CHARACTERIZATION OF MILLET AND SORGHUM FLOUR POLYPHENOLS FROM NORTHERN IVORY COAST: CAPACITIES AT PROTECTING LDL AGAINST OXIDATION MEDIATED BY Cu²⁺ AND AAPH.

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ABSTRACT

Millet and sorghum, the more used food in the northern of Ivory Coast are known for their polyphenol contents. In this study, total phenolic contents from white and red sorghums and millet were determined. The aim of this study was to analyze their antioxidant capacity to protect LDL against oxidation generated by Cu²⁺ / AAPH compared to red wine and olives oil wastewater. Red sorghum which had about 3 times higher phenolic compounds than white sorghum and millet, showed a specific antioxidant activity (SAA) of 168 while white sorghum and millet had a SAA respectively of 97 and 66. In a compared study, we found that red sorghum protection is 6 times higher 46 min than red wine 6.85 min and olives oil wastewater 7.15 min. Sorghum and millet, from its phenolic compounds has a good potential as natural food to prevent the oxidation of LDL therefore to play an antiarteriosclerotic role in pathophysiological conditions.

KEYWORDS: sorghum, millet, polyphenols, LDL, specific antioxidant activity.

1-INTRODUCTION

Millet (Pennisetum glaucum) and sorghum (Sorghum bicolor (L.) Moench) are an important staple food in developing countries of the semi-arid tropics and serve as one of the main sources of energy and protein in these populations [1]. Several reports have shown that sorghum [2] and millet [3] are inexpensive and nutritionally comparable or even superior to major cereals. In Africa, India and China, sorghum grain come third among cereals for human consumption, superseded only by rice and wheat [4]. Sorghum particularly could also play an important role in the production of ethanol and other bio-industrial products such as bioplastics, especially in dry areas where other crops are not easily grown [5]. Several potential health and pharmaceutical benefits of sorghum have been reported. These include slow digestibility, cholesterol-lowering, antioxidant, anti-
inflammatory, and anti-carcinogenic properties [6-7]. Cereal polyphenols are important phytochemicals with one or more aromatic rings, with hydroxyl groups in different patterns [8]. They exhibit significant antioxidant activity and could be as important as vitamin E in preventing oxidative damage in tissues [9] by reducing lipid oxidation [10] and/or inhibiting free radicals production [11].

The anthocyanins are the major class of flavonoids studied in sorghum. In general, this class of compounds contributes to the blue, purple, and red colors in plants. Sorghum anthocyanins are unique since they do not contain the hydroxyl group in the 3-position of the C-ring (fig 1A) and thus are called 3-deoxyanthocyanins. This unique feature increases their stability at high pH compared to the common anthocyanins [12] (fig 1B), which render these compounds as potential natural food colorants. The two common sorghum 3-deoxyanthocyanidins are the yellow apigeninidin and the orange luteolinidin [12-13].

![Structures of Sorghum anthocyanins](image)

\[
R_1 = H, R_2 = H, R_3 = H: \text{apigeninidin} \\
R_1 = H, R_2 = \text{Glucose}, R_3 = H: \text{Apigeninidine-5-glucoside} \\
R_1 = H, R_2 = H, R_3 = \text{CH}_3: 7-\text{O-methyl apigeninidin} \\
R_1 = \text{OH}, R_2 = H, R_3 = H: \text{Luteolinidin} \\
R_1 = \text{OH}, R_2 = \text{Glucose}, R_3 = H: \text{Luteolinindin-5-glucoside} \\
R_1 = \text{OH}, R_2 = \text{CH}_3, R_3 = H: 5-\text{methoxyluteolinidin} \\
\]

**Fig. 1 (A). Structures of Sorghum anthocyanins**

![Structures of six common Anthocyanins.](image)

\[
R_1 = \text{OH}, R_2 = H: \text{cyaniding} \\
R_1 = R_2 = H: \text{pelargonidin} \\
R_1 = R_2 = \text{OCH}_3: \text{malvidin} \\
R_1 = R_2 = OH: \text{delphinidin} \\
R_1 = O\text{CH}_3, R_2 = H: \text{peonidin} \\
R_1 = O\text{CH}_3, R_2 = \text{OH}: \text{petunidin} \\
\]

**Fig. 1 (B). Structures of six common Anthocyanins.**

The antioxidant activity of fruits and cereals is mainly correlated with their phenolics and carotenoids content [14]. Interest in polyphenol antioxidants has increased considerably due to their high capacity to scavenge free radicals associated with various diseases. Notably, some studies have reported that cereals polyphenols inhibit lipid peroxidation and LDL oxidation [15-16].

In Ivory Coast and specifically the northern part of the country, there is a great variety of cereal (millet and sorghum) with polyphenol contents whose characterization could present some potential for additional health benefits to consumers.
The aim of this study was (i) to determine the polyphenols contents of white, red sorghums and millet found in northern Ivory coasts, (ii) to compare their antioxidant activity by the ability of their phenolic compounds to protect LDL against oxidation mediated by Cu²⁺ or AAPH [2-2’azobis (2-amidinopropane) hydrochloride] and (iii) to compare their protective effect depending on the food portion and antioxidant activity than red wine (RW) and olive oil wastewaters (OOWW) and Palme oil (HP).

The global crisis of food requires some developing countries to enhance their agricultural products. In this context, sorghum and millets have different potentials:

- a substitute for rice whose import is too expensive and wheat for people allergic to gluten.
- Transformation into malt may also find application in industries of beer instead of malt barley if the activities are sufficient and amylase in the preparation of weaning foods (boiling) with low viscosity [17]

2- MATERIAL AND METHOD

2.1. Materials and Chemicals

Local varieties of sorghum (Sorghum bicolor (L.) Moench) and millet (Pennisetum glaucum) grains were purchased from local market and cleaned to remove debris.

CuCl₂, butylated hydroxytoluene (BHT) and Folin Ciocalteu reagent were obtained from Sigma Aldrich (Saint Quentin Fallavier, France), while gallic acid and methanol for high-performance liquid chromatography (HPLC) ultra gradient grade purchased from Merck (Darmstadt, Germany). AAPH was from Biovalley (Conches, France). Other chemicals used are either reagent grade or HPLC grade.

The red wine (RW) phenolic compounds were prepared and analyzed by (Institut National de la recherche Agronomique, Narbonne, France) from a Cabernet-Sauvignon grape variety. This preparation involved three steps: phenolic adsorption on an ADS-4 preparative column (a stationary phase from Applexion, Epone, France), alcoholic desorption (ethanol/water, 46:4, v/v), and eluent concentration by gentle evaporation. The concentrated residue was then sprayed to obtain a dry powder. One liter of red wine produced 1.3 g of dry powder containing 112 mg/g of total Catechins plus proanthocyanins (expressed as catechin, with only 1.0% of catechin and epicatechin), 64 mg/g of total anthocyanins with 85% quercetin and quercetin-3-glucoside, and 8.7 mg/g total phenolic acids with 19.5% caffeyl tartaric acid.

Olive oil wastewater (OOWW) was the aqueous phase obtained after olive crushing and separation of the lipid phase by centrifugation (Clermont l’Hérault, France). One kilogram of olive produced 1 L of OOWW, containing 7 g/L of total phenols with 0.4 g/L of hydroxytyrosol. This resulted in concentrations 10-20 times higher than olive oil itself. The phenol content of these natural mixtures was expressed as µmol of gallic equivalent/L (µGAE/L), i.e. 1 mg/L of RW phenolics and OOWW corresponded to 13.7 and 14.2 µGAE/L, respectively.

Tô is a very common dish in Mali and Ivory Coast, as well as in Burkina Faso made from millet or sorghum flour and water. It is cooked with a whip and then used as a paste eaten with a sauce.

2.2. Samples Extraction and Polyphenol Extraction

Sorghum and millet grains were powdered in a ‘Disk Mill’ (Glen Mills Inc., Clifton, NJ, USA) and the whole flours was employed in the study. Extraction procedure involved the addition of 50 mL ethanol and 50 mL water acidified by acetic acid (pH 2.6) to 10 g of sample to obtain an ethanol/water/H⁺ extract (EWH⁺). Samples were shaken during three hours at room temperature and centrifuged at 3000 g for 10 min. Supernatants were then concentrated on a Rota vapor at 40°C to a final volume of 25 mL. This EWH⁺ extract was used for HPLC and spectral characteristic analyses.

Then, 2 mL EWH⁺ extract was added 8 mL chloroform and 10 mL water. Samples were then vortex and centrifuged at 3000 g for 10 min to obtain about 11 mL of the polyphenol aqueous extract (EWH⁻PC). Determination of polyphenol content – The amount of the total phenolics in (EWH⁻PC) was determined with Folin-Ciocalteu reagent according to a modification colorimetric proposed by Waterhouse method and measured at 765 nm [18-19] using gallic acid as a standard. 200 µL of diluted (EWH⁻PC) extracts and 100 µL Folin reagent diluted in 1.4 mL of HPLC water were placed into tubes and then 300 µL of Na2CO₃ were added so as to obtain a final volume of 2 mL, the sample were incubated at 40°C for 30 minutes. Each sample was performed in triplicate. The absorbance was measured at 765 nm using a spectrometer Beckmann.
Quantification was obtained by reporting the absorbance in the calibration curve of gallic acid used as standard phenol (concentration range 0,0074 - 0,029 mM eq gallic acid/L). The results were expressed in micromole of gallic acid equivalent for liter of extract.

2.3. LDL Isolation and Oxidation Studies.
LDL was isolated from fresh human plasma [20-21]. LDL oxidizability measurements were monitored at 234 nm and 245 nm for Cu$^{2+}$- and AAPH- oxidation respectively. Isolated LDL was diluted to 1 µmol. L$^{-1}$ to investigate the antioxidant efficiency of the EWH$^+$-PC extract. LDL was added with various EWH$^+$-PC concentrations to be tested and then oxidized either by 5 µmol. L$^{-1}$-Cu$^{2+}$, or by 5 mmol. L$^{-1}$ AAPH after a tenfold dilution in oxygenated PBS (phosphate-saline buffer) as previously described [20-21], pH 7.4. Thus, we determined the kinetic profile of peroxidation which was principally characterized by the lag time of oxidation (Tlag), the maximal rate of conjugated diene (CD) production [R$\rho$ (CD)] and the maximal accumulation of oxidation products (CD$_{max}$) [19]. The “relative” Tlag, designated by rTlag = Tlag / Tlag$^-$ × 100, (Tlag$^-$ being the Tlag in presence of a given antioxidant concentration, Tlag$^-$ in its absence) was then plotted versus increasing concentrations of the different tested compounds, producing a linear relationship. The expression of the antioxidant capacities used the notion of specific antioxidant activity (SAA) considered as the regression coefficient of this linear relationship and expressed as µmol$^{-1}$. L for purified compounds or as µGAE$^{-1}$. L (µM$^{-1}$ of gallic acid equivalent) for EWH$^+$-PC, as previously described [22]. The Cu$^{2+}$/apoB and AAPH / apoB molar ratios of 50:1 and 50,000:1 were chosen because they allow us to make the Tlag independent of apoB concentration.

2.4. Statistical Analysis
All analyses were carried out in triplicate or more. Results are expressed as mean ± standard deviation (SD). Data were compared on the basis of the mean values. Statistical analyses of the data were carried out by one-way analysis of variance (ANOVA) using the Stata Software V10.0 (Stata Corp, 2007 edition). A probability of P < 0.05 was considered to be significant.

RESULTS
3.1. Total Phenolic Content and Specific Antioxidant Activity (SAA)
Total phenolic contents and specific antioxidant activity (SAA) of sorghums, millet, red wine (RW) and olive oil wastewater (OOWW) are reported in Table 1. Total phenolic contents and specific antioxidant activity (SAA) of Tô red sorghum (Tô RS), Tô white sorghum (Tô WS), Tô millet (Tô M) are reported in Table 2.

<table>
<thead>
<tr>
<th>Cereal</th>
<th>Total phenols As µGAE L$^{-1}$</th>
<th>Total phenols As µGAE L$^{-1}$/g flour</th>
<th>SAA (µM$^{-1}$)</th>
<th>SAA (µM$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Sorghum</td>
<td>34</td>
<td>272</td>
<td>168</td>
<td>47</td>
</tr>
<tr>
<td>White Sorghum</td>
<td>10.3</td>
<td>82.4</td>
<td>97</td>
<td>39</td>
</tr>
</tbody>
</table>

Table1: Total phenolic contents and specific antioxidant activity (SAA) of sorghums, millets, red wine (RW) and olive oil wastewater (OOWW)
<table>
<thead>
<tr>
<th>Cereal</th>
<th>Total phenols As µ GAE L⁻¹</th>
<th>Total phenols As µ GAE L⁻¹ /g flour</th>
<th>SAA (µM⁻¹) (Cu²⁺)</th>
<th>SAA (µM⁻¹) (AAPH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Sorghum</td>
<td>15.4</td>
<td>123</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>White Sorghum</td>
<td>2.4</td>
<td>19.2</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Millet</td>
<td>6</td>
<td>48</td>
<td>3</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Red sorghum which had about 3 times higher phenolic compounds than white sorghum and millet, showed a specific antioxidant activity (SAA) of 168 µGAE⁻¹×L while white sorghum and millet had a SAA respectively of 97 and 66 µGAE⁻¹×L.

A difference, with respect to the polyphenol contents of the varieties (red, white sorghum and millets), is observed and it was found that the red variety contained higher proportion of polyphenols than did white variety and millet. The noticeable difference between white and red varieties could be due to the presence of red pigments, such as anthocyanins, which are generally polymerized phenolics present in red cultivars.

### 3.2. Effects of EWH⁺-PC on Cu²⁺ - and AAPH- mediated LDL oxidation

Effects of EWH⁺-PC on the Cu²⁺ - and AAPH-mediated oxidative modification of LDL were shown in figure 2 (A and B) under standard conditions (0.1 µmol L⁻¹ -LDL in PBS at 37°C ) with OD = 234 nm for 5 µmol L⁻¹ - Cu²⁺ (A) and 245 nm for 5 mmol L⁻¹ - AAPH (B), using increasing concentrations of the EWH⁺-PC for red sorghum. Comparable results were obtained for white sorghum and millet and were not shown.
Fig 2. (A and B). Effect of polyphenols from Sorghum extracts on low density lipoprotein (LDL): A) Cu^{2+} - mediated and B) AAPH-mediated oxidation in vitro.

1: LDL without Sorghum polyphenols.
2 to 4: LDL with increasing concentrations of Sorghum polyphenols (5 µM to 25 µM) which was took as an example.

Compared with the control LDL (LDL without antioxidant, numbered 1 on figure 2A and 2B), we noticed that phenolic compounds prolonged the lag time with a dose-dependant effect under the two oxidation systems (table 3).

Table 3: Tlag foods based on the specific antioxidant activity (SAA) and serving against oxidation generated by Cu^{2+} / AAPH. (RW, OOWW, Tô RS, Tô WS, Tô M)

<table>
<thead>
<tr>
<th>FOOD</th>
<th>Total phenols As µGAE/serving</th>
<th>Total phenols As µGAE/L blood</th>
<th>Protection(min) (Cu^{2+})</th>
<th>Protection(min) AAPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>RW</td>
<td>4.45</td>
<td>0.89</td>
<td>6.85</td>
<td></td>
</tr>
<tr>
<td>OOWW</td>
<td>4.97</td>
<td>0.994</td>
<td>7.15</td>
<td></td>
</tr>
<tr>
<td>Tô RS</td>
<td>154</td>
<td>30.8</td>
<td>46</td>
<td>37</td>
</tr>
<tr>
<td>Tô WS</td>
<td>32</td>
<td>6.4</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Tô M</td>
<td>81.5</td>
<td>16.3</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

3. DISCUSSION

The antioxidant phytochemicals in grains, vegetables and fruits have received increased attention recently for their potential role in prevention of human diseases as well as in food quality improvement [23 - 24]. Phenolics are considered as a major group of compounds that contribute to the antioxidant activities of grains. Sorghum and barley are two important food grains reported to contain significant quantities of phenolic compounds [25]. Despite this, Sorghum grain and its products have not been explored extensively for their phytochemical attributes. Recently, it has become widely accepted that diet may play an important role in health promotion and disease prevention. Objective data are required by consumers and health care professionals to improve daily diets and, consequently, reduce the risk of chronic diseases such as coronary heart disease (CHD), which has an annual cost of $50-100 billion in the United States and has few opportunities to affect a cure after it is
established [26]. Growing evidence suggests that LDL oxidation and reactive oxygen species (ROS) attacks may play a causal role in the pathogenesis of atherosclerotic complications including CHD [27-28].

The present study showed that EWH+-PC prolonged the LDL lag time in a dose dependent manner in both Cu²⁺- and AAPH-mediated oxidation mediated systems. Red sorghum which had about 3 times higher phenolic compounds than white sorghum and millet, showed a highest specific antioxidant activity (SAA) of 168 while white sorghum and millet had a SAA respectively of 97 and 66 in copper mediated oxidation. The SAA of our polyphenol extracts are about 2 fold increase higher in the copper- than in the AAPH-system. (Table 1)

It is noted that LDL oxidation is a complex, multistep procedure involving both lipid and protein fractions through different mechanisms [28]. Free radical-mediated chain reaction is a possible mechanism involved in LDL oxidation. The effectiveness of EWH+-PC antioxidants may depend on the diffusion of the antioxidants to either lipid or protein fractions of LDL, the capacity of antioxidants to directly react with and quench free radicals in the system, the chelating potency of EWH+-PC to reduce the availability of transition metals including Cu²⁺ that may act as catalysts to generate the first few radicals that initiate the oxidative chain reaction, directly react with and convert the peroxides to less reactive compounds, and inducing the activity of antioxidative defense in vivo.

This result suggests the potential of sorghum and millet antioxidants in suppressing LDL oxidation in vivo. The reduction of LDL oxidation in vivo may delay the progress of atherosclerosis and reduce the risk of heart diseases.

We have also during this study compare the effect of flour transformed into To in terms of protection in time of LDL oxidation based on the portion and specific antioxidant activity (SAA) with those of red wine and olives oil wastewater. We found that red sorghum protection is 6 times higher 46min than red wine 6.85 min and olives oil wastewater 7.15 min. While white sorghum and millet gave us similar results compared with red wine and olives oil wastewater respectively 8 and 5 minutes.

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. This has potential applications in foods for diabetics.

REFERENCES


