.4	39.0	45.0
V39	0.82	0.76	51	49	nd	13.7	17.9	37.4	0.6	1.6	47.7	47.3
V40	0.72	1.01	40	47	nd	17.1	25.8	53.1	1.1	1.5	50.0	34.1
V41	0.73	0.7	30	52	7.7	8.7	31.7	39.5	1.1	2.1	52.7	46.3
V42	0.74	0.55	27	38	7.3	10.8	7.9	58.8	1.3	1.5	52.2	37.8
V43	0.83	1.05	26	53	1.0	12.7	23.9	51.7	1.0	1.4	49.6	11.7
V44	0.45	0.63	17	40	6.1	11.9	18.3	53.8	0.4	1.5	54.2	42.1
V45	0.98	0.77	41	46	nd	9.8	42.3	51.0	0.9	1.2	49.3	37.2
V46	0.63	0.71	16	38	nd	8.7	39.8	65.7	1.2	1.6	57.1	36.2
V47	1.10	0.96	36	46	nd	13.1	47.1	96.2	1.0	2.3	54.7	33.1
V48	0.58	0.61	20	40	nd	12.6	13.4	51.5	1.2	1.0	50.0	34.8
V49	0.46	0.81	25	46	nd	12.7	67.5	82.6	0.4	1.3	52.6	28.3
V50	1.71	1.01	67	52	1.7	7.1	32.3	58.8	1.0	1.4	59.2	39.2
Mean	0.88	0.92	42	41	2.49	8.49	38.21	79.90	0.90	1.05	45.3	25.5
Range	0.5-3	0.5-2.9	16-80	14-70	0-13	0.1-30	8-93	16-184	0.4-1.3	0.1-2	36-59	1-63
Std error	0.05		2		0.4		4		0.05		2	

[a] Gallic acid equivalents; [b] Antioxidant activities (µmol Trolox equivalents/g of fresh matter); [c] PAL = phenylalanine ammonia lyase, [d] POX = peroxidase, [e] mono-PPO = monophenolase activity of polyphenol oxidase (PPO), [f] *o*-diphenolase activity of PPO. Enzymes activities are expressed in terms of specific activities (Units/mg of protein). g- = ungerminated sorghum; g+ = germinated sorghum. Data is from Dicko et al. (2005a, 2006a).

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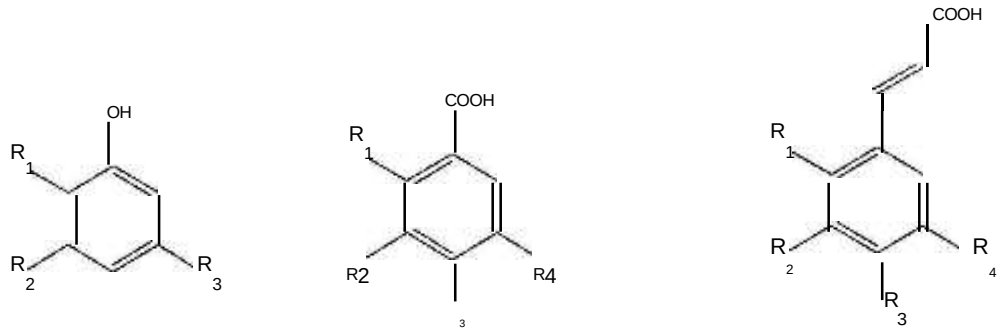


Simple phenols

Phenol: $R_1 = R_2 = R_3 = H$
Catechol: $R_1 = OH$, $R_2 = R_3 = H$
Resorcinol: $R_2 = OH$, $R_1 = R_3 = H$
Phloroglucinol: $R_2 = R_3 = OH$, $R_1 = H$

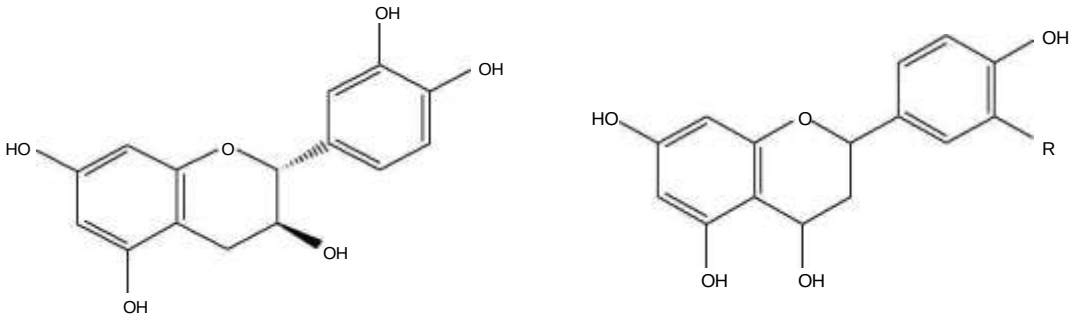
Hydroxybenzoic acids

Gallic: $R_1 = H$, $R_2 = R_3 = R_4 = OH$
Gentisic: $R_1 = R_4 = OH$, $R_2 = R_3 = OH$
Salicylic: $R_1 = OH$, $R_2 = R_3 = R_4 = H$
p-(OH)-Benzoic: $R_1 = R_2 = R_4 = H$, $R_3 = OH$
Protocatechuic: $R_1 = R_4 = H$, $R_2 = R_3 = OH$
Syringic: $R_1 = H$, $R_2 = R4 = OCH_3$, $R_3 = OH$
Vanillic: $R_1 = R_4 = H$, $R_3 = OH$; $R_2 = OCH_3$

Hydroxycinnamic acids

p-Coumaric: $R_1 = R_2 = R_4 = H$, $R_3 = OH$
Caffeic : $R_1 = R_4 = H$, $R_2 = R_3 = OH$
Ferulic: $R_1 = R_2 = H$, $R_4 = OCH_3$, $R_3 = OH$
Sinapic : $R_1 = H$, $R_2 = R_4 = OCH_3$, $R_3 = OH$

Figure 2. Structures of monophenols found in sorghum (Reed, 1995; Towo et al., 2003; Awika et al., 2004a).



Flavan-3-ol
catechin

Flavan-4-ols
Apiforol (leucoapigeninidin) : R = H
Luteoforol (leucoluteolinidin) : R = OH

Figure 3. Structure of some sorghum flavanols (Melake-Berhan et al., 1996; Hagerman, 2005).

compounds in sorghum varieties.

Simple phenols and phenolic acids

Simple phenols are relatively rare in plants. Catechol and resorcinol were reported in sorghum grain; however their concentrations were not given (Watt and Breyer-Brandwijk, 1962; Czarnota et al., 2003; Towo et al., 2003). These compounds are undesired in food products because they are carcinogenic, hepatotoxic and goitrogenic (Gaitan et al., 1989, Reed, 1995). Interestingly, if present they may be removed by food processing like heating (Gaitan et al., 1989). Phenolic acids in sorghum include hydroxybenzoates and hydroxycinnamates (Figure 2). They are found in free form or bound as esters, and are concentrated in the outer layers of the grain (Waniska, 2000; Awika et al., 2004a). The most abundant phenolic acids in sorghum are ferulic acid and p-coumaric acid (Hahn et al., 1983; Verbruggen et al., 1993).

Flavonoids

Flavonoids sensu latu constitute the largest class of phenolic compounds with more than 3000 structures, possessing in common a flavylium unit (C_6-C_3-C_6) (Iacobucci and Sweeny, 1983). Sorghum contains flavonoids such as flavanols (flavan- 3-ols, flavan-4-ols, etc., Figure 3), flavanones, flavones and anthocyanins (Haslam, 1998; Awika, 2004a). The flavan-4-ols apiforol

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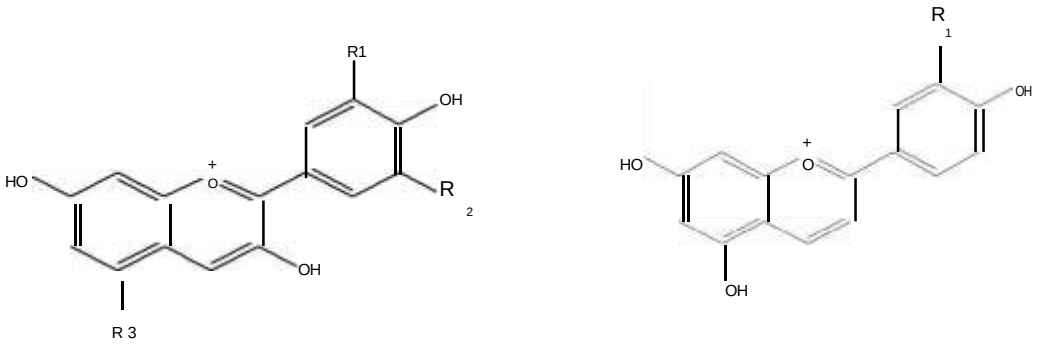


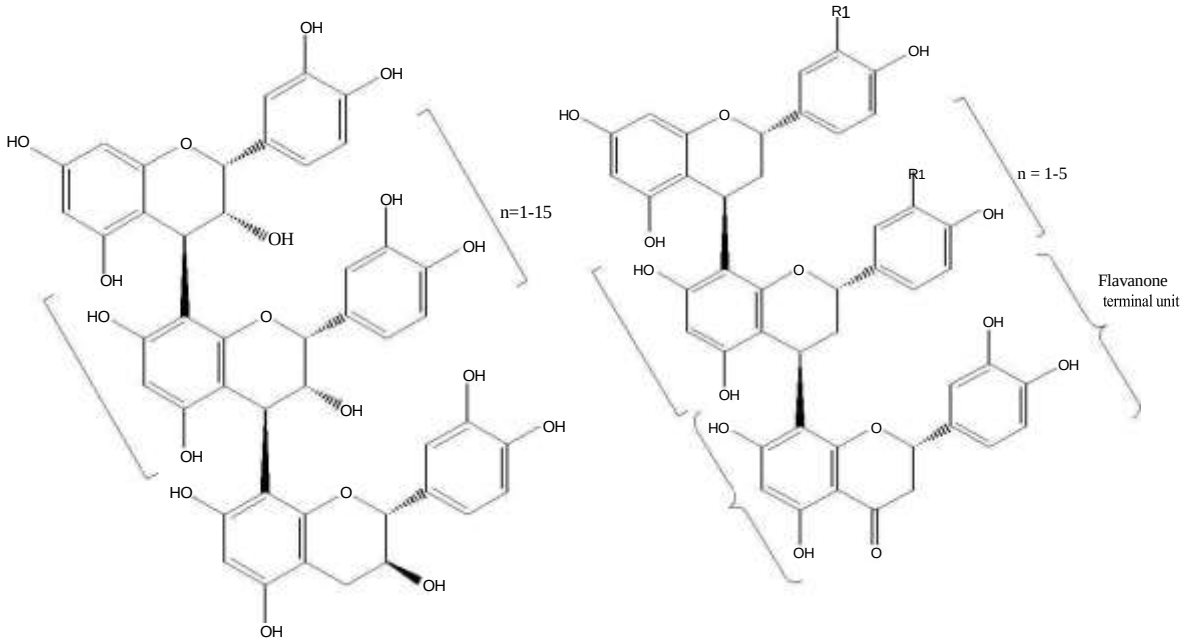
Anthocyanidins

Fisetinidin : $R_1 = R_2 = OH, R_3 = H$
Cyanidin : $R_1 = OH ; R_2 = H, R_3 = OH$
Pelargonidin : $R_1 = R_2 = H, R_3 = OH$
Peonidin : $R_1 = OCH3 ; R_2 = H, R_3 = OH$
Malvidin : $R_1 = R_2 = OCH_3, R_3 = OH$
Delphinidin $R_1 = R_2 = R_3 = OH$
Petunidin $R_1 = OCH3 ; R_2 = R_3 = OH$

3-Deoxyanthocyanidins

Apigeninidin : $R_1 = H$
Luteolinidin : $R_1 = OH$

Figure 4. The main sorghum anthocyanidins and 3-deoxyanthocyanidins (Kouda-Bonafos et al., 1996; Palé et al., 1997; Awika et al., 2004a, 2004b).



Proanthocyanidins

Pro-3-deoxyanthocyanidins

R_1 = H, pro-apigeninidins
R_1 = OH, pro-luteolinidins

Figure 5. Structure of sorghum polyflavans (Krueger et al. 2003; Chen and Hagerman, 2004; Awika and Rooney, 2004a).

(pro-apigeninidin or leuco-apigeninidin) and tuteoforol (proluteolinidin or leuco- luteolinidin) are abundant in sorghum (Dicko et al., 2005a), and precursors of apigeninidin and luteolinidin, respectively (Ferreira and Slade, 2002; Haslam, 1998; Hagerman, 2005). Sorghums with a black pericarp have higher levels of flavan-4-ols and anthocyanins than the other varieties (Dykes et al., 2005).

The most abundant anthocyanins in sorghum grain are 3-deoxyanthocyanidins, e.g. apigeninidin and luteolinidin (Figure 4) (Bate- Smith, 1969; Kouda et al., 1994, 1996; Palé et al., 1997; Awika et al., 2004b). The red color of

the grain's pericarp is essentially due to the presence of 3-deoxyanthocyanidins (Bate-Smith, 1969). Like most anthocyanidins, these compounds are used as natural food colorants (Morazzoni and Magistretti, 1990; Coultate, 1996; Awika et al., 2004a). As a food (E163) or pharmaceutical (Myrtocyan) additive, these phenolic compounds represent a world market of 250 millions US dollars (Morazzoni and Magistretti, 1990; Coultate, 1996). 3-Deoxyanthocyanidins have interesting food applications because of their thermal and color stability (Iacobucu and Sweeny, 1983, Awika et al., 2003, 2004b). The 3-deoxyanthocyanidins (3-DAs), are particularly abundant in notably red sorghum grain (Dicko et al., 2005a), but rare or absent in other plants (Palé et al., 1997; Awika and Rooney, 2004a; Awika et al., 2004b). 3-DAs are of interest because they are more stable in organic solvents as well as in acidic solutions than anthocyanidins commonly found in fruits, vegetables and other cereals (Kouda-Bonafos et al., 1996; Palé et al., 1997; Awika et al., 2004b). This has been suggested as a potential advantage of sorghum as a viable commercial source of anthocyanins (Awika and Rooney, 2004a).

Polyflavans

The term polyflavan is referred to phenolic compounds formed by polymers of flavylium units in which, some hydrogen groups are substituted with hydroxyl groups (Krueger et al., 2003, Awika and Rooney, 2004a). Most polyflavans are often called condensed tannins, but this generic name is sometimes confusing because it does not give a structural definition of the compounds. The polyflavans found in sorghum are essentially polymers of flavan-3-ols (proanthocyanidins) and pro-3-deoxyanthocyanidins (Figure 5). Sorghum proanthocyanidins consist of flavan-3-ol units linked by C-C (type B proanthocyanidins) and occasionally C-O-C (type A proanthocyanidins) bonds ranging from one to fifteen (Krueger et al., 2003, Awika and Rooney, 2004a). The most abundant polyflavans in sorghum are homopolymers of catechin/epicatechin with uniform B-type interflavan bonds (Krueger et al., 2003). Not all sorghum varieties contain these polyflavans because their content is genetically governed by B1-B2 genes (Serna-Salvidor and Rooney, 1995; Butler, 1992; Waniska, 2000). In general, varieties with pigmented testa layers contain proanthocyanidins (Waniska, 2000, Dicko et al., 2002a, 2005a).

The main pro-deoxyanthocyanidins found in sorghum are pro-apigeninidins and pro-luteolinidins (Figure 5). Although present in sorghum, these polyflavans are very rare in other plants (Stafford, 1990). Hydrolysis of pro-apigeninidins and pro-luteolinidins yields apigeninidins and luteolinidins, respectively (Hagerman, 2005).

Role of phenolic compounds in plants

Phenolics play an important role in plant metabolism, but also protect the plant against stresses. For instance, it has been recently shown that flavonoids, such as cate chin, regulate the auxin transport in plants, and, therefore, play an important role in plant development (Brown et al., 2001). Several studies have shown that the plant resistance to both biotic (pathogens and predators) and abiotic (UV-radiation, drought, etc.) stresses is related to phenolic compounds (Parr, 2000, Dicko et al., 2005b). All classes of phenolic compounds (hydroxybenzoic acids, hydroxycinnamic acid derivatives, flavonoids, polyflavans, etc.) are involved in the resistance mechanisms. Sorghum 3-deoxyanthocyanidins are phytoalexins (plant-microbe interaction) or allelochemicals (plant-plant interaction), involved in plant resistance to biotic stresses such as fungi and parasitic plant invasion (Weiergang et al., 1996; Lo et al., 1999; Parr and Bolwell, 2000; Weir et al., 2004). Proanthocyanidins, 3- deoxyanthocyanidins and flavan-4-ols prevent losses from premature germination and damage due to mold (Waniska, 2000). Hydroxycinnamic acids are constituent of plant cell- wall polymers such as lignin, suberin and cutin. These polymers are physical barriers against invading predators, drought and several other stresses (Parr and Bolwell, 2000).

Health related properties of phenolic compounds

Condensed tannins, e.g. proanthocyanidins, may bind to proteins, carbohydrates and minerals, thereby affecting the nutritional and functional value of the bound constituents. Of major nutritional concern is the ability of proanthocyanidins to bind strongly to large proteins and to proline-rich proteins, thereby reducing their digestibility (Butler, 1992) . Proanthocyanidins may be antinutritional through direct interference within the animal body of the digestive processes or inhibition of hydrolytic enzymes through formation of complexes (Nguz et al., 1998). One of the most undesired effects of phenolic compounds is their pro-oxidant activity, which can lead to mutagenicity and carcinogenicity (Stoewsand et al., 1984; Morton, 1992). This pro- oxidant activity depends strongly on the type of phenolic compounds (Awad et al., 2000; Rietjens et al., 2001; Awad, 2002; van der Woude et al., 2002).

The earlier idea of classification of proanthocyanidins as antinutritional factors is now questioned because they also are believed to have health benefits for humans (Hagerman et al., 1998; Waniska, 2000, Parr and Bolwell, 2000; Clifford, 2000; Awika, 2004a). Sorghum proanthocyanidins are unlikely to bind minerals (Waniska, 2000) and high molecular weight proanthocyanidins (DP >3) do not cross the gastrointestinal cell monolayer (Deprez et al., 2001) . Bioavailability of iron in sorghum for human subjects was found to be affected more by phytic

acid than by the proanthocyanidins content of the grains (Radhakrishnan and Sivaprasad, 1980). Gomez-Cordoves and co- workers (2001) demonstrated the effective therapeutic effect of sorghum proanthocyanidins against human melanoma. The binding of proanthocyanidins with proteins participates in their antibacterial activity (Murdiati and McSweeney, 1987; Scalbert, 1991). Proanthocyanidins have been shown to inhibit the growth of human immunodeficiency virus 1 (HIV-1), influenza virus, and herpes simplex virus by blocking their entry in the host cells (Chan and Kim, 1998; Okuda et al.; 1991, Lu et al., 2004; Hamauzu et al., 2005). The anti-HIV-1 activity is of high interest in Africa, and in Burkina Faso in particular, where HIV-1 prevalence is a major concern. The mechanism of proanthocyanidins toxicity against microbes is related to inhibition of hydrolytic enzymes, interactions to inactivate microbial adhesions and cell envelope transport proteins, and non-specific interaction with carbohydrates (Cowan, 1999). Among sorghum flavanols, the flavan-4-ols (Figure 5) have particular therapeutic interest because of their antitumor activity (Ferreira and Slade, 2002). Flavan-4-ols revealed strong host mediated antitumor activity, which is due to the enhancement of immune response of the host animals through the actions on tumor cells and some immunocytes (Okuda et al., 1991; Ferreira and Slade, 2002).

A number of highly reactive oxygen species such as singlet oxygen ($^1O_2$), superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), nitric oxide radical ($NO^{\bullet}$), and alkyl peroxyl ($ROO^{\bullet}$) are regularly produced in the human body (Langseth, 1995). These radicals can damage lipids, proteins and DNA and participate in pathogenesis and ageing (Ryan and Robards, 1998; Santos-Buelga and Scalbert, 2000; Parr and Bolwell, 2000). Phenolic compounds, together with other natural compounds (vitamins C and E, and carotenoids), contribute to the defense by scavenging free radicals, by inhibiting oxidative enzymes such as lipooxygenase and cyclooxygenase and by chelating metal ions (Shi et al., 2001). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals (Bors et al., 2001). In general, phenolic compounds possessing ortho-hydroxyls, found for instance in caffeic acid and in the B-ring of some flavonoids (catechin, quercetin, luteolinidin, etc.), have higher antioxidant activities than the others (Natella et al., 1999; Shi et al., 2001). Antioxidant activities of phenolic compounds have been suggested to exert beneficial pharmacological effects on neurological disorders on the basis of in vitro observations (Moosmann and Behl, 1999). Epidemiological studies have shown that consumption of some phenolic compounds is associated with a reduced risk for developing chronic diseases, such as coronary heart disease, cancer, diabetes, and Alzheimers's disease, linked to their free radical scavenging activities (Ames et al., 1993; Block et al., 1992; Hertog et al., 1993; Temple, 2000; Joshipura et al., 2001; Willett, 2002; Khokhar and Magnusdottir, 2002; Yang et al., 2004). Another interesting property of phenolic compounds, notably hydroxyanthraquinones and hydroxynaptho-quinones, is their cathartic effect (Clifford, 2000). Cathartic compounds are believed to give a better feeling and help to deal with difficult emotions and eliminate them.

Relevance of phenolic compounds for sorghum food quality

Among phenolic compounds of particular interest in sorghum are proanthocyanidins (PAs), 3-deoxyanthocyanidins (3-DAs) and flavan-4-ols. This is because of their importance in human nutrition and because of their agronomic properties in grain preservation. For instance, the content of PAs is important in assessing the food grade quality of sorghum because of their possible antinutritional effect linked to the inhibition of hydrolytic enzymes (Butler, 1992). For the preparation of infant porridges, low PAs containing varieties, may be more convenient by avoiding enzyme-mediated oxidation of endogenous phenolic compounds into colored products or the inhibition of hydrolytic by PAs. On the other hand, food-based sorghum varieties rich in PAs can be suggested to obese people and diabetic patients by analogy with the 50% weight loss observed with animals (rabbits, pigs, etc.) fed with sorghums containing high levels of PAs (Ambula et al., 2001). This is supported by the observation that in certain cultures in Africa, people prefer to consume PAs containing sorghums because they have a longer "residence power" in the stomach (Awika and Rooney, 2004a). The low digestibility of high PAs containing sorghums through the inhibition of hydrolytic enzymes, together with their high antioxidant activities may be interesting from a nutritional standpoint for obese persons.

While most plants, including other cereals, are lacking 3-deoxyanthocyanidins, e.g. apigeninidins and luteolinidins, sorghum is unique in containing a relatively high level of these compounds (Awika et al., 2004b, Dicko et al., 2005a). Reports on the levels of phenolic compounds in sorghum varieties show a high inter-varietal difference of contents among varieties screened (Subramanian et al., 1992; FAO, 1995; Iwuoha and Aina, 1999; Bvochora et al., 1999, Dicko et al., 2002a, 2005a). Flavan-4-ols, present in only rare sorghum varieties (Audilakshmi et al., 1999, Dicko et al., 2005a), are of particular interest for grain storage (Melake-Berhan et al., 1996) and for treating various cancers (Ferreira and Slade, 2002). The contents of these compounds among sorghum varieties from Burkina Faso vary more than in other sorghums for instance from Nigeria, India and

Zimbabwe (Dicko et al., 2002a, 2005a). The majority of sorghum varieties do not contain high amounts of PAs, which is an advantage for weaning foods preparation.

Despite their importance for nutrition, the effect of germination on PAs, 3-DAs, and flavan-4-ols in sorghum varieties has been scarcely studied. Previous reports on 2-4 varieties indicated that the analyzable content of phenolic compounds may increase (Nwanguma and Eze, 1996) or decrease upon germination (Iwuoha and Aina, 1997). It was recently shown that, on average, germination does not affect the total phenolic compounds content of the fifty selected varieties (Dicko et al., 2005a). This could be related to the fact that during germination, both enzymes involved in phenolic compounds biosynthesis as well as enzymes involved in their degradation are activated (Dicko et al., 2006a). A clear decrease in PA, 3-DA and flavan-4-ol contents was observed. The decrease in extractable PAs and DAs upon germination could be due to leaching of water-soluble PAs and DAs, which are located in the pericarp and testa (Beta et al., 1999; Waniska et al., 2000) or due to formation of insoluble complexes between PAs and proteins (Hagerman et al., 2001).

Recently, Awika and co-workers (2003) reported methods to determine antioxidant activities of sorghum grains. It was also shown that independent of germination, there are large inter-varietal differences in antioxidant activities among sorghum varieties (Table 2) (Dicko et al., 2005a, Dykes et al., 2005). Phenolic compounds and antioxidant activities were well correlated before germination, but germination lowered this correlation (Dicko et al., 2005a). Although white sorghum varieties have lower antioxidant activities than red varieties (Dicko et al., 2005a), these activities are among the most important sources of natural antioxidants (Pellegrini et al., 2003). Since sorghum is a staple food in Africa, it can be tentatively inferred that it is the main potential source of natural antioxidants for people relying on it as a main source of energy. Cardiovascular disease (CVD) is one of the most important diseases in Western countries. Epidemiological studies suggest that the consumption of whole cereal grains, including sorghum lowers the mortality from CVD, linked probably to their antioxidant properties (Kushi et al., 1999; Awika and Rooney, 2004a). This implies that several sorghum varieties could be candidate to be processed into food not only for Africa, but also for other countries. For sorghum varieties displaying high levels of antioxidant activities, it would be interesting to perform a further analysis of the nature of phenolic compounds contained in these varieties and to study their antioxidant or pro-oxidant properties. A thorough analysis of the phenolic composition of these selected varieties may allow discovering new flavonoids displaying anti- HIV-1 activities (Li et al., 1998; Kitamura et al., 1998; Yao et al., 2004) . The same analysis could allow finding lignans and other bioactive phenolic compounds in sorghum grains.

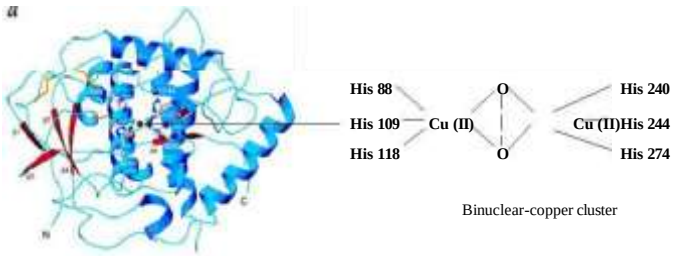


Figure 6. Structure of potato PPO and its binuclear copper-cluster (Klabunde et al., 1998).

SORGHUM PHENOLIC ENZYMES BIOCHEMISTRY

Role of phenylalanine ammonia lyase in the biosynthesis of phenolic compounds

The biosynthesis of phenolic compounds in plant (Figure 1) is initiated by the shikimic acid pathway (Tomas-Barberan and Espin, 2001; Heldt, 2005). This pathway continues with the production of phenylalanine, which is subsequently deaminated by the enzyme phenylalanine ammonia lyase [EC 4.3.1.5, PAL]. PAL can deaminate both L-phenylalanine and L-tyrosine into cinnamate derivatives (Rosler et al., 1997; Heldt, 2005). The released ammonia is refixed by glutamine synthetase [L-glutamate-ammonia ligase, EC 6.3.1.2] to produce glutamine (Singh et al., 1998; Heldt, 2005).

PAL is inhibited by its products, e.g. trans-cinnamates (Heldt, 2005). Apart from PAL, the main other important enzymes in phenolic synthesis are cinnamate-4-hydroxylase [EC 1.14.13.11]; 4-coumarate CoA ligase [EC 6.2.1.12], acetyl CoA carboxylase [EC 6.4.1.2]; chalcone synthase [EC 2.3.1.74); and chalcone-flavanone isomerase (EC 5.5.1.6] (Hrazdina, 1992, Haslam, 1998). PAL is indirectly associated with the synthesis of phenol polymers, including lignin and suberin (Parr and Bolwell, 2000; Heldt, 2005). In several fruits and vegetables, a high plant PAL activity has been associated with the accumulation of anthocyanins and other phenolic compounds (Tovar et al., 2002). In barley, PAL activity has been associated with the response to pathogen challenge (Shiraishi et al., 1995) and to light (Baztan and Torres, 1988). The inhibition of PAL activity in barley induces the susceptibility to fungal attack (Carver et al., 1994). PAL activity has been detected in the green shoots and leaves (Stafford, 1969, Mohan et al., 1988) of sorghum. In sorghum, the infection of the plant with pathogen involved a very rapid accumulation of PAL mRNA (Cui et al., 1996). The presence of PAL activity in sorghum grain and its activation upon germination was assessed (Dicko et al., 2006a). Although PAL is indirectly involved in the synthesis of almost all phenolic compounds, its activity was not correlated with the contents in phenolic compounds in both

ungerminated and germinated varieties (Dicko et al., 2006a). This lack of correlation may be due to the presence of phenolic oxidizing enzymes in the grain.

Polyphenol oxidases

Polyphenol oxidases [monophenol, 3,4, L-dihydroxyphenylalanine: oxygen oxidoreductase, EC 1.14.18.1, PPOs] are type-3 copper containing oxidases that occur in plants as monomeric, dimeric and tetrameric structures, and as several isoforms (Kowalski et al., 1992; Martinez and Whitaker, 1995; Sheptovitsky and Bruvig, 1996; Jolivet et al., 1998; Klabunde et al., 1998; Timothy et al., 2001; Chazarra et al., 2001). In sorghum like in wheat, PPO isoenzymes displaying monophenolase activity were found exclusively in the endosperm while those having only o-diphenolase activity were localized in the pericarp (Mayer and Harel, 1979; Marsh and Galliard, 1986; Hatcher and Kruger, 1993, Dicko et al., 2005b). The crystal structure of a potato PPO has been solved (Klabunde et al., 1998). The active site of PPO is constituted of a binuclear copper-cluster (Figure 6). The copper atoms are linked to each other through oxygen atoms and bound to the polypeptide chain through three histidine residues. PPO may catalyze a regioselective aerobic two electrons transfer oxidation of monophenols (monophenolase or cresolase activity) to o-diphenols and their subsequent dehydrogenation to the corresponding o-quinones (diphenolase or catecholase activity) (Martinez and Whitaker, 1995; Timothy et al., 2001). The o-quinones produced may undergo non-enzymatic cyclization or polymerization reactions to yield melanin-like pigments (Martinez and Whitaker, 1995; Rodakiewicz-Nowak and Ito, 2003). Not all PPO isoenzymes can perform the hydroxylation step and even those possessing that activity may loose it during extraction or storage (Sanchez-Ferrer et al., 1995). The catecholase activity is a shared property of all PPOs.

PPOs are involved in various protection mechanisms, including human pigmentation and the browning of fruits and vegetables. The defense function of PPO in plants is attributed to the modification of endogenous phenolic compounds, notably allelochemicals or phytoalexins into o-quinones, which are toxic to the invading pathogens and pests (Mayer and Harel, 1979; Luthra et al., 1988; Kowalski et al., 1992; Weir et al., 2004). PPO is suggested to be indirectly involved in auxin biosynthesis because the o-quinones produced by PPO can react with tryptophan to form indole-3-acetic acid (Mayer and Harel, 1979) . PPO was also found both in latent and active forms in the photosystem-II of the thylacoid membrane-protein complex, suggesting a function in photosynthesis (Sheptovitsky and Brudvig, 1996).

In sorghum, PPO is present in the leaves (Stafford and Dresler, 1972; Vaughn and Duke, 1981) and in the grain (Glennie, 1981, Dicko et al., 2002a, 2002b, 2006a). Three PPO isoforms with different molecular masses were found in the leaves, although no molecular masses were given (Stafford and Dresler, 1972) . PPO activity in sorghum leaves has been associated in response to fungal invasion (Luthra et al., 1988; Gowda et al., 1989). However, until now no further purification and characterization of sorghum PPO has been performed.

Sorghum varieties display different monophenolase and o-diphenolase activities (Dicko et al., 2002a, 2002b, 2006a) . However, the inter-varietal difference of PPO activity and the effect of germination on the occurrence of PPO isoenzymes in sorghum varieties are unknown. Like most other plant PPOs (Martinez and Whitaker, 1995), sorghum PPOs are more active with o-diphenols than with monophenols. Germination decreases the o-diphenolase activity and slightly increases the monophenolase activity. Zymography revealed that germination does not induce new PPO isoenzymes in sorghum grain (Dicko et al., 2006a). As reported for wheat (Hatcher and Kruger, 1993), a decrease of o-diphenolase activity of PPO was observed (Dicko et al., 2006a). This might be related to its localization in the pericarp, where it can leach out during germination or form complexes with phenolic compounds. Since both activities of PPO can be kinetically controlled for the synthesis of o-diphenols or o- quinones, sorghum varieties possessing these activities may find interesting applications in the food and chemical industries (Dubey et al., 1998; Espin et al., 2001).

Peroxidases

Peroxidases [hydrogen peroxide, oxidoreductase, EC 1.11.1.7, POXs] are ubiquitous enzymes found in bacteria, fungi, plants and animals (Krylov and Dunford, 1996, Dunford, 1999). They have in common that they accept hydrogen peroxide or hydroperoxide analogs as oxidant and form water as by-product. Most POXs studied to date contain ferric protoporphyrin IX as prosthetic group and act through a high-valence iron-oxo species. Besides these heme-containing POXs, selenium-, manganese-, vanadium- and flavin-containing POXs are known (Adam et al., 1999). Based on sequence similarity, numbers of calcium ions and origins, POXs have been divided in three major classes (Welinder, 1992). Class I includes yeast cytochrome c POXs, ascorbate POXs and bacterial catalase-POXs. Cytochrome c POXs are found in the mitochondrial electron transport chain, where they probably protect against toxic peroxides. Ascorbate POXs are the main enzyme responsible for hydrogen peroxide removal in chloroplasts and cytosol of higher plants (Dalton, 1993). Bacterial catalase-POXs have both classical POX and catalase activities (Welinder, 1991; Fraaije et al., 1996). Class II comprises secretory fungal POXs such as lignin POXs and manganese-dependent POXs (Reddy and

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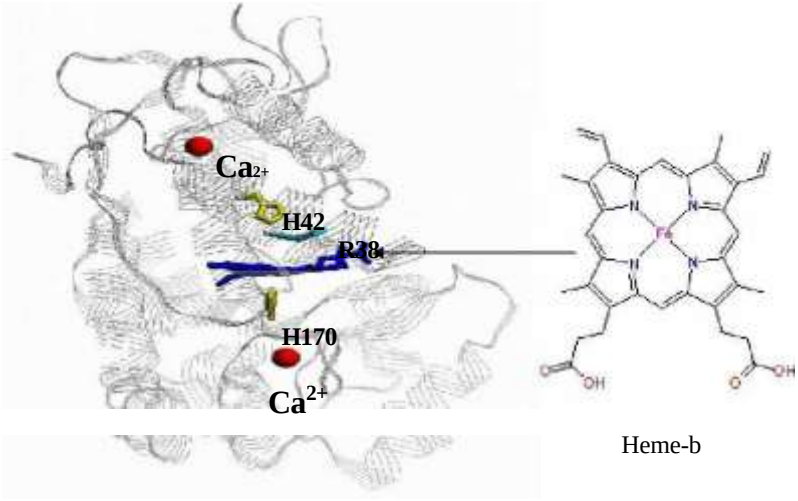

Figure 7. Three-dimensional structure of horseradish peroxidase. Shown are the heme, the structural calcium ions, and the three key amino-acid residues of the active site: Arg + 2 His. Source: Dunford (1999) and Silaghi-Dumitrescu (2005).

Souza, 1994). Class III consists of the secretory plant POXs found in e.g. horseradish, barley, and wheat.

Plant POXs have many different physiological functions including the removal of hydrogen peroxide from chloroplasts and cytosol; the oxidation of toxic compounds; the biosynthesis of cell walls (lignin and suberin); defense responses towards wounding and other stresses; indole-3-acetic acid regulation; ethylene biosynthesis; etc. (Dunford, 1999; Welinder et al., 2002; Duroux and Welinder, 2003). POXs occur in plants as several isoenzymes or glycoforms, with different cellular localization (Dunford, 1999; Duroux and Welinder, 2003). While in the five chromosomes of Arabidopsis 73 POX genes were found (Duroux and Welinder, 2003), 138 POX genes and 14 POX pseudogenes were annotated in the twelve chromosomes of rice (Oryga sativa japonica) (Passardi et al., 2004). Considering the phylogenetic linkage between rice and sorghum (Paterson, 2003), and the fact that the genome of sorghum (750 Mb) is twice as large as that of rice, even more POX genes are expected in sorghum. Moreover, the ongoing project of sorghum genome sequencing (http://peroxidase.isb-sib.ch/index.php) has allowed us to currently identify 160 stretches of sorghum peroxidase genes (dicko et al., 2006b). Plant secretory POXs generally have the following structural properties in common (Henriksen et al., 1998; Dunford, 1999; Welinder et al., 2002; Duroux and Welinder, 2003; Veitch, 2004):

monomeric glycoprotein structure of 300-310 amino-acids
putative N-glycosylation sites: Asn-X-Ser/Thr (X≠Pro)
non-covalently bound iron(III)-protoporphyrin-IX (type-b heme)
key catalytic amino-acid residues around the heme prosthetic group: His^{42}, His^{170}, Arg^{38}, Asn^{70}, Asp^{238}, Phe^{41} and Pro^{139} (horseradish peroxidase numbering)
four structural calcium ion binding sites
Cys^{97-301}, and $Cys^{177-209}$ (horseradish peroxidase numbering)
structural water molecules extending from the heme pocket to the distal calcium-binding site

The crystal structures of POXs from peanut (Schuller et al., 1996), horseradish (Gajhede et al., 1997); barley (Henriksen et al., 1998), and Arabidopsis (Mirza et al., 2000; Østergaard et al., 2000) have been elucidated. The cationic horseradish (HRP-C) isoenzyme is the most studied POX (Figure 7), because of its availability and its catalytic performance.

POXs oxidize reducing substrates (AH) either in presence of hydrogen peroxide (classical POX cycle) or molecular oxygen (oxidase cycle). Recently, it was shown (Berglund et al., 2002) that HRP-C is essentially in five oxidation states during catalysis (native enzyme, compound-I, compound-II, Compound-III, and ferro-peroxidase). In each of these states, the enzyme has a different conformation, especially at the heme environment (Berglund et al., 2002). Compound-I is obtained by two-electron oxidation of the resting enzyme by H_2O_2 and is stable (Hiner et al., 2002). In contrast to the generally assumed concept of irreversible formation of Compound–I, Rietjens and co-workers proposed that the formation of Compound I can be a reversible reaction via an uptake of a water molecule (van Haandel et al., 1998, van Haandel, 2000). The one-electron-reduction of Compound-I by AH produces Compound-II. Another electron from AH is required to reduce Compound-II to the native enzyme (Dunford, 1999). Formation of Compound-I (k_1) and the regeneration of the native enzyme (k_3) are generally rate-limiting steps in POX catalysis (Dunford, 1999) . Compound-III is formed from

conserved disulphide bridges: Cys$^{11-91}$, Cys$^{44-49}$, Compound-II with excess of H_2O_2 or upon reaction of the

ferrous-enzyme (oxyperoxidase) with O_2 (Dunford, 1999, Berglund et al., 2002). It is important to note that an excess of H_2O_2 can irreversibly inactivate POX because Compound-III is unstable (van Haandel, 2000). In addition, Compound-III can also react with an excess of H_2O_2 to produce verdohemoprotein called P-670; in this case H_2O_2 acts as a suicidal substrate (Dunford, 1999; Hernandez- Ruiz et al., 2001; Sakharov and Sakharova, 2002). The POX catalytic cycle yields free radicals ($A^{\bullet}$), which can undergo polymerization reactions, coupling with molecular oxygen, etc. In the oxidase cycle, POX transfers one electron to molecular oxygen, which in turn is transferred to a substrate; this is termed the monooxygenase activity (Dawson, 1988). The monooxygenase catalytic route of POX is performed via the formation of the ferrous form (Fe^{2+}) of the enzyme (ferro-POX) (Gazarian et al., 1998). Native POX or Compound III may also perform catalase or dismutase reactions (EC 1.11.1.6) by decomposition of H_2O_2 to water and oxygen (Hiner et al., 2001).

Some POXs are able to oxidize auxin in the absence of H_2O_2, thus display indole-3-acetic acid (IAA)-oxidase activity (Christensen et al., 1998, Gazarian et al., 1998; Dunford, 1999). POXs from non-plant sources are unable to oxidize IAA in the absence of H_2O_2, because they are lacking the IAA-binding site localized in the distal domain near the heme pocket (Savitsky et al., 1999). The IAA-oxidase activity of POX isoforms is related to their physiological role in auxin metabolism (Hiner et al., 2001; Marco et al., 1999).

POX activities have been detected in the leaves (Stafford and Bravinder-Bree, 1972; Vaughn and Duke, 1981; Luthra et al., 1988) and grains (Glennie, 1981) of sorghum. POX activities in sorghum varieties and the occurrence of isoenzymes have been reported (Ollitrault et al., 1989; Bvochora et al., 1999, Dicko et al., 2002a; Dicko et al., 2006a). There is a strong inter-varietal difference of POX activity among sorghum varieties, before and after germination (Dicko et al., 2002a, 2006a). Germination increased POX activity due to the activation of already present isoenzymes and/or de novo synthesis of POX isoenzymes (Dicko et al., 2006a) . The cationic POX is the most abundant POX isoform in sorghum, and it is ubiquitously present in all varieties, in both ungerminated and germinated grains (Dicko et al., 2002a; 2006a) . In other cereals such as barley, wheat and maize, the cationic isoenzymes are also the most abundant, accounting for 80-90% of total POX activity (Johansson et al., 1992; Converso and Fernandez, 1995; Billau et al., 1999).The major cationic POX in sorghum grain has been purified and characterized to the molecular level (Dicko et al., 2006b). Mass spectrometry analysis showed that the enzyme consists of two glycoforms with molecular masses of 34227 and 35629 Da and it contains a type-b heme. Chemical deglycosylation allowed the estimation of sugar contents of 3.0% and 6.7% (w/w) in glycoform I and II, respectively, and a mass of the apoprotein of 33246 Da. The enzyme is localized in the chromosome 1 of sorghum. The sequence of sorghum POX has a high sequence identity with barley BP1 (85%), rice Prx23 (90%), wheat WSP1 (82%) and maize POX (58%), indicative for a common ancestor. In spite of the conserved active sites, the sorghum cationic POX differs from the archetypically known barley POX BP1 in being active with aromatic compounds above pH 5. Another interesting POX isoenzyme in sorghum is an anionic POX (pI 3.1), which is expressed only in germinated grain (Dicko et al., 2006a). This germination-associated isoenzyme may play a role in cell wall synthesis and protect the seed against pathogens (Passardi et al., 2004). Purification and characterization of this anionic isoenzyme will give more insight in its specificity for phenolic compounds that are involved in plant growth.

Cationic POXs are generally more active on phenolic compounds than laccases and anionic POXs and are promising biocatalysts (Wallace and Fry, 1999). In view of this, the utilization of the sorghum cationic POX in biocatalysis could be a future challenge. Sorghum POX may be applied in food biotechnology for the modification of carbohydrate-containing hydroxycinnamates (Oudgenoeg et al., 2001, 2002; Boeriu et al., 2004; Regalado et al., 2004). In addition, POX catalyzed reactions are useful for (bio) chemical, clinical and biocatalytic applications (Adam et al., 1999; Regalado et al., 2004).

Role of phenolic enzymes in food

Contents of phenolic compounds and phenolic oxidizing enzymes are strongly associated with food quality (Deshpande et al., 1986; Hilhorst et al., 1999; 2002; Tomas-Barberan and Espín, 2001) . Enzymes involved in the biosynthesis and oxidation of phenolic compounds have been shown to be determinants for the quality of plant-derived foods (Tomas-Barberan and Espin, 2001). The presence of PAL activity in sorghum grain and its activation upon germination was assessed (Dicko et al., 2006a). Although PAL is indirectly involved in the synthesis of almost all phenolic compounds, its activity was not correlated with the contents in phenolic compounds in both ungerminated and germinated varieties (Dicko et al., 2006a). This lack of correlation may be due to the presence of phenolic oxidizing enzymes in the grain.

PAL, POX and PPO were more activated during germination in red grains than in white ones, indicating that the phenolic biosynthesis and catabolism processes are relatively more important in red grains than in white grains (Dicko et al., 2006a). In wheat, the di-PPO activity was also higher in red grains than in white ones (Mayer and Harel, 1979; Hatcher and Kruger, 1993).

Phenolic compounds are potential substrates of POX

Table 3. Sorghum varieties as potential source of phenolic compounds or phenolic oxidizing enzymes.

(Bio)chemical component	Germination requirement	Utilizations	References
Total phenolic compounds	no	antioxidant, food additive, antimicrobial	1-3
Proanthocyanidins	no	antioxidant, antimicrobial and antiviral agent (including HIV1)	4-6
3-deoxyanthocyanidins	no	food colorant	7, 8
Flavan-4-ols	no	anti-cancer agent	9, 10
Polyphenol oxidase	no	synthesis of *o*-diphenols and *o*-quinones	11, 12
Peroxidase	yes	synthesis of various organic compounds, functional modification of carbohydrates and proteins	13, 14, 15-17

1 2 3 4 5 6

Awika et al. (2003); Pellegrini et al. (2003); Tomas-Barberan and Espín (2001); Scalbert (1991); Chan and Kim (1998); Lu et al. (2004); Awika and Rooney (2004a), Kouda-Bonafos et al. (1996); Ferreira and Slade (2002); Okuda et al. (1991); Dubey et al., 1998; Espin et al. (2001); Guerra and Ferraz (2003); Adam et al. (1999); Oudgenoeg et al. (2001, 2002); Boeriu et al. (2004).

and PPO and their oxidation products (benzoquinones and polymeric compounds) affect food quality (Matheis and Whitaker, 1984, Martinez and Whitaker, 1995) . PPO and POX work synergistically because PPO may generate H_2O_2 during the course of catalysis (Richard-Forget et al., 1997) and POX on other hand can generate O_2 (Matheis and Whitaker, 1984). Both PPO and POX generate benzoquinones, which are also new substrates of POXs. Because of their electrophilic nature, these quinones undergo secondary reactions, such as cross-linking with amino acid side groups in proteins (Anderson and Morris, 2001). PPO and POX are known to influence product properties during and after food processing (Matheis and Whitaker, 1984; Haslam, 1998, Feillet et al., 2000). They affect post harvest degradation of food by causing browning and the development of off-flavors in raw and unblanched cereals (March and Galliard, 1986; Hatcher and Kruger, 1993). In wheat the appearance of colored products in the flour is attributed to the oxidation of endogenous phenolic compounds by PPO (March and Galliard, 1986; Hatcher and Kruger, 1993). PPO and POX are generally admitted to be the most determinant enzymes for the preservation and organoleptic qualities of fruits and vegetables (Matheis and Whitaker, 1984; Parr, 2000, Tomas-Barberan and Espin, 2001). In analogy with what was found in wheat dough (Hilhorst et al., 1999), POX may mediate the thickness of sorghum flour during "tô" preparation by cross-linking carbohydrates containing endogenous hydroxycinnamate derivatives (Dicko et al., 2002a; 2006a). Low POX and PPO activities to avoid beer darkness and haze occurrence by the oxidation of endogenous phenolic compounds may be other criteria for selection of sorghum for industrial brewing (Clarkson et al., 1992).

For couscous preparation, the formation of a gel, mediated by POX via the cross-linking of macromolecules, is not desired. In most African countries, bakers do not use composite sorghum/wheat flour. However, acceptable bread can be produced with 30-50% sorghum substitution for wheat (Carson et al., 2000, Dicko et al., 2006c). Addition of sorghum flour possessing high POX activities (Hilhorst et al., 1999, Baik et al., 2003) could lead to the cross-linking of sorghum glucuronoarabinoxylans. These polysaccharides are known to contain ferulic acid (Verbruggen et al., 1993; 1996) and may make addition of a higher percentage of sorghum flour acceptable, in analogy with the action of POX in wheat bread (Hilhorst et al., 1999). These criteria may give directions for selecting sorghum varieties for bread making.

Sorghum grain as potential source of bioactive components

The investigation of several constituents within sorghum varieties has shown their wide (bio) chemical diversity. This large (bio) chemical diversity could be rationally exploited. Among the phenolic compounds and phenolic enzymes studied, some are of particular interest as bioactive constituents or biocatalysts. Therefore, it is useful to identify some varieties as sources of specific (bio) molecules. For instance, varieties containing a high level of total phenolic compounds could be novel sources of antioxidants. Varieties containing high levels of PAs could be candidate to isolate these compounds because they are currently gaining attention as antibacterial and anti-HIV1 agents (Chan and Kim, 1998). Varieties containing high levels of 3- DAs will be of interest for the isolation of apigeninidins and luteolinidins that are currently commercialized as food colorants and for various other industrial utilizations (Morazzoni and Magistretti, 1990; Coultate, 1996). As reviewed above, some sorghum varieties could be used as sources of specific biochemical (Table 3).

CONCLUSION

Sorghum is a tropical cereal that has great diversity in its content of phenolic compounds as well as phenolic related enzymes. Phenolic compounds, phenylalanine

ammonia lyase, polyphenol oxidase and peroxidase are effective biochemical determinants for sorghum use as food or as source of bioactive components. Research on endogenous bioactive components such as phenolic compounds and phenolic enzymes, will contribute to unleash the capacity of sorghum to be the cornerstone of food security in Africa as well as in many developing countries.

ACKNOWLEDGEMENT

The organization for the prohibition of chemical weapons (OPCW) via the International Foundation for Science (Sweden) is acknowledged for supporting the research carried out by Dr. M. H. DICKO.

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