Review

Sorghum and millet phenols and antioxidants

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Abstract

Sorghum is a good source of phenolic compounds with a variety of genetically dependent types and levels including phenolic acids, flavonoids, and condensed tannins. Most sorghums do not contain condensed tannins, but all contain phenolic acids. Pigmented sorghums contain unique anthocyanins that could be potential food colorants. Some sorghums have a prominent pigmented testa that contains condensed tannins composed of flavan-3-ols with variable length. Flavan-3-ols of up to 8–10 units have been separated and quantitatively analyzed. These tannin sorghums are excellent antioxidants, which slow hydrolysis in foods, produce naturally dark-colored products and increase the dietary fiber levels of food products. Sorghums have high concentration of 3-deoxyanthocyanins (i.e. luteolinidin and apigenidin) that give stable pigments at high pH. Pigmented and tannin sorghum varieties have high antioxidant levels that are comparable to fruits and vegetables. Finger millet has tannins in some varieties that contain a red testa. There are limited data on the phenolic compounds in millets; only phenolic acids and flavones have been identified.

Keywords: Sorghum; Millet; Phenols; Phenolic acids; 3-Deoxyanthocyanins; Condensed tannins; Antioxidants; Health benefits

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1. Introduction

All sorghums (Sorghum bicolor (L.) Moench) contain phenolic acids and most contain flavonoids. Only varieties with a pigmented testa have condensed tannins. The types and quantities of phenols present in the grain are genetically controlled. There are a number of myths about sorghum that exist in the scientific community. These myths significantly and adversely affect sorghum use in food and industrial products. These myths originate from the misconceptions that consumption of sorghum with tannins is toxic to animals and perhaps to humans. The confusion exists because numerous publications allege that sorghums with tannins cause a problem when fed to livestock. We will discuss which sorghums have condensed tannins and their effects on livestock rations and human foods widely consumed in many areas of the world. In addition, we show that sorghums have a large number of bioactive compounds relevant to human health. We will also summarize the limited information available on the millet phenols and their contribution to health.

2. Overview of sorghum genetics and kernel structure relevant to tannins and phenols

Sorghum genetics relevant to tannins and phenols has been reviewed by Rooney and Miller (1982). The pericarp color of the sorghum kernel is controlled by the }R and }Y genes. A pericarp is white when }Y is homozygous recessive (rryy or _R_yy), whereas a yellow pericarp has recessive }R and dominant }Y genes (rr}Y_). When both }R and }Y genes are dominant, the pericarp is red. The intensifier gene }I affects the intensity of the pericarp color and is most apparent in red sorghums.

Pericarp thickness is controlled by the }Z gene. A pericarp is thick when the gene is homozygous recessive (zz) and thin when it is dominant (ZZ). Sorghums with a thick pericarp have starch granules in the mesocarp; sorghums with a thin pericarp do not contain starch granules in that area (Earp and Rooney, 1982; Earp et al., 2004b). Secondary plant color is controlled by the }P and }Q genes. Plants with homozygous dominant }PQ genes produce purple- or red-pigmented plants, while recessive }pq genes produce tan-pigmented plants. All of the aforementioned genes affect phenol content.

Pericarp color is not a reliable indicator of tannins in sorghums. Boren and Waniska (1992) investigated tannin content in a wide variety of sorghums varying in pericarp color. They showed that pericarp color and its intensity is not a good indicator of tannin content. It is erroneously believed that all sorghums with a red/brown pericarp contain tannins. Sorghums with white, yellow, red, or brown color pericarp may or may not have tannins depending upon the presence of a pigmented testa (Fig. 1), which is controlled by the }B1 and }B2 genes. Sorghums with a pigmented testa must have both dominant genes (B1_B2_). The spreader gene }S controls the presence of brown pigments, possibly tannins, in the epicarp and endocarp when a pigmented testa is present. (Blakeley et al., 1979). Testa color is controlled by the }Tp gene. A testa is purple when }Tp is homozygous recessive (tptp) and brown when it is dominant (Tptp). The levels of condensed tannins are highest in sorghums containing dominant B1_B2_}SS genes; these sorghums have high bird and mold resistance.

Sorghum varieties are divided into three groups based upon their genetics and chemical analyses (Rooney and Miller, 1982). Type I sorghums (b1b1b2_b1B2_, b1b1b2b2) do not have a pigmented testa, and contain low levels of phenols and no tannins. Types II and III both have a pigmented testa and contain tannins. The tannins in Type II sorghums (B1_B2_}ss) are extracted with acidified methanol (1% HCl methanol) while the tannins in Type III sorghums (B1_B2_}S) are extracted with either methanol or acidified methanol when using the vanillin/HCl assay. Earp et al. (2004a) showed that the tannins in the Type II sorghums are deposited differently in the testa layer. In the grain of Type II sorghums, tannins are deposited in the vesicles within the testa layer, whereas the tannins in type III are deposited along the cell walls of the testa and some are present in the pericarp. This may explain why acid is required to disrupt the structure of the vesicles, which releases tannins in Type II sorghums (Earp et al., 2004a).

3. Kernel structure relevant to tannins and phenols in millets

McDonough and Rooney (2000) reviewed the structure of the major millets. Millets are separated botanically into utricles and caryopses. In a utricle, the seed is separated by the pericarp, which is attached only at one point. Thus, the pericarp is easily removed. Finger (Eleusine coracana (L.) Gaertn.), proso (Panicum miliaceum (L.)) and foxtail (Setaria italica (L.) P.Beauv.) millets are utricles. In a caryopsis, the pericarp is attached strongly to the seed. Pearl (Pennisetum glaucum (L.) R.Br.), fonio (Digitaria exilis (Kippist) Stapf), and teff (Eragrostis tef (Zucc.) Trotter) are caryopses. Millet germplasm collections vary in kernel color, size, shape, and other characteristics. Some
varieties of finger millet have a dark brown or red structure that is most likely a pigmented testa, which contains condensed tannins. Finger millets with dark brown or red color have higher phenol and tannin levels than white varieties (Ramachandra et al., 1977). Teff, and sometimes pearl millets, were reported to have tannins and a pigmented testa, but detailed structure evaluations are not reported. Proso and fonio millets do not have a pigmented testa, but it is unclear if the germplasm has been carefully evaluated. Most likely, the tannins reported in white varieties were not condensed tannins, but phenols that gave background noise in the determinations.

4. Phenols in sorghums and millets

4.1. Phenolic acids

All sorghums and millets contain phenolic acids, which are located in the pericarp, testa, aleurone layer, and endosperm (Hahn et al., 1984; McDonough et al., 1986). Phenolic acids consist of two classes: hydroxybenzoic and hydroxycinnamic acids. Hydroxybenzoic acids are directly derived from benzoic acid and include gallic, p-hydroxybenzoic, vanillic, syringic, and protocatechuic acids, among others. The hydroxycinnamic acids have a C₆–C₃ structure and include coumaric, caffeic, ferulic, and sinapic acids. The phenolic acids reported in sorghum and millets are listed in Table 1.

Hahn et al., (1983) identified free and bound phenolic acids in sorghum (Table 2). Free and bound phenolic acids are extracted in methanol and in boiling 2 M HCl, respectively. Free phenolic acids are found in the outer layers of the kernel (pericarp, testa, and aleurone), whereas the bound phenolic acids are associated with the cell walls (Hahn et al., 1984). According to Hahn et al. (1983), the phenolic acids in sorghum are present mostly in bound form with ferulic acid being dominant (24–47%). In addition, gallic acid is found only in bound form (12.9–46.0 µg/g, dry wt), whereas cinnamic acid is found only in free form (2.0–10.7 µg/g, dry wt) with the exception of one variety (SC0719, red pericarp with pigmented testa), which is also reported to contain cinnamic acid in bound form only (19.7 µg/g, dry wt) (Hahn et al., 1984). The other

<table>
<thead>
<tr>
<th>Phenolic acids detected in sorghum and millet grains</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenolic acid</strong></td>
</tr>
<tr>
<td>Hydroxybenzoic acids:</td>
</tr>
<tr>
<td>Gallic</td>
</tr>
<tr>
<td>Protocatechuic</td>
</tr>
<tr>
<td>p-Hydroxybenzoic</td>
</tr>
<tr>
<td>Gentisic</td>
</tr>
<tr>
<td>Salicylic</td>
</tr>
<tr>
<td>Vanillic</td>
</tr>
<tr>
<td>Syringic</td>
</tr>
<tr>
<td>Hydroxycinnamic acids:</td>
</tr>
<tr>
<td>Ferulic</td>
</tr>
<tr>
<td>Caffeic</td>
</tr>
<tr>
<td>p-Coumaric</td>
</tr>
<tr>
<td>Cinnamic</td>
</tr>
<tr>
<td>Sinapic</td>
</tr>
</tbody>
</table>

*Reported only in sorghums.

<table>
<thead>
<tr>
<th>Free and bound phenolic acid composition of some sorghum varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenolic acid</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Gallic</td>
</tr>
<tr>
<td>Protocatechuic</td>
</tr>
<tr>
<td>p-Hydroxybenzoic</td>
</tr>
<tr>
<td>Vanillic</td>
</tr>
<tr>
<td>Caffeic</td>
</tr>
<tr>
<td>p-Coumaric</td>
</tr>
<tr>
<td>Ferulic</td>
</tr>
</tbody>
</table>

*Source: Data from Hahn et al. (1983).*

¹Values are expressed as µg/g, dry weight basis.

²Value not available.

³ND = not detected.
phenolic acids are present in free (54.1–230.4 mg/g, dry wt) and bound (276.7–622.9 mg/g, dry wt) forms.

In general, ferulic, p-coumaric, and cinnamic acids are the major phenolic acids in millets (Table 3) (McDonough and Rooney, 2000; McDonough et al., 1986). The literature on the proportion of free and bound phenolic acids in millets is limited. For finger millet, Subba Rao and Muralikrishna (2002) reported that phenolic acids were present mostly in free form (71%). They also identified ferulic acid as the major bound phenolic acid (18.60 mg/100 g), whereas protocatechuic acid was reported as the major free phenolic acid (45.0 mg/100 g). They did not detect bound gallic, protocatechuic, or vanillic acids.

### 4.2. Flavonoids

Many sorghum flavonoids have been isolated and identified (Table 4). The anthocyanins are the major class of flavonoids studied in sorghum. In general, this class of compounds contributes the blues, purples, and reds in plants. The six common anthocyanidins are cyanidin, delphinidin, malvidin, pelargonidin, petunidin, and peonidin (Fig. 2). Unlike these common anthocyanidins, sorghum anthocyanins are unique since they do not contain the hydroxyl group in the 3-position of the C-ring (Fig. 2) and thus are called 3-deoxyanthocyanidins. This unique feature increases their stability at high pH compared to the common anthocyanidins (Awika et al., 2004a, b; Gous, 1989; Nip and Burns, 1971). Spergularidin (Pate et al., 1997; Seitz, 2004; Wu and Prior, 2005), 7-methoxyapigeninidin (Seitz, 2004, and 7-methoxyluteolinidin (Seitz, 2004).

Sorghums with a black pericarp have the highest levels of 3-deoxyanthocyanidins (Awika et al., 2004a, b; Dykes et al., 2005; Gous, 1989), which are concentrated in the bran (Awika et al., 2005). Using the pH differential method of Fuleki and Francis (1968), Awika et al. (2004b) reported the anthocyanin content of a black sorghum bran was 3–4 times higher than the whole grain and had at least twice the levels of anthocyanins (10.1 mg/g) compared to red (3.6 mg/g) and brown (3.6 mg/g) sorghum brans. In addition, Awika et al. (2004a, b) reported luteolinidin and apigeninidin represented 36–50% of the total anthocyanin content in black (Tx430 Black) and brown (Hi Tannin) sorghum brans using HPLC (Table 5). They also reported that apigeninidin represented 19% of the total anthocyanin in a red sorghum (Tx2911) and detected trace amounts of luteolinidin. These data suggest that black sorghum bran is a major source of 3-deoxyanthocyanidins for potential use in natural food colorants.

Table 5
Phenolic acid composition of some millets

<table>
<thead>
<tr>
<th>Phenolic acid</th>
<th>Finger</th>
<th>Pearl</th>
<th>Prosa</th>
<th>Teff</th>
<th>Fonio</th>
<th>Foxtail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folin/Ciocalteu</td>
<td>0.55–0.59</td>
<td>0.19–0.33</td>
<td>0.05–0.10</td>
<td>0.09–0.15</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td>Vanillin/HCl</td>
<td>0.17–0.32</td>
<td>0.05</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Phenolic acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protocatechuic</td>
<td>23.1</td>
<td>11.8</td>
<td>—d</td>
<td>25.5</td>
<td>—d</td>
<td>—d</td>
</tr>
<tr>
<td>Gentisic</td>
<td>61.5</td>
<td>96.3</td>
<td>—d</td>
<td>15</td>
<td>—d</td>
<td>21.5</td>
</tr>
<tr>
<td>p-Hydroxybenzoic</td>
<td>8.9</td>
<td>22</td>
<td>—d</td>
<td>25.5</td>
<td>—d</td>
<td>—d</td>
</tr>
<tr>
<td>Vanillic</td>
<td>15.2</td>
<td>16.3</td>
<td>—d</td>
<td>54.8</td>
<td>—d</td>
<td>87.1</td>
</tr>
<tr>
<td>Caffeic</td>
<td>16.6</td>
<td>21.3</td>
<td>—d</td>
<td>3.9</td>
<td>—d</td>
<td>10.6</td>
</tr>
<tr>
<td>Syringic</td>
<td>7.7</td>
<td>17.3</td>
<td>—d</td>
<td>14.9</td>
<td>—d</td>
<td>93.6</td>
</tr>
<tr>
<td>Coumaric</td>
<td>56.9</td>
<td>268.2</td>
<td>—d</td>
<td>36.9</td>
<td>—d</td>
<td>2133.7</td>
</tr>
<tr>
<td>Ferulic</td>
<td>387</td>
<td>679.7</td>
<td>—d</td>
<td>285.9</td>
<td>—d</td>
<td>765.8</td>
</tr>
<tr>
<td>Cinnamic</td>
<td>35.1</td>
<td>345.3</td>
<td>—d</td>
<td>46</td>
<td>—d</td>
<td>781.7</td>
</tr>
</tbody>
</table>

Source: Adapted from McDonough and Rooney (2000).

| a Lemma and palea attached. |
| b Values are expressed on mg/100 mg catechin equivalents, dry weight basis. All millets have phenols but only finger millet has condensed tannins. Pearl millet gives a reading with the vanillin assay, but does not contain condensed tannins. |
| c mg phenolic acid/mg samples, as is moisture basis. |
| d Value not available. |
Table 4
Flavonoids and proanthocyanidins reported in sorghum grains

<table>
<thead>
<tr>
<th>Compound</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthocyanins</strong></td>
<td></td>
</tr>
<tr>
<td>5-Methoxyapigenidin 7-glucoside</td>
<td>Wu and Prior (2005)</td>
</tr>
<tr>
<td>7-Methoxyluteolinidin</td>
<td>Wu and Prior (2005)</td>
</tr>
<tr>
<td>Luteolinidin 5-glucoside</td>
<td>Nip and Burns (1971), Wu and Prior (2005)</td>
</tr>
<tr>
<td>5-Methoxyapigenidin 7-glucoside</td>
<td>Seitz (2004)</td>
</tr>
<tr>
<td>7-Methoxyapigenidin</td>
<td>Seitz (2004)</td>
</tr>
<tr>
<td><strong>Flavan-4-ols</strong></td>
<td></td>
</tr>
<tr>
<td>Luteoforol</td>
<td>Bate-Smith (1969)</td>
</tr>
<tr>
<td>Apiforol</td>
<td>Watterson and Butler (1983)</td>
</tr>
<tr>
<td><strong>Flavones</strong></td>
<td></td>
</tr>
<tr>
<td>Apigenin</td>
<td>Gujer et al. (1986), Seitz (2004)</td>
</tr>
<tr>
<td>Luteolin</td>
<td>Seitz (2004)</td>
</tr>
<tr>
<td><strong>Flavacanes</strong></td>
<td></td>
</tr>
<tr>
<td>Eriodictyol</td>
<td>Kambal and Bate-Smith (1976)</td>
</tr>
<tr>
<td>Eriodictyol 5-glucoside</td>
<td>Gujer et al. (1986)</td>
</tr>
<tr>
<td>Naringenin</td>
<td>Gujer et al. (1986)</td>
</tr>
<tr>
<td><strong>Flavanols</strong></td>
<td></td>
</tr>
<tr>
<td>Kaempferol 3-rutinoside-7-glucuronide</td>
<td>Nip and Burns (1969)</td>
</tr>
<tr>
<td><strong>Dihydroflavonols</strong></td>
<td></td>
</tr>
<tr>
<td>Taxifolin</td>
<td>Gujer et al. (1986)</td>
</tr>
<tr>
<td>Taxifolin 7-glucoside</td>
<td>Gujer et al. (1986)</td>
</tr>
<tr>
<td><strong>Proanthocyanidin monomers/dimers</strong></td>
<td></td>
</tr>
<tr>
<td>Catechin</td>
<td>Gupta and Haslam (1978), Gujer et al. (1986)</td>
</tr>
<tr>
<td>Procyandin B-1</td>
<td>Gupta and Haslam (1978), Gujer et al. (1986)</td>
</tr>
<tr>
<td><strong>Proanthocyanidin polymers</strong></td>
<td></td>
</tr>
<tr>
<td>Epicatechin- (epicatechin)_catechin</td>
<td>Gupta and Haslam (1978), Gujer et al. (1986)</td>
</tr>
<tr>
<td>Prodelphinidin</td>
<td>Brandon et al. (1982), Krueger et al. (2003)</td>
</tr>
<tr>
<td>Proapigenidin</td>
<td>Krueger et al. (2003)</td>
</tr>
<tr>
<td>Proluteolinidin</td>
<td>Krueger et al. (2003)</td>
</tr>
</tbody>
</table>

Red pericarp sorghums have flavan-4-ol compounds, such as luteoforol and apiforol, which are produced from flavanones (i.e., naringenin and eriodictyol) and may be precursors of sorghum anthocyanidins (Wharton and Nicholson, 2000). Flavan-4-ols may play an important role in mold resistance, as several studies report a correlation between flavan-4-ols concentration and mold resistance in sorghums (Audilakshmi et al., 1999; Jambunathan et al. 1990, 1991; Melake-Berhan et al., 1996; Menkir et al., 1996). However, selecting sorghums for concentration of flavan-4-ols has been ineffective in creating resistance to molds.

Flavan-4-ol levels vary among sorghum genotypes. Gous (1989) reported that black pericarp sorghums have the highest levels of flavan-4-ols (11.8–13.6 abs/ml/g) compared to red pericarp sorghums (8.7–9.0 abs/ml/g), which was confirmed by Dykes et al. (2005). Dicko et al. (2005) reported that red-pigmented plant sorghums with a red pericarp and pigmented glumes generally have higher levels of flavan-4-ols (0.20–0.42%, w/w, cyanidin, dry wt.) than the other varieties studied. Dykes et al. (2005) also reported that purple/red-pigmented plant sorghums with a thick pericarp have higher levels of flavan-4-ols (4.3–9.3 abs/ml/g) than purple/red-pigmented plant sorghums with a thin pericarp (3.0–3.6 abs/ml/g) and tan-pigmented plant sorghums (2.3–2.7 abs/ml/g).

Other flavonoids isolated and identified in sorghum grains include the flavones apigenin and luteolin, which are predominant in tan-pigmented plant sorghums (Seitz, 2004). Flavanones, eriodictyol (Kambal and Bate-Smith, 1976; Yasumatsu et al., 1965) and eriodictyol 5-glucoside (Gujer et al., 1986) have been reported. The flavanone, naringenin, was also reported (Gujer et al., 1986) and was found as a major peak in the Tx2911 red sorghum (Awika, 2003). The flavonol, kaempferol 3-rutinoside-7-glucuronide (Nip and Burns, 1969) and the dihydroflavonols taxifolin (Gujer et al., 1986), and taxifolin 7-glucoside (Gujer et al., 1986) have also been isolated.

The only millet flavonoids reported are flavones (Fig. 3). Hilu et al. (1978) identified eight flavones in the leaves of finger millet: orientin, iso-orientin, vitexin, isovitexin, saponarin, violanthin, lucenin-1, and tricin. Reichert (1979) detected glucosylvitexin, glucosylorientin, and vitexin in pearl millet in the ratio of 29:11:4; they were responsible for the yellow–green discoloration of millet flour at basic pH. Luteolin and tricin were reported in Japanese barnyard millet (Echinochloa crus-galli (L.) P. Beauv.) (Watanabe, 1999). Sartelet et al. (1996) detected glucosylvitexin, glucosylorientin, and vitexin, orientin, isoorientin, vitexin, isovitexin, saponarin, violanthin, lucenin-1, and tricin. Reichert (1979) detected glucosylvitexin, glucosylorientin, and vitexin in pearl millet in the ratio of 29:11:4; they were responsible for the yellow–green discoloration of millet flour at basic pH. Luteolin and tricin were reported in Japanese barnyard millet (Echinochloa crus-galli (L.) P. Beauv.) (Watanabe, 1999). Sartelet et al. (1996) reported the presence of apigenin (150 mg/kg) and luteolin (350 mg/kg) in fonio; 10% of apigenin and 80% of luteolin were present in free form while the remaining percentages of those compounds were bound as O-glycosylflavonenes.

### 4.3. Condensed tannins

Sorghums with the B₁-B₂ gene contain tannins, which are the major phenolic compounds in those varieties (Hahn et al., 1984). These compounds confer some resistance to molds and deterioration of the grain (Waniska et al., 1989). Tannin levels vary among genotypes. In general, type II and III sorghums have tannin levels of 0.02–0.19 mg/100 mg and 0.4–3.5 mg/100 mg catechin equivalents, respectively (Earp et al., 1981). Recent data on tannin content of Type II and III sorghums are presented in Table 6. The tannins in these sorghums are of the...
condensed type. Tannic acid, a hydrolysable tannin, has never been found in sorghum.

Condensed tannins, also known as proanthocyanidins or procyanidins, are high-molecular weight polyphenols that consist of polymerized flavan-3-ol and/or flavan-3,4-diol units linked mainly by C4-C8 interflavan bonds, and thus are called B-type proanthocyanidins (Fig. 4a). There are also the A-type proanthocyanidins where the flavan-3-ol units are linked by C4-C8 interflavan bonds and by an additional ether bond between C2-C7, which have been identified mostly in cranberries (Foo et al., 2000; Gu et al., 2003). The proanthocyanidins in tannin sorghums are the B-type with (-)-epicatechin as extension units and catechin as terminal units (Gu et al., 2002, 2003; Gupta and Haslam, 1978); however, some diversity of proanthocyanidins in sorghum has been reported. For example, prodelphinidin and heteropolyflavan-3-ols with both A- and B-type interflavan linkages (Fig. 4b) consisting of procyanidin or prodelphinidin as extension and terminal units have been reported (Brandon et al., 1982; Gujer et al., 1986; Krueger et al., 2003). Glucosylated heteropolyflavans with proluceolinidin or proapigenidin as extension units and the flavonones eriodictyol or eriodictyol-5-0-glucoside as terminal units (Fig. 4c) have also been reported (Gujer et al., 1986; Krueger et al., 2003). Other flavan-3-ols identified in sorghum include catechin and procyanidin B-1 (Gujer et al., 1986; Gupta and Haslam, 1978).
Finger millet is the only millet reported to contain condensed tannins. Ramachandra et al. (1977) reported that brown finger millets contain tannins (0.12–3.47% catechin equivalents) compared to white finger millets (0.04–0.06%). Reports on structural characterization of millet proanthocyanidins are lacking.

5. Methods of analysis of sorghum phenols

Many methods have been used to measure phenolic compounds and these have been reviewed in several papers (Hagerman et al., 1997; Naczk and Shahidi, 2004; Rohr et al., 2000; Schofield et al., 2001; Shahidi and Naczk, 1995). In this review, only major methods used in the analysis of sorghum and millet phenols are discussed.

5.1. Conventional methods

The Bleach Test is a good method to qualitatively identify sorghum with tannins (Waniska et al., 1992); a rough idea of the amount of tannin sorghums present can be obtained by calculating the percentage of kernels containing the pigmented testa. It is a good method to determine whether a sample contains a mixture of tannin and non-tannin sorghums. For tannin sorghums, bleaching dissolves the pericarp and turns the pigmented testa black; non-tannin sorghums do not turn black. This method is inexpensive, relatively quick to perform, and effective when used with appropriate standards. However, it does not measure tannin content or differentiate between Type II and Type III sorghums (Waniska et al., 1992). Also, it can yield false-positives on Type I sorghums that have been molded, weathered, or damaged by insect bites. Therefore, care must be used when evaluating the bleached samples (Dykes et al., 2002; Waniska et al., 1992). Duodu (personal communication, 2006) has indicated that some varieties of finger millet give a positive Bleach Test for the presence of a pigmented testa.

Colorimetric methods (Table 7) give an estimate of phenol content and are rapid and economical to perform compared to other methods requiring expensive equipment. Total phenol content has been measured using the Folin–Ciocalteu assay (Kaluza et al., 1980; Singleton and Rossi, 1965) or the Prussian Blue assay (Price and Butler, 1977). These methods are based on oxidation–reduction reactions, are not specific to a class of phenols, and suffer from interference by the amino acid tyrosine (Hahn and Rooney, 1986; Ring, 1984) and non-phenolics such as ascorbic acid (Hagerman et al., 1997). The Folin–Denis assay and the ferric ammonium citrate assay of the International Organization for Standardization (ISO) have been used to measure total phenols and tannins in sorghum using tannic acid as the standard (Bate-Smith and Rasper, 1969; Beta et al., 1999; Maxson and Rooney, 1972). Values were given in tannic acid equivalents, which led some scientists to conclude erroneously that tannin sorghums contain tannic acid/hydrolysable tannins.

Condensed tannins are measured using the vanillin/HCl or the butanol/HCl assays. The modified vanillin/HCl method of Price et al. (1978) involves the condensation of...
the aromatic aldehyde vanillin (4-hydroxy-3-methoxy benzaldehyde) with monomeric flavanols and their oligomers to form a red adduct that absorbs at 500 nm. Type I sorghums give low tannin values due to the interference of other non-tannin phenolics (Table 6) (Waniska and Rooney, 2000), which was also observed in millets (Table 3) (McDonough and Rooney, 2000). Sorghums that do not have a pigmented testa contain non-tannin phenolics that react with the reagents and give some “tannin values” that are not really tannins (Dykes et al., 2005; Earp et al., 1981; Hahn and Rooney, 1986). These values are widely reported as tannins in the literature and give rise to the myth that all sorghums have tannins (Rooney, 2005). Thus, significant confusion exists. The vanillin/HCl assay does not measure tannin content accurately. The lack of an appropriate standard for condensed tannins is a major problem due to the heterogeneity of these compounds (Schofield et al., 2001). The preparation of a pure tannin standard from sorghum or other tannin-containing material (i.e. quebracho) is difficult and time-consuming (Butler, 1989; Hagerman and Butler, 1980). The standard mostly used for the vanillin/HCl assay is catechin but it gives values that are unrealistically high (Rohr et al., 2000; Schofield et al., 2001). In sorghum, the tannins reside mainly in the pigmented testa, which is only a portion of the outer covering that comprises approximately 5–6% (dry weight) of the kernel. Thus, catechin equivalent values of 5–6%

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**Fig. 4.** Structures of proanthocyanidins reported in sorghum: (a) Polyflavan-3-ol with B-type interflavan linkages (Gupta and Haslam, 1978; Gu et al., 2002); (b) heteropolyflavan-3-ols with A- and B-type interflavan linkages (Krueger et al., 2003); (c) glucosylated heteropolyflavans with a flavanone as the terminal unit (Gujer et al., 1986; Krueger et al., 2003).
<table>
<thead>
<tr>
<th>Method</th>
<th>Standard</th>
<th>Reagents</th>
<th>Extraction</th>
<th>Time</th>
<th>Assayed compounds</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prussian blue</td>
<td>Catechin</td>
<td>FeCl$_3$ in HCl</td>
<td>Methanol</td>
<td>1 min</td>
<td>Phenolic compounds</td>
<td>Price and Butler (1977)</td>
</tr>
<tr>
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<td>Catechin or gallic acid</td>
<td>Folin–Ciocalteu reagent</td>
<td>Methanol</td>
<td>1 h</td>
<td>Phenolic compounds</td>
<td>Kaluza et al. (1980)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1% HCl in methanol</td>
<td>2 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folin–Denis</td>
<td>Tannic acid</td>
<td>Folin-Denis reagent</td>
<td>Water</td>
<td>5 h</td>
<td>All reducing compounds</td>
<td>Maxson and Rooney (1972)</td>
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<tr>
<td>Vanillin</td>
<td>Catechin</td>
<td>4% HCl, 1% vanillin in methanol</td>
<td>Methanol</td>
<td>24 h</td>
<td>Leucoanthocyanidins, proanthocyanidins (tannins)</td>
<td>Nip and Burns (1971)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1% HCl in methanol</td>
<td>20 min</td>
<td></td>
<td>Price et al. (1978)</td>
</tr>
<tr>
<td>Acid butanol</td>
<td>Purified proanthocyanidins</td>
<td>HCl in butanol</td>
<td>Methanol</td>
<td>20 min</td>
<td>Proanthocyanidins</td>
<td>Porter et al. (1986), Watterson and Butler (1983)</td>
</tr>
<tr>
<td>International</td>
<td>Tannic acid</td>
<td>Ferric ammonium citrate, NH$_3$</td>
<td>Dimethylformamide</td>
<td>1 h</td>
<td>Proanthocyanidins</td>
<td>ISO 9648 (1988)</td>
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<td>Standardization</td>
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<td>Organization (ISO)</td>
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<tr>
<td>Protein precipitation</td>
<td>Tannic acid, sorghum tannins</td>
<td>FeCl$_3$, alkaline detergent</td>
<td>Methanol</td>
<td>15 min</td>
<td>Proanthocyanidins</td>
<td>Hagerman and Butler (1978)</td>
</tr>
<tr>
<td>Relative-degree of</td>
<td></td>
<td></td>
<td>Methanol; 1% HCl in methanol</td>
<td>15 min</td>
<td>Leucoanthocyanidins, anthocyanidins, proanthocyanidins</td>
<td>Butler (1982)</td>
</tr>
<tr>
<td>polymerization</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Source:** Adapted from Waniska and Rooney (2000).

*A standard is not reportedly used for this method.*
(Table 6) are much too high. Therefore, tannin values from this assay are only relative indices of tannin content among samples. The butanol/HCl assay also measures tannin content and involves the depolymerization of condensed tannins in boiling acidic butanol to yield anthocyanidins (Porter et al., 1986).

Sorghum tannins precipitate proteins and assays based on protein binding have been used to measure tannin content (Hagerman and Butler, 1978; Hagerman et al., 1997; Schofield et al., 2001). Bovine serum albumin is the most widely used protein for these assays (Rohr et al., 2000). The relative degree of polymerization of tannins is determined by the ratio of the anthocyanidin formation from the butanol/HCl assay to the color production from the vanillin assay carried out in glacial acetic acid (Butler, 1982). Flavan-4-ols are measured by a modification of the butanol/HCl assay as described by Butler (1982) or by Gous (1989). Total anthocyanin content is determined using the pH differential method of Fuleki and Francis (1968).

5.2. Instrumental methods

High-performance liquid chromatography with UV–vis, photodiode array, or fluorescence detection is the best method to separate, identify, and quantify sorghum and millet phenolic acids and flavonoids (Awika et al., 2004a, b; Gujer et al., 1986; Hahn et al., 1983; Seitz, 2004; Subba Rao and Muralikrishna, 2001, 2002). Reversed-phase HPLC has been used to measure condensed tannins, but separation could only be accomplished on tannins up to tetramers and these were not separated according to their degree of polymerization (Prior and Gu, 2005). Normal-phase HPLC with fluorescence detection allows the separation and quantification of tannins according to their degree of polymerization (Awika et al., 2003a; Gu et al., 2002; Hammerstone et al., 1999). Tannins up to decamers were successfully separated; tannins with higher degrees of polymerization were shown as a single peak (Awika et al., 2003a; Gu et al., 2002). This is helpful in determining the distribution of tannins in a sample since sorghum varieties differ in the distribution of oligomeric and polymeric tannins (Table 8). This method also confirmed that sorghums without a pigmented testa have no tannins (Fig. 5).

Determination of the proportion of constituent units and the average degree of polymerization of sorghum proanthocyanidins has been achieved by thiolytic degradation (Gu et al., 2002, 2003). In this reaction, the proanthocyanidin extension units react with benzyl mercaptan to yield the corresponding benzylthioether while the terminal unit is released as a free flavan-3-ol.

Sorghum and millet phenols are difficult to identify and characterize due to lack of standards. Methods used to structurally characterize these compounds include mass spectrometry, $^1$H and $^{13}$C nuclear magnetic resonance

<table>
<thead>
<tr>
<th>Table 8</th>
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<tbody>
<tr>
<td>Procyanidin content and composition of tannin sorghum grains and bran</td>
</tr>
<tr>
<td><strong>DP</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
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<td>9</td>
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<tr>
<td>10</td>
</tr>
<tr>
<td>P</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>% Oligomers</td>
</tr>
</tbody>
</table>

Source: Adapted from Awika et al. (2003a).

a: mg/g, obtained by normal-phase HPLC with fluorescence detection.

b: Values are means ± standard deviation from two separate extractions.

c: Degree of polymerization.

d: Mixture of polymers with DP > 10.

e: Oligomers (DP < 10) as percent of total.

Fig. 5. Normal-phase HPLC profiles of proanthocyanidins in tannin sorghum (Sumac) grains compared to non-tannin sorghum (BTx 378 and BTx 3197) grains. DP, degree of polymerization; P, polymers with DP > 10 (Adapted from Dykes et al., 2004).
spectrum, and infrared spectroscopy (Gujer et al., 1986; Krueger et al., 2003; Watanabe, 1999; Wu and Prior, 2005).

6. Effect of phenols on food quality and processing properties of sorghum and millet

In sorghum, pericarp color, secondary plant color, endosperm color, and the presence of a pigmented testa are factors affecting the color and acceptability of food products (Waniska and Rooney, 2000). A large number of traditional food products (i.e., porridges, alcoholic and non-alcoholic beverages) are prepared using tannin sorghums. They are readily accepted by local people because these sorghums are well adapted, produce consistent crops and are often preferred for food production. Sometimes special procedures are followed to enhance food quality. Often, particular foods, especially beer and porridges, are prepared from tannin sorghums.

Tannins bind to proteins, carbohydrates, and minerals and thus reduce digestibility of these nutrients. To reduce these negative effects, decortication, fermentation, germination, and chemical treatments (i.e., HCl, formaldehyde, and alkali) are used (Beta et al., 2000a, b; Bvochora et al., 2005; Chibber et al., 1978; Dicko et al., 2005; Osman, 2004; Price et al., 1979; Reichert et al., 1980). Tannin sorghums with red/brown pericarps are often used in the production of opaque beers as the dark color imparted to the beer by the pericarp pigments is desirable. Tannins affect malt amylase activity but alkaline or formaldehyde treatments during malting allows brewers to avoid problems (Beta et al., 2000a; Dewar et al., 1997).

Sorghums with a pigmented pericarp provide a unique opportunity to produce special food products with a natural, attractive dark color, high levels of dietary fiber, and antioxidants with a variety of phenols. Black and tannin sorghum brans have been added into yeast-leavened bread formulas for production of food products with potential health benefits. For example, good-quality breads containing tannin sorghum bran have high phenols, antioxidant activity, and dietary fiber levels with a natural dark-brown color and excellent flavor (Gordon, 2001; Rooney and Waniska, 2000). Healthy bread mixes containing tannin sorghum bran, barley flour, and flaxseed hulls have also been developed (Rudiger, 2003). The addition of black and tannin sorghum bran to maize masa produced purple and reddish-brown tortillas, respectively. The same was observed with tortilla chips (Cedillo-Sebastian 2005). Whole grain of these sorghums produces acceptable tortilla chips. For instance, black sorghums without tannins produce tortilla chips with a more intense blue/black color than those made with blue maize, and tannin sorghums produced dark tortillas and chips (Zelaya et al., 1999). Extrusion of black or tannin sorghums in a high-temperature, high-pressure short barrel friction extruded, cooked and expanded the product, which made a tasty whole grain snack after drying and flavoring (Perez-Gonzalez, 2005).

Processing tannin or black sorghums into food products affects phenol levels. For example, processing tannin sorghum bran to produce cookies, and breads decreased tannin content by 52% and 72%, respectively; the loss was mainly from the high-molecular weight tannins (Awika et al., 2003a). Awika et al. (2003a) also reported that extrusion of tannin sorghum caused an 85% decrease in polymeric tannins while the lower molecular weight tannins increased by 29–478%. The decrease in tannin level does not mean that the tannin is lost; it means that during processing, the tannins bind to other molecules (i.e., proteins, carbohydrates, minerals) making them difficult to extract. Phenol levels of maize tortillas containing black or brown sorghum bran decreased by 33–38% and 47–50%, respectively (Cedillo-Sebastian, 2005). Frying into tortilla chips reduced phenol levels by 52–55% (black sorghum bran) and 60–66% (tannin sorghum bran) compared to the tortillas (Cedillo-Sebastian, 2005). Thus, processing affects the extractability of phenolic compounds and phenol levels.

Pearl millet changes color reversibly from gray to yellow–green at alkaline pH; at acidic pH they are gray to creamy white, due to glucosylvitexin, glucosylorientin, and vitexin (Reichert, 1979). Decortication and steeping in water, especially in acidic solution (2 M HCl), decreases the color of the millet porridges (Akingbala, 1991; Reichert, 1979). In Namibia, sour, low pH pearl millet porridge is preferred because of its white color.

The tannins of finger millet are significantly reduced by decortication (Ramachandara et al., 1977), which increased protein digestibility. Fermentation of finger millet decreased measurable phenol and tannin levels by 20% and 52%, respectively (Antony and Chandra, 1998). Malting finger millet for 96 h decreased bound caffeic, coumaric, and ferulic acid levels by 45%, 41%, and 48%, respectively, (Subba Rao and Muralikrishna, 2001). On the other hand, the level of gallic, vanillic, coumaric, and ferulic acids in free form increased after 96 h of malting (Subba Rao and Muralikrishna, 2002).

6.1. Feeding properties of tannin sorghum

The tannins in tannin sorghums provide a degree of resistance to bird predation in the field. Hence, the terms sometimes used for the tannin sorghums are “bird proof” or “bird resistant.” Birds can and do consume all sorghums and are not adversely affected by condensed tannins. In sorghum nurseries with white, red, and tannin sorghums, birds eat white sorghum first and then red sorghums before eating the Type II tannin sorghums and finally the Type III tannin sorghums (Rooney, 2005). Birds consume tannin sorghums when no other food is available, but definitely prefer other sorghums when given a choice (Bullard and Gebrekidan, 1989; Bullard and York, 1985, 1996). The nutritional effects of tannin sorghums have been reviewed by Awika and Rooney (2004), Butler (1989), Butler and Rogler (1992), and Nyachoti et al. (1997).
Tannins in sorghum do not cause toxicity problems in animals consuming the grain, but the feed efficiency of livestock fed tannin sorghums is decreased depending on the animal species, the method of processing the grain, and the diet fed (Hahn et al., 1984). The feed efficiency can be reduced by 10–30% compared to non-tannin sorghums; animals generally consume more feed to produce about the same or less weight gains (Hahn et al., 1984; Rooney, 2005).

Tannin sorghums have a reputation of being toxic since early publications reported the tannins in sorghum as tannic acid (Armstrong et al., 1974; Elkin et al., 1978a, b; Kondos and Foale, 1983). Investigators observed that tannic acid fed to chickens and rats adversely affected their health and performance (Armstrong et al., 1974; Chang and Fuller, 1964; Glick and Joslyn, 1970a, b; Rayudu et al., 1970; Rostagno et al., 1973; Vohra et al., 1966). Even though tannic acid has never been found in sorghums, these early publications are often used to indicate that sorghum is unacceptable for use in weaning and other food products. Tannin sorghums are not preferred for feeding because they decrease feed efficiency. However, they are used in livestock feeds effectively, provided the price is reduced compared to non-tannin sorghums.

The low protein digestibility of tannin sorghum may not be solely due to tannins. Elkin et al. (1996) showed that sorghums with equal levels of tannins have different digestibilities, which suggests that tannins are only partially responsible for low protein digestibility. Recent information in feeding trials clearly indicates that the tannin sorghums do not cause problems in livestock rations. For example, Jacob et al. (1996) did not observe any adverse effects in broiler chicks fed diets containing 1.3% catechin equivalents (dry wt basis). In fact, they observed that the highest incidence of leg abnormalities were in chicks fed maize diets supplemented with methionine. They concluded that methionine, not tannins, was involved in the etiology of leg abnormalities. Ambula et al. (2003) reported tannin sorghums containing 5% catechin equivalents did not cause any adverse affects in laying hen performance.

Al-Mamary et al. (2001) observed that rabbits fed diets containing low-tannin sorghums (1.4% catechin equivalents) did not affect growth rate, food consumption, or feed conversion ratio compared to the control. However, rabbits fed diets with high-tannin sorghums (3.5% catechin equivalents) had significantly reduced weight gain, feed conversion ratio, and slightly higher food consumption when compared to the control. Sorghum containing tannins is not toxic to animals or humans and can be used effectively in feed and foods.

6.2. Contribution of sorghum/millet phenols to health

The total phenol content of sorghum is significantly correlated with antioxidant activity (Awika et al., 2003b; Dicko et al., 2005; Dykes et al., 2005). These findings are in contrast to those of Kamath et al. (2004) who did not find a correlation between phenols and antioxidant activity, simply because they used only white sorghums, which are quite low in phenols. Sorghums with a pigmented testa increase antioxidant activity (Awika et al., 2003b; Dicko et al., 2005; Dykes et al., 2005) due to the presence of tannins, which are more potent antioxidants than monomeric phenolic compounds (Hagerman et al., 1998). Dicko et al. (2005) reported a correlation between antioxidant activity and tannins (r = 0.79); Dykes et al. (2005) reported a strong correlation between antioxidant activity and flavan-4-ol levels (r = 0.88) among non-tannin sorghums with a red pericarp. Sorghums containing condensed tannins have consistently shown the highest antioxidant activity in vitro. They approach or exceed the antioxidant levels of fruits and vegetables (Table 9).

Factors affecting the type of phenols and their composition are partially understood in sorghum. Our partial understanding, however, could enable the breeding of non-tannin and tannin sorghums with significantly higher levels of antioxidants. Dicko et al. (2005) and Dykes et al. (2005) reported that antioxidant activity was increased when sorghums had purple/red secondary plant color, a black or dark-red, thick pericarp, and a pigmented testa with a spreader gene. Additionally, decortication of sorghum to produce sorghum bran increases sorghum phenols and antioxidant activity 3–5 times over the original grain (Awika et al., 2003b, 2005).

Several health benefits of sorghum have been reported (Awika and Rooney, 2004). For example, Klopfenstein et al. (1981) reported that guinea pigs fed low tannin sorghum had significantly lower cholesterol levels than those fed whole wheat, rolled oats, or pearl millet diets. In

Table 9
Antioxidant activity (ORAC) of sorghum grain and bran compared to common fruits and vegetables

<table>
<thead>
<tr>
<th>Commodity</th>
<th>ORAC (μmol TE/g, dry wt)</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>Tannin sorghum (grain)</td>
<td>868</td>
<td>Awika et al. (2003b)</td>
</tr>
<tr>
<td>Tannin sorghum (bran)</td>
<td>3124</td>
<td>Awika et al. (2003b)</td>
</tr>
<tr>
<td>Black sorghum (grain)</td>
<td>219</td>
<td>Awika et al. (2003b)</td>
</tr>
<tr>
<td>Black sorghum (bran)</td>
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<td>Awika et al. (2003b)</td>
</tr>
<tr>
<td>Red sorghum (grain)</td>
<td>140</td>
<td>Awika et al. (2003b)</td>
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<tr>
<td>Red sorghum (bran)</td>
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</tr>
<tr>
<td>White sorghum (grain)</td>
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<td>Sweet pepper, green</td>
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<tr>
<td>Radishes</td>
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<td>Wu et al. (2004)</td>
</tr>
<tr>
<td>Potatoes, russet</td>
<td>63</td>
<td>Wu et al. (2004)</td>
</tr>
</tbody>
</table>

*Sumac variety.*
addition, Rooney et al. (1992) reported that sorghum and pearl millet brans were excellent bulking agents compared to wheat. They also reported that millet brans have better cholesterol-lowering properties than wheat, white sorghum, or brown sorghum brans. Rats fed sorghum, proso millet or buckwheat at 30% (w/w) of the total diet increased HDL cholesterol without changing total cholesterol level (Cho et al., 2000). Lee and Pan (2003) reported that fish feed diets containing tannin sorghum distillery residues gave better blood-thinning activities in gray mullet than the control diet during the winter season.

Tannin sorghums are slowly digested. Some cultures in Africa prefer tannin sorghums since it contributes a longer period of satiety or fullness compared to other cereals, which could be due to its slow digestibility (Awika and Rooney, 2004). This has potential applications in foods for diabetics.

Sorghum and millet have anti-carcinogenic properties. For example, Van Rensburg (1981) and Chen et al. (1993) reported that populations consuming sorghum and millet had lower incidences of esophageal cancer compared to those consuming wheat or maize. However, Morton (1970, 1972) reported that there was an association between high tannin sorghum consumption and human esophageal cancer, but these studies were criticized due to inadequate experimental design (Awika and Rooney, 2004; Yu and Swaminathan, 1987). Grimmer et al. (1992) showed that polymeric tannins from sorghum had higher anti-mutagenic activity than the lower molecular weight tannins. Gomez-Cordoves et al. (2001) showed that sorghum tannins increased melanoenic activity without increasing total melanin and reduced the formation of human melanoma colony cells. A recent study by Turner et al. (2006) showed that black and tannin sorghum bran reduced colon carcinogenesis in rats. In their study, rats fed diets containing black or tannin sorghum bran had fewer aberrant crypts than those fed diets containing cellulose or white sorghum bran. The reduction in colon carcinogenesis could be due to the antioxidant activity of the black and tannin sorghum bran (Turner et al., 2006). Studies are needed to determine which compounds are responsible for the anti-carcinogenic effects of sorghum.

The antioxidant activity of millet phenols and their health benefits have also been reported. For instance, in Japanese barnyard millet, the antioxidant activity of luteolin was nearly equal to that of quercetin; however, the activity of tricin was lower than luteolin (Watanabe, 1999). Finger millet is a potent source of antioxidants and has potent radical-scavenging activity that is higher than that of wheat, rice, and other millets; these results corresponded to their phenolic content (Sripriya et al., 1999). The brown or red variety of finger millet, which is commonly available, had higher activity (94%) than the white variety (4%) using the DPPH method (Sripriya et al., 1996). Kodo millet quenched DPPH by nearly 70% higher than other millets (15–53%); white millet varieties had lower activity (Hegde and Chandra, 2005). Luteolin, a flavone present in sorghum and millets, has been reported to have antioxidant, anti-inflammatory, cancer-preventive, anti-arrhythmic properties (Duke, 1992; Watanabe, 1999). Tricin has been reported to have anti-tumor and anti-leukemic properties (Lee et al., 1981; Watanabe, 1999).

7. Conclusion

Sorghum contains large quantities of phenolics and other compounds of use in human foods to prevent deterioration of health. Different phenols with varying properties exist, but relatively little effort to demonstrate the potential of these compounds in human health has been made. Of all the cereals, sorghum has the potential to be bred specifically to produce high levels of different phenols that can be easily concentrated by simple processes. These special sorghums have reasonable grain yields and agronomic characteristics that make them productive and economical to produce. The future is bright, but significant investment in evaluation and feeding trials to demonstrate their health promoting properties is required. Millets, with the exception of finger millet, are not as promising. However, they have been the subjects of little if any research.

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