Antioxidant properties of 3-deoxyanthocyanidins and polyphenolic extracts from Côte d’Ivoire’s red and white sorghums assessed by ORAC and in vitro LDL oxidisability tests

Marie-Annette Carbonneau, Moctar Cisse, Nathalie Mora-Soumille, Sofiane Dairi, Maxence Rosa, Françoise Michel, Céline Lauret, Jean-Paul Cristol, Olivier Dangles

To cite this version:

Marie-Annette Carbonneau, Moctar Cisse, Nathalie Mora-Soumille, Sofiane Dairi, Maxence Rosa, et al.. Antioxidant properties of 3-deoxyanthocyanidins and polyphenolic extracts from Côte d’Ivoire’s red and white sorghums assessed by ORAC and in vitro LDL oxidisability tests. Food Chemistry, Elsevier, 2014, 145, pp.701-709. 10.1016/j.foodchem.2013.07.025. hal-02634650

HAL Id: hal-02634650
https://hal.inrae.fr/hal-02634650
Submitted on 27 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Antioxidant properties of 3-deoxyanthocyanidins and polyphenolic extracts from Côte d’Ivoire’s red and white sorghums assessed by ORAC and in vitro LDL oxidisability tests

Marie-Annette Carbonneau a,*, Moctar Cisse a, b, Nathalie Mora-Soumille c, Sofiane Dairi a, Maxence Rosa c, François Michel a, d, Céline Lauret a, Jean-Paul Cristol a, d, Olivier Dangles c

a UMR 204 NUTRIPASS, University Institute of Clinical Research, 641, Av. Doyen Gaston Giraud, 34093 Montpellier Cedex 5, France
b University of Avignon, INRA, UMR 408, 84000 Avignon, France
c INPHB, 1093 Yamoussoukro, Côte d’Ivoire
d Department of Biochemistry, Lapeyronie Hospital, CHRU 371, Av. Doyen Gaston Giraud, 34295 Montpellier Cedex 5, France

Keywords: 3-Deoxyanthocyanidins, apigeninidin, luteolinidin, Sorghum polyphenols, Oxygen radical absorption capacity, Low density lipoprotein oxidation, Vitamin E

A B S T R A C T

Red sorghum is a source of phenolic compounds (PCs), including 3-deoxyanthocyanidins that may protect against oxidative stress related disease such as atherosclerosis. HPLC was used to characterise and quantify PCs extracted from red or white sorghum whole grain flour. Antioxidant activity was measured by an oxygen radical absorbance capacity assay and against LDL-oxidisability, and further compared to that of synthesised 3-deoxyanthocyanidins (i.e., luteolinidin and apigeninidin). Phenolic content of red and white sorghums was evaluated as 3.90 ± 0.01 and 0.07 ± 0.01 mmol gallic acid equivalents L⁻¹, respectively. Luteolinidin and apigeninidin were mainly found in red sorghum. Red sorghum had almost 3 and 10 times greater specific antioxidant activity compared to luteolinidin and apigeninidin, respectively. Red sorghum PCs and the two 3-deoxyanthocyanidins were also effective at preventing LDL vitamin E depletion and conjugated diene production. Red sorghum flour exhibits antioxidant capacity suggesting that it may be a valuable health-promoting food.

1. Introduction

Sorghum bicolor (L.) Moench is one of the most important cereal crops in the world. Sorghum is the staple food in several countries, notably in Africa. In West Africa, non-germinated sorghum grain is generally used for the preparation of “tò” (thick porridge) and couscous (granulated food). Malted sorghum is often used for infant food (thin porridge), for production of a local beer (“dolo”) and non-fermented beverages.

Sorghum contains significant quantities of phenolic acids like hydroxybenzoic and hydroxycinnamic acids. Moreover, anthocyanins are the major flavonoid class in sorghum. In general, these pigments contribute to the blue, purple, and red colours in plants (Awika, Rooney, & Waniska, 2004; Yoshida, Mori, & Kondo, 2009), and possess antioxidant and anti-inflammatory properties that may be relevant in the prevention of chronic diseases (Tsuda, 2012). Sorghum anthocyanidins (anthocyanin aglycones) are unique since they do not display a hydroxyl group in the C-ring 3-position (Fig. 1) and thus are called 3-deoxyanthocyanidins (3-DAs). This unique feature increases their chemical stability at neutral pH compared to the common anthocyanidins. The two common sorghum 3-DAs, namely apigeninidin [1] and luteolinidin [2], are especially abundant in sorghum grains but rare or absent in other plants (Awika et al., 2004). These polyphenols could present a potential for additional health benefits to consumers, for instance by reducing the incidence of oesophageal and gastrointestinal cancers as shown by epidemiological studies (Isaacson, 2005) or in vitro inhibition of cell proliferation (Awika, Yang, Browning, & Faraj, 2009). On the other hand, the inhibition of LDL oxidation has been widely used to analyse the antioxidant activity of natural products (mainly composed of phenolic acids, flavanols or flavonols) in relation to protection against cardiovascular disease (Cartron, Carbonneau, Fouret, Descomps, & Léger, 2001; Monde et al., 2011). However, no data are available concerning the effects of sorghum polyphenols and particularly the effects of 3-DAs on the inhibition of LDL oxidation.

The aim of this work was to synthesise red sorghum anthocyanidins (3-DAs) and compare their oxygen radical absorption capacity (ORAC) and their ability to protect low-density lipoprotein (LDL) against in vitro oxidation – initiated by Cu²⁺ ions or the diazo compound 2,2'-azobis (2-amidinopropane dihydrochloride (AAPH) – with that of red and white sorghum polyphenolic compounds (PCs). LDL oxidation was monitored by conjugated diene formation
Sorghum bicolor (L.) Moench grains were purchased from Côte d’Ivoire at several markets in Abidjan and the interior of the country, then mixed and cleaned to remove debris. CuCl₂, butylated hydroxytoluene (BHT) and the 2N Folin–Ciocalteu reagent were obtained from Sigma Aldrich (Saint Quentin Fallavier, France), while gallic acid and ultra-gradient grade methanol for high-performance liquid chromatography (HPLC) were purchased from Merck (Darmstadt, Germany). AAPH was from Biovalley (Conches, France). Other chemicals used are either reagent grade or HPLC grade. Trolox (6-hydroxy-2,3,7,8-tetramethylether-2-carboxylic acid) and 2′,7′-dichlorofluorescein were from Aldrich and Merck, respectively.

2.2. Chemical synthesis 3′,4′-dihydroxycacetophenone

A mixture of activated zinc powder (5 g, 76 mmol), co-chloro-3′,4′-dihydroxycacetophenone (5 g, 27 mmol), THF (120 mL) and acetic acid (30 mL) was vigorously stirred for 3 days at room temperature. After filtration and concentration under reduced pressure, 100 mL of EtOAc were added. The organic layer was washed with water (3 × 100 mL), dried over Na₂SO₄ and evaporated. The crude product was purified by column chromatography (SiO₂, cHex/EtOAc, 1:1 v/v) to give compound 3′,4′-dihydroxycacetophenone as a white amorphous powder; yield, 90%; Rₜ (cHex/EtOAc, 1:1), 0.47; ¹H-NMR (CDCl₃), δ 7.67 (1H, d, J = 2.0, H₂), 7.55 (1H, dd, J = 2.0 and 8.3 Hz, H₃), 6.56 (1H, d, J = 8.3 Hz, H₄), 6.19 (1H, s, OH), 5.99 (1H, s, 10H), 2.53 (3H, s, 3H, COCH₃); HPLC-DAD - Rₜ 14.2 min; [m/z] 276 Da.

2.2. Chemical synthesis 3′,4′-dihydroxycacetophenone

A mixture of activated zinc powder (5 g, 76 mmol), co-chloro-3′,4′-dihydroxycacetophenone (5 g, 27 mmol), THF (120 mL) and acetic acid (30 mL) was vigorously stirred for 3 days at room temperature. After filtration and concentration under reduced pressure, 100 mL of EtOAc were added. The organic layer was washed with water (3 × 100 mL), dried over Na₂SO₄ and evaporated. The crude product was purified by column chromatography (SiO₂, cHex/EtOAc, 1:1 v/v) to give compound 3′,4′-dihydroxycacetophenone as a white amorphous powder; yield, 90%; Rₜ (cHex/EtOAc, 1:1), 0.47; ¹H-NMR (CDCl₃), δ 7.67 (1H, d, J = 2.0, H₂), 7.55 (1H, dd, J = 2.0 and 8.3 Hz, H₃), 6.56 (1H, d, J = 8.3 Hz, H₄), 6.19 (1H, s, OH), 5.99 (1H, s, 10H), 2.53 (3H, s, 3H, COCH₃); HPLC-DAD - Rₜ 14.2 min; [m/z] 276 Da.

2.2. Chemical synthesis 3′,4′-dihydroxycacetophenone

A mixture of activated zinc powder (5 g, 76 mmol), co-chloro-3′,4′-dihydroxycacetophenone (5 g, 27 mmol), THF (120 mL) and acetic acid (30 mL) was vigorously stirred for 3 days at room temperature. After filtration and concentration under reduced pressure, 100 mL of EtOAc were added. The organic layer was washed with water (3 × 100 mL), dried over Na₂SO₄ and evaporated. The crude product was purified by column chromatography (SiO₂, cHex/EtOAc, 1:1 v/v) to give compound 3′,4′-dihydroxycacetophenone as a white amorphous powder; yield, 90%; Rₜ (cHex/EtOAc, 1:1), 0.47; ¹H-NMR (CDCl₃), δ 7.67 (1H, d, J = 2.0, H₂), 7.55 (1H, dd, J = 2.0 and 8.3 Hz, H₃), 6.56 (1H, d, J = 8.3 Hz, H₄), 6.19 (1H, s, OH), 5.99 (1H, s, 10H), 2.53 (3H, s, 3H, COCH₃); HPLC-DAD - Rₜ 14.2 min; [m/z] 276 Da.
3.4,5,7-Tetrahydroxyflavilium chloride = luteolinidin [2]. Same procedure as for [1] replacing 4-hydroxyacetophenone by 3,4-dihydroxyacetophenone. After deacetylation, reacidification and purification on reversed-phase silica gel, [2] was obtained as a HPLC-pure red powder: overall yield, 71% \( 1\% \) ( nBuOH/HOAc/H2O, 3:2:1), 0.77; The purity of [2] was carefully checked by reverse-phase HPLC-DAD with detection at 280 nm. The sole peak displayed a UV/Vis spectrum typical of anthocyanins: \( \lambda_{max} \) 269 min, \( \lambda_{max} \) 485 nm. UV/Vis (pH 3 citrate buffer), \( c \) = 19000 M\(^{-1}\) cm\(^{-1}\) at 481 nm. \( 1\)-H-NMR (DMSO-d\(_6\)/0.2 M TFA), \( \delta \) 7.99 (1H, d, \( J = 8.7 \) Hz, H4), 8.08 (1H, d, \( J = 8.7 \) Hz, H7), 7.91 (1H, \( J = 8.8 \) Hz, H4), 7.78 (1H, s, H2), 7.06 (1H, d, \( J = 8.8 \) Hz), 6.96 (1H, s, H6), 6.78 (1H, s, H3); \( 1\)-C-NMR (DMSO-d\(_6\)/0.2 M TFA), \( \delta \) 172.3 (C2), 172.2 (C6), 160.1 (C1), 160.0 (C0), 157.2 (C4), 149.7 (C4), 148.0 (C5), 126.4 (C6), 122.2 (C11), 118.0 (C11), 116.1 (C2), 113.4 (C10), 110.5 (C1), 103.0 (C0), 96.1 (C0); HRMS-ESI, \( m/z (M^+) \) calculated for C\(_{15}\)H\(_{11}\)O\(_{5}\)Na, 271.0601; found, 271.0606.

2.3. Whole flour sample preparation, polyphenol extraction and quantification

2.3.1. Preparation of whole flour and sample extraction

Samples of sorghum grain (collected on different markets and mixed) were powdered in a ‘Disk Mill’ (Glen Mills Inc., Clifton, NJ) and the whole flour was employed in this study. The extraction procedure involved the addition of 50 mL EtOH and 50 mL H\(_2\)O acidified by acetic acid (pH 2.6) to 10 g of whole flour to obtain an acidic ethanol/water extract (EWH\(^{+}\)). The sample was shaken for 3 h at room temperature and centrifuged at 3000 g for 10 min. The supernatant was then concentrated on a Rotavapor at 40 °C to a final volume of 25 mL. The extraction procedure was carried out at least in triplicate. The EWH\(^{+}\) method, like the acidic ethanol/water extract (EWH\(^{+}\)). The sample was shaken for 3 h at room temperature and centrifuged at 3000 g for 10 min. The supernatant was then concentrated on a Rotavapor at 40 °C to a final volume of 25 mL. The extraction procedure was carried out at least in triplicate. The EWH\(^{+}\) method, like the acidic ethanol/water extract (EWH\(^{+}\)–PC), this procedure was carried out for removing the more hydrophobic compounds (CHCl\(_3\) phase) and was systematically applied prior to the ORAC and LDL oxidation tests.

2.3.2. Determination of phenol content

The phenolic amount in EWH\(^{+}\)–PC was determined with the Folin-Ciocalteu reagent according to a modified procedure (Waterhouse, 2001), using gallic acid as a standard: 200 \( \mu \)L of diluted PCs and 100 \( \mu \)L of Folin-Ciocalteu reagent reagent diluted in 1.4 mL H\(_2\)O were placed into tubes and 300 \( \mu \)L of 1 M Na\(_2\)CO\(_3\) were added, to obtain a final volume of 2 mL. Samples were incubated at 40 °C for 30 min. Measurements were performed in triplicate. Absorbance values were measured at 765 nm using a UVicron XL spectrophotometer (BIO-TEK Instruments SAS, Colmar, France). Quantification was obtained by reporting the absorbance measured on the calibration curve of gallic acid used as a standard (concentration range: 0.7–3.0 mmol L\(^{-1}\)). The results were expressed in millimol of gallic acid equivalent per litre (mmol GA\(_E\) L\(^{-1}\)).

Separation and quantification of the EWH\(^{+}\) extract compounds were carried out by HPLC with a diode array detector (DAD) on a reversed phase column Lichrospher 100 RP-18 (250 \( \times \) 4.6 mm; 5 \( \mu \)m) protected by a pre-column of the same phase. The oven was thermostated at 30 °C for all tests. The injection volume was 5 \( \mu \)L. The mobile phase consisted of a linear gradient between solvent A: H\(_2\)O/H\(_2\)CO\(_3\) (95:5, v/v) and solvent B: H\(_2\)O/H\(_2\)CO\(_3\)/MeCN (15:5:80, v/v/v), from 0% solvent B to 80% solvent B. The flow rate was 0.25 mL/min. Identification was achieved by comparison of both retention times and absorption spectra obtained for each eluted peak with those of suitable standards. Concentrations were calculated from the chromatogram peak areas with suitable external standards. Particularly, caffeic acid (commercial) and luteolinidin (chemically synthesised) were used for the quantification of phenolic acids and anthocyanidins, respectively.

2.3.3. ORAC test

ORAC values of EWH\(^{+}\).PCs and 3-DAs were measured on a Perkin–Elmer fluorescence spectrometer by inhibition of 2,7′-dichlorofluorescein oxidation according to the method previously reported (Ishimoto et al., 2012) with slight modifications. Briefly, all samples and reagents were dissolved in 10 mM phosphate/150 mM NaCl buffer (PBS) at pH 7.4; 50 \( \mu \)L test samples or 50 \( \mu \)L of standard solutions (0–20 \( \mu \)M) 100 \( \mu \)L 2,7′-dichlorofluorescein solution (50 nM), and 100 \( \mu \)L AAPH solution (20 mM) were added to the wells of a 96-well plate. The fluorescence was recorded at 37 °C every 1 min for 100 min at respective excitation and emission wavelengths of 485 and 535 nm. A calibration curve was generated by using the correlation between the period of time needed to obtain 50% fluorescence decay and the Trolox concentration. ORAC levels were expressed as mole of Trolox equivalents (TE) per mole of antioxidant standard or mole of GA\(_E\) (extracts). Gallic and caffeic acids were used as controls. Their respective ORAC values of 1.5 ± 0.4 and 5.3 ± 0.3 mol TE mol\(^{-1}\) (means ± SEM; \( n = 13 \)) were similar to those previously reported Ishimoto et al. (2012).

2.4. LDL oxidation studies

2.4.1. Human LDL preparation and oxidation

LDL was isolated from fresh human plasma obtained from the “French Blood Establishment”, in accordance with ethical rules of that establishment (Cartron et al., 2001) by sequential ultracentrifugation. LDL purity was controlled by electrophoresis and was obtained without serum albumin contamination (data not shown). LDL oxidisability was monitored at 234 nm and 245 nm for Cu\(^{2+}\) and AAPH-induced oxidation, respectively. Isolated LDL was diluted to 1 \( \mu \)mol apol L\(^{-1}\) to investigate the antioxidant efficiency of PCs. LDL was incubated with various PC concentrations (expressed as mmol GA\(_E\) L\(^{-1}\) for red and white sorghum PC extracts, luteolinidin and apigeninidin, for comparison, and as mmol L\(^{-1}\) for all pure standard compounds), then oxidised in the presence of either 5 \( \mu \)M Cu\(^{2+}\), or 5 mM AAPH after a tenfold dilution in a pH 7.4 oxygenated PBS as previously described (Cartron et al., 2001). The kinetic oxidation profile was principally characterised by the lag time \( t_{lag} \) during which the accumulation of conjugated dienes is very slow. The relative \( t_{lag} \) defined as \( r_{lag} = \frac{t_{lag}}{t_{lag}^{TE} (\%)} \times 100 \) (\( t_{lag} \) being the \( t_{lag} \) in the presence of antioxidant, \( t_{lag}^{TE} \) in its absence), was then plotted vs. increasing concentrations of the different tested compounds, producing a linear relationship. The specific antioxidant activity (SAA) was defined as the slope of this linear relationship, as previously described Cartron et al. (2001) and was expressed as mmol GA\(_E\) L\(^{-1}\) or as mmol L\(^{-1}\) depending on the concentration expression (Cartron et al., 2001; Monde et al., 2011).

2.4.2. LDL-vitamin E analyses

EWH\(^{+}\)–PC and 3-DAs’ (luteolinidin and apigeninidin) influence on the time course of in vitro LDL \( \alpha \)-tocopherol (Vit E) consumption...
was determined after Vit E extraction by hexane/ethyl acetate (3/1; v/v) from oxidized LDL. For this, we measured the extracted products by means of an HPLC method with a Lichrocart® 125-4 (5-μm particle size) column (Merck, France), with α-tocopherol as an internal standard, as previously described by us Monde et al. (2011).

3. Results and discussion

Sorghum and barley are two important dietary grains reported to contain significant quantities of phenolic compounds (Folliad, Traoré, Vaksman, & Kouressy, 2004). However, sorghum and its products have not been explored extensively for their phytochemical and biological effects. Epidemiological studies have clearly demonstrated that a diet containing whole grain cereals can provide numerous health benefits due to the high content of bioactive compounds (Anderson, 2003), cancer (Chatenoud et al., 2012) and diabetes (Venn & Mann, 2004). We have investigated the antioxidant properties of PCs of red and white sorghum extracts in comparison with the two 3-DAs, luteolinidin and apigeninidin, which are commonly found in red sorghums but have a restricted distribution in nature (Awika et al., 2004).

3.1. Chemical synthesis of 3-DAs

4′,5,7-Trihydroxyflavylum chloride (apigeninidin, [1]) and 3′,4′,5,7-tetrahydroxyflavylum chloride [luteolinidin, (2)] could be extracted and purified from sorghum in small amounts and time- and solvent-consuming procedures or obtained at a high cost from commercial sources. As an alternative, in this work, [1] and [2] were efficiently synthesised, according to an essentially two-step route (Fig. 1) that is much simpler than the previously reported procedure (Sweeney & Iacobucci, 1981). First, 2,4,6-trihydroxybenzaldehyde has to be converted into 2,4-diacetoxy-6-hydroxy-benzaldehyde, not only to ensure solubility in EtOAc (the most convenient solvent for the subsequent condensation step) but also to deactivate the substrate toward oligomerisation (via acid-catalysed aromatic electrophilic substitution) leading to unidentified red pigments. This strategy was already applied to the synthesis of a natural anthocyanin (Dangles, & El Hajji, 1994). However, to our knowledge, this is the first time that the chemical synthesis of [1] and [2] is reported in detail. The satisfying yield and regioselectivity of the acylation step may be attributed to the strong hydrogen bond between the carbonyl group and one of the adjacent OH groups, which is thus relatively nonreactive. The flavylium chromophore was constructed via acid-catalysed aldo condensation followed by cyclisation and subsequent dehydration. Flavylum chlorides thus formed precipitated and were therefore easily isolated for subsequent deacetylation by sodium methyate. Re-acidification and purification on reversed-phase silica afforded pure pigments [1] and [2]. The H- and 13C-NMR spectra of [1] were in agreement with the literature (Kouda-Bonafos, Narco, & Ancian, 1996). The NMR data of [2] were consistent with those of already reported analogues (Bjoroy, Rayyan, Fossen, Kalsberg, & Anderson, 2009; Dangles & El Hajji, 1994; El Hajji, Dangles, Figueiredo, & Brouillard, 1997).

3.2. Polyphenol extraction, quantification and antioxidant activity

Phenolic content of Côte d’Ivoire’s sorghum varieties were evaluated as 3.90 ± 0.01 and 0.07 ± 0.01 mmol GAE L⁻¹ in EWH⁺-PCs of red and white sorghums, respectively. They were also expressed in whole flour as 9.1 ± 0.9 and 0.17 ± 0.02 mg GAE g⁻¹ on a dry matter basis, under our experimental extraction conditions. Moreover, 1 mol of apigeninidin [1] and 1 mol of luteolinidin [2] are equivalent to 1.06 ± 0.03 and 1.96 ± 0.12 mol GAE, respectively; and 1 mol of [1 + 2] is equivalent to 1.35 ± 0.01 mol GAE (n = 5).

The major polyphenolic compounds were analysed by HPLC-DAD (Fig. 2) and presented in Table 1. As shown in Fig. 2C, red sorghum contains 3-DAs (luteolinidin and apigeninidin) and some derivatives (mono-, di-methyl ethers and unidentified glycosides). However, white sorghum does not contain 3-DAs nor flavanols (catechin and epicatechin) (data not shown). The 3-DA content is not so high in the red sorghum extract (6.5% of total phenols for luteolinidin and its methylated derivatives, 2.2% for apigeninidin and its methylated derivatives), while flavanols and quercetin derivatives are major compounds, as shown in Table 1. Stilbenoids trans-piceid and trans-resveratrol previously quantified as low as 0.2–1 μg g⁻¹ in red sorghum grains (Bröhan, Jerkovic, & Collin, 2011) were not looked for in our study.

To evaluate the antioxidant capacity of sorghum polyphenol extracts, we firstly determined their oxygen radical absorbing capacity (ORAC) in comparison with pure synthetic 3-DAs. Luteolinidin [2] and apigeninidin [1], with ORAC values of 4.9 ± 0.4 and 4.5 ± 0.3 TE mol⁻¹, respectively, were in the same range as potent flavonoids (e.g., quercetin and myricetin) but less potent than catechin or epicatechin, which show remarkable ORAC levels (Ishimoto et al., 2012). Moreover, an equimolar addition of the two compounds [1 + 2] gave an ORAC value (4.7 ± 0.3 TE mol⁻¹) equivalent to that of each of the compounds taken in isolation, without any synergy. All these results are expressed as mean ± SD (n = 5–8). While devoid of a catechol group, apigeninidin is only slightly less potent than luteolinidin. This is unexpected, as the catechol group is typically put forward as the major determinant of the radical-scavenging capacity, owing to the relative stability of the corresponding semiquinone radical (Dangles, 2012).

As expected, 1 g of red sorghum is much more effective (ca. a factor 25) at scavenging peroxyl radicals than the same amount of white sorghum (ORAC values = 103.8 ± 2.1 μmol TE g⁻¹ and 4.2 ± 0.2 μmol TE g⁻¹; respectively). However, this gain in efficiency is lower than what could be anticipated from the richness of PCs in red sorghum (a factor ca. 54 in comparison with white sorghum based on the Folin–Ciocalteu test). Hence, when data are expressed in terms of GAE (instead of dry mass), the red sorghum EWH⁺-PC extract with an ORAC value of 1.93 ± 0.04 μmol TE g⁻¹ came up as less effective than the white sorghum EWH⁺-PC extract (ORAC value = 4.32 ± 0.15 μmol TE g⁻¹) and the two pure 3-DAs. This suggests that, although poor in polyphenols, white sorghum contains other antioxidants that could be intrinsically more potent at scavenging peroxyl radicals than those of red sorghum. In other words, for a fixed concentration expressed in μmol GAE L⁻¹, i.e. for a given capacity at reducing MoVI (the basis of the Folin–Ciocalteu test), antioxidants from white sorghum are better peroxyl radical scavengers (the basis of the ORAC test) than those extracted from red sorghum.

Finally, on a whole flour dry weight basis, EWH⁺-PCs of red sorghum displayed ORAC activities in the range of those previously found for different sorghum varieties (Awika et al., 2009; Bröhan et al., 2011), showing that the EWH⁺-PC extraction method used in this study was as efficient as other extraction techniques previously used for easily extractable (loosely bound) compounds, e.g., 1% HCl/methanol (Awika et al., 2009), or acetone/water (7/3) (Bröhan et al., 2011).
Fig. 2. HPLC profiles of acidified ethanol/water (pH 2.6) extract obtained from red sorghum whole flour. Detection at 280 and 360 nm (A and B): (1) gallic acid; (2) protocatechuic acid; (3) chlorogenic acid; (4) caffeic acid; (5 and 6) luteolin derivatives; (7) p-coumaric acid; (8) apigenin derivatives; (9) luteolin derivative; (10 and 11) flavanols; (12) quercetin derivative; (13) quercetin; (14, 15 and 16) hydroxycinnamic acid derivatives. Detection at 480 nm (C): (a) luteolinidin; (b) apigeninidin; (c) 5-methoxy-luteolinidin; (d) 7-methoxy-luteolinidin; (e) 5,7-dimethoxy-luteolinidin, (f) 7-methoxy-apigeninidin; (g) 5,7-dimethoxy-apigeninidin; (h) anthocyanidin derivatives.
The antioxidant effects of red and white sorghum EWH+-PCs and 3-DAs in comparison with some standard phenolic acids or flavonoids, assessed by in vitro LDL oxidation.

Table 1

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>Red sorghum μg g⁻¹ dry weight</th>
<th>White sorghum μg g⁻¹ dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>280</td>
<td>2.0</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>280</td>
<td>5.3</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>330</td>
<td>10.5</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>330</td>
<td>10.0</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>330</td>
<td>6.8</td>
</tr>
<tr>
<td>Flavonols</td>
<td>280</td>
<td>266.8</td>
</tr>
<tr>
<td>Quercetin derivatives / Quercetin</td>
<td>360</td>
<td>74.3</td>
</tr>
<tr>
<td>Luteolinidin + derivatives</td>
<td>480</td>
<td>26.5</td>
</tr>
<tr>
<td>Apigeninidin + derivatives</td>
<td>480</td>
<td>9.0</td>
</tr>
<tr>
<td>Luteolinidin</td>
<td>74.0; 100.0</td>
<td>12 (0.99)</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>54.0; 24 (0.76)</td>
<td>18.6 (0.99)</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>155.5; 8 (0.99)</td>
<td>156.2 (0.99)</td>
</tr>
<tr>
<td>Rutin</td>
<td>216.8; 10 (0.92)</td>
<td>156.2 (0.99)</td>
</tr>
</tbody>
</table>

3.3. Polyphenol protective effect on human LDL oxidisability

The inhibition of Cu²⁺- or AAPH-induced human LDL oxidation was then investigated in vitro at different concentrations of red and white sorghum EWH⁺-PC and of luteolinidin and apigeninidin. In both oxidation protocols, the concentrations of sorghum extracts were expressed in μmol GAE L⁻¹ for red and white sorghums, luteolinidin and apigeninidin (un-sterilized values) and as μmol 1⁻¹ L for all pure compounds, (sterilized values).

\[ \text{SAA} = \frac{\text{r} \cdot \text{AAPH-induced oxidation} - 1}{\text{Cu²⁺-induced oxidation}} \]

For comparison, for red and white sorghums, luteolinidin and apigeninidin, concentrations were expressed as μmol GAE L⁻¹. Concentrations for all pure compounds were expressed in μmol L⁻¹.

\[ \text{SAA} = \text{specific antioxidant activity, defined as the slope of the relationship between relative } t_{lag} \text{ and concentrations of tested compounds; SAA were expressed as } \mu\text{mol GAE L}^{-1} \text{ for red and white sorghums, luteolinidin and apigeninidin (un-sterilized values) and as } \mu\text{mol L}^{-1} \text{ for all pure compounds, (sterilized values).} \]

\[ \text{r} = \text{correlation coefficient; } p < 0.01; \text{ } \rho < 0.05 \]

Table 2

Antioxidant effects of red and white sorghum EWH⁺-PCs and 3-DAs in comparison with some standard phenolic acids or flavonoids, assessed by in vitro LDL oxidation.

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>Red sorghum μmol L⁻¹</th>
<th>White sorghum μmol L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luteolinidin</td>
<td>74.0; 100.0</td>
<td>12 (0.99)</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>54.0; 24 (0.76)</td>
<td>18.6 (0.99)</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>155.5; 8 (0.99)</td>
<td>156.2 (0.99)</td>
</tr>
<tr>
<td>Rutin</td>
<td>216.8; 10 (0.92)</td>
<td>156.2 (0.99)</td>
</tr>
</tbody>
</table>

For comparison, for red and white sorghums, luteolinidin and apigeninidin, concentrations were expressed as μmol GAE L⁻¹. Concentrations for all pure compounds were expressed in μmol L⁻¹.

\[ \text{SAA} = \text{specific antioxidant activity, defined as the slope of the relationship between relative } t_{lag} \text{ and concentrations of tested compounds; SAA were expressed as } \mu\text{mol GAE L}^{-1} \text{ for red and white sorghums, luteolinidin and apigeninidin (un-sterilized values) and as } \mu\text{mol L}^{-1} \text{ for all pure compounds, (sterilized values).} \]

\[ \text{r} = \text{correlation coefficient; } p < 0.01; \text{ } \rho < 0.05 \]

\[ \text{Cu²⁺-induced oxidation SAA; } n (r) \]

\[ \text{AAPH-induced oxidation SAA; } n (r) \]

For comparison, for red and white sorghums, luteolinidin and apigeninidin, concentrations were expressed as μmol GAE L⁻¹. Concentrations for all pure compounds were expressed in μmol L⁻¹.

\[ \text{SAA} = \text{specific antioxidant activity, defined as the slope of the relationship between relative } t_{lag} \text{ and concentrations of tested compounds; SAA were expressed as } \mu\text{mol GAE L}^{-1} \text{ for red and white sorghums, luteolinidin and apigeninidin (un-sterilized values) and as } \mu\text{mol L}^{-1} \text{ for all pure compounds, (sterilized values).} \]

\[ \text{r} = \text{correlation coefficient; } p < 0.01; \text{ } \rho < 0.05 \]

Results are reported as the means of two separate analyses.

sites on LDL (chlorogenic acid possibly via its quinic moiety since caffeic acid is much less efficient). Owing to its catechol nucleus, the critical determinant of metal chelation and radical-scavenging capacity (Dangles, 2012; Yeomans, Linseisen, & Wolfram, 2005), luteolinidin is fairly active, although slightly less than chlorogenic acid and the two flavonols, whereas apigeninidin (no catechol nucleus) is weakly active. However, although luteolinidin is probably a much better metal chelator than apigeninidin, the ORAC test suggests that it may not be that much more efficient at scavenging peroxyl radicals. Finally, on the basis of the SAA values obtained, the observation that the red sorghum EWH⁺-PCs are better inhibitors of LDL oxidation than white sorghum EWH⁺-PCs and both individual 3-DA components may be interpreted as follows: (a) red sorghum contains other efficient phenolic antioxidants also present in the white variety but in very low concentrations (Table 1); and/or (b) efficient synergisms could take place between some red sorghum phenolic compounds.

The data of AAPH-induced LDL oxidation largely confirm those of the copper-mediated process. Luteolinidin is an average inhibitor, typically less potent than common polyphenols having a catechol nucleus. Surprisingly, apigeninidin even displays a weak pro-oxidant activity, as evidenced by a small negative SAA value. Catechin and, to a lesser extent, rutin are the most effective free radical scavengers in this system.

To understand the observed protective effect of these polyphenols, their influence on the time course of LDL-vitamin E (Vit E) consumption under Cu²⁺-mediated oxidation was investigated as...
shown in Fig. 3 for red sorghum EWH+-PCs, in parallel with CD production. Comparable kinetic curves were obtained for luteolinidin and apigeninidin, individually or in combination, and for white sorghum EWH+-PCs (figures not shown). As shown in Table 3, Vit E consumption and CD production are delayed in a dose-dependent manner by the different EWH+-PCs. Firstly, initial Vit E oxidation rate \( R_{i(VitE)} \) is about twice lower when PC concentration is twice higher, pointing out a direct protection of Vit E by EWH+-PCs. However, the very beginning of the CD propagation phase (i.e., the end of the inhibition period corresponding to the starting time \( t_{lag} \) of detectable CD production) does not coincide with the period of time required for total Vit E oxidation \( t_{VitE} \) for EWH+-PCs and 3-DAs. Unlike white sorghum EWH+-PCs for which \( t_{VitE} \approx t_{lag} \) (Table 3), red sorghum EWH+-PCs and 3-DAs still protect LDL from oxidation after complete consumption of Vit E. The most efficient antioxidant is the red sorghum EWH+-PC extract, which is able to reduce significantly (\( p < 0.05 \)) Vit E consumption from \( R_{i(VitE)} = 0.46–0.08 \text{ mol L}^{-1} \text{ min}^{-1} \) at a concentration of 4 \( \mu \text{mol GAE L}^{-1} \), whereas 15 \( \mu \text{mol GAE L}^{-1} \) of white sorghum EWH+-PC extract are required for the same protection (Table 3). Moreover, the LDL-Vit E protection afforded by red sorghum EWH+-PCs is significantly higher than that of the luteolinidin and apigeninidin mixture (\( R_{i(-vitE)} = 0.22 \text{ mol L}^{-1} \text{ min}^{-1} \) for a total 6 \( \mu \text{mol GAE L}^{-1} \) concentration of DAs) (Table 3).

LDL oxidation is a complex, multistep mechanism involving both lipid and protein fractions (Pinchuk, Gal, & Lichtenberg, 2001). In particular, AAPH-derived peroxyl radicals and lipid oxyl radicals produced by copper-induced decomposition of lipid hydroperoxide traces trigger the typical free radical chain reaction of lipid peroxidation. The effectiveness of antioxidants at inhibiting LDL peroxidation mainly depends on their diffusion to either lipid or protein fractions, their capacity to directly reduce lipid oxyl and peroxyl radicals, their affinity for transition metal ions including Cu\(^{2+}\) (formation of inert metal-antioxidant complexes), their capacity to regenerate endogenous antioxidants such as \( \alpha \)-tocopherol (vitamin E), and the fate of the antioxidant-derived aryloxyl radicals.

Red sorghum EWH+-PCs and 3-DAs not only protect vitamin E but also protect LDL after consumption of vitamin E. This suggests that red sorghum polyphenols are first able to inhibit the onset of LDL oxidation by chelation of copper ions and possibly by partial regeneration of vitamin E (although no lag phase in vitamin E

Fig. 3. Time course of LDL-vitamin E degradation induced by \textit{in vitro} Cu\(^{2+}\)-mediated oxidation, in parallel with CD production, with or without addition of red sorghum EWH+-PCs. LDL oxidation was induced by 5 \( \mu \text{mol L}^{-1} \) copper ions at 37 °C. Vitamin E was analysed by an HPLC method (left y-axis) and CD production was monitored at 234 nm (right y-axis). Red sorghum EWH+-PC extract (2 and 4 \( \mu \text{mol GAE L}^{-1} \)) was added to LDL before oxidation, in comparison to LDL alone. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
consumption is observed; see Fig. 3). After vitamin E consumption, red sorghum polyphenols and/or some of their oxidation products liable to diffuse into the core of the LDL particles, could prolong the lag phase of CD production by a direct scavenging of the propagating lipid peroxyl radicals.

As suggested by the pro-oxidant activity of apigeninidin in AAPH-induced LDL oxidation, the arylxyl radicals formed from apigeninidin may be reactive enough to re-initiate lipid peroxidation by H-atom abstraction from PUFA moieties, as already evidenced in α-tocopherol mediated LDL peroxidation (Litwinienko & Ingold, 2007). It is also known that the flavone apigenin participates in peroxidase-catalysed oxidation of glutathione (GSH) by H2O2 whereas luteolin is inhibitory. In the first case, apigenin arylxyl radicals are reactive enough to abstract the thiol H-atom of GSH, thereby inducing a cascade leading to O2− reduction. In the second case, the luteolin semiquinone is rapidly oxidised to the o-quinone, which simply undergoes conjugation with GSH (Galati, Moridani, Chan, & O’Brien, 2001).

It must also be kept in mind that in neutral conditions 3-DAs are liable to diffuse into the core of the LDL particles, could prolong the lag phase of CD production by a direct scavenging of the propagating lipid peroxyl radicals. The low correlation between the inhibition of LDL oxidation and ORAC test by the sorghum polyphenols suggests that, when PCs are screened for their antioxidant activity, more realistic structured models involving lipids and proteins should be preferred to the ORAC assay.

It is now well known that a flavonoid-rich diet (onion, cereals or beverages (green and black tea, wine) permits the detection of low concentrations of flavonoid metabolites in the plasma, thus confirming their partial bioavailability (Cartron et al., 2003; Crozier, Jagnathan, & Clifford, & M. N., 2009). However, these metabolites could also exhibit antioxidant (e.g., anti-inflammatory) effects in relation to the prevention of chronic diseases (Halliwell, Rafferty & Jenner, 2005). Particularly, PCs are able to delay the in vitro LDL-vitamin E oxidation (Cartron et al., 2001; Monde et al., 2011) could as well exert direct in vivo beneficial antioxidant effects within the eosophagel (Awika et al., 2009) and gastrointestinal tracts by protecting dietary polyunsaturated lipids and vitamin E and/or by scavenging potential carcinogens. It is perhaps on this aspect that more investigations on the biological activity of dietary polyphenols should focus in the future.

4. Conclusion

In this study, we have investigated the composition of easily extractable polyphenolic compounds of red and white sorghum whole flour from Côte d’Ivoire, in order to determine their antioxidant activities in comparison to synthesised 3-DAs, luteolinidin and apigeninidin. The results of our study show that the sorghum extracts possess a significant free radical-scavenging activity (ORAC test). For a given phenolic concentration (expressed in μmol GAEL−1), white sorghum could even be more potent than red sorghum. However, when a more physiological test was used (LDL oxidisability), the red sorghum extract came up as more efficient than white sorghum and 3-DAs. Its effectiveness is of the order of the potent inhibitors of LDL oxidation quercetin and its 3-O-glycoside rutin. When PCs are screened for their antioxidant activity, more realistic structured models involving lipids and proteins should be preferred to the ORAC assay.

Polyphenols in plants can be classified as easily extractable (loosely bound) or poorly extractable (strongly bound). Recent investigations have demonstrated that the polyphenolic content of plant foods is often underestimated because significant amounts remain in the extraction residues and thus escape quantification (Durazzo, Turfani, Azzini, Maiani, & Carcea, 2013). It could also be interesting to evaluate the antioxidant activity of poorly extractable (strongly bound) PCs that could be present in red sorghum whole flour.

References


