Anti-nutritional metabolites in six traditional African cereals

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Summary
This article reports on the biochemical investigation of six African cereals (fonio, teff, sorghum, African rice, finger millet, pearl millet). These cereals play an important role in food security in many African and Asian regions, despite not being internationally traded, with the exceptions of sorghum and, partially, of pearl millet. Nowadays, crop breeders and research institutions are becoming concerned with improving the productivity and the nutritional quality of these cereals for reasons connected with the problems of human population growth and climate change. However, in these species less is known about the presence and content of anti-nutritional components that may impact human health, such as polyphenols, in particular tannins, phytate and the goitrogenic C-glycosyl flavones. In this work, these compounds were quantified and identified by means of biochemical assays or HPLC and LC-MS analysis. Obtained results showed that each of the six analysed cereals contained different levels of anti-nutritional or harmful goitrogenic compounds. In particular, all three examined pearl millet lines showed the sharply highest content of C-glycosyl flavones, slightly highest content of phytic acid and a relevant average level of polyphenols. On the other side, the Fonio lines presented the lowest level of C-glycosyl flavones and condensed tannins.

Keywords
African crops; cereals; nutraceutical compounds; anti-nutritional factors; genetic improvement

Mankind nutrition is largely dependent on two dozen crops, with rice, wheat and maize accounting for providing some 60 % of total energy value in food and feed. Africa is the centre of origin, and still today the major producing area, for a number of typical cereal grains, representing staple food for millions of people [1]. These traditional African grains, in a world threatened by global warming, have great advantages: they contribute to agricultural diversification and improvement in land use, possess economic potential and, moreover, provide for the diversification and improvement of the human diet, possessing several interesting nutritional properties [2, 3]. Some of the African cereal grains studied in this work (fonio, teff, African rice, finger millet), usually cultivated in restricted areas, are considered "Neglected and Underutilized Species" (NUS) or orphan cereals. Recently, local varieties of African cereals are being involved in breeding programs in order to increase the yield [4].

Fonio *(Digitaria exilis)* is one of the oldest cereals originating from Africa. It has exceptional content of amino acids such as methionine (350 g·kg^{-1} of total N), cysteine or leucine. In addition to the role of food, it improves the structure and fertility of poor and degraded soils on which it is grown [5].

Teff (*Eragrostis tef)* was originally domesticated in Ethiopia. Nowadays, it is normally ground into flour, which is used to make "injera", a traditional fermented Ethiopian pancake [6]. Compared to other cereals, teff is more tolerant to extreme environmental conditions and, moreover, its seeds can be easily stored under local storage conditions without losing viability since the grains are resist-

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ant to attack by storage pests [3].

Sorghum *(Sorghum bicolor*) represents one of the chief cereal grains consumed in Asia and Africa. The nutrient composition of sorghum indicates that it is a good source of energy, proteins, carbohydrates, vitamins and minerals. Sorghum is the only cereal among those analysed in the present work that has been already subjected to some investigations concerning its nutraceutical value [7, 8].

African rice (*Oryza glaberrima*) is generally cultivated only in northern part of the West African coast. It shows certain negative features with respect to the other domesticated species of rice, the Asian *O. sativa*. Its seed scatters easily, the grain is brittle and difficult to mill and, most importantly, the yields are lower. On the other side, African rice is more resistant to diseases and pests [9].

Finger millet (*Eleucine coracana*), also known as "Ragi", is widely grown in the cooler, higher altitude regions of Africa and Asia. It is the second most important millet in Africa and constitutes a staple food for many poor communities in the semi-arid areas of Africa and Asia [10, 11].

Finally, pearl millet (*Pennisetum glaucum*) is the most important of several unrelated millet species. Its peculiar feature is the presence in the grain of C-glycosyl-flavones (CGFs), the compounds that, if ingested in large quantities, can be goitrogenic, i.e. cause goiter onset. Vitexin, glycosyl-orientin and glycosyl-vitexin are the three CGFs so far identified in pearl millet [12].

Overall, these species are well adapted to local environmental conditions, such as drought and specific local pathogens, and are often endowed with good or excellent nutritional qualities [13]. Moreover, they are particularly rich in minerals but their bioavailability is usually low due to the presence of anti-nutritional factors such as phytate or polyphenols. Since most information regarding their nutritional qualities is already available, the work focused the attention on the content of compounds with anti-nutritional properties in the above mentioned traditional African cereal species. In particular, levels of phytic acid, polyphenols, condensed tannins and CGFs were determined in this study. Mineral availability may be adversely affected by high polyphenol and phytic acid contents in food grains [14], although these compounds may have relevant anti-oxidant activity and other positive effects on human health. Condensed tannins, a subgroup of polyphenols, may form a less digestible complex with dietary proteins and may bind and inhibit endogenous protein such as digestive enzymes. These compounds confer some resistance to moulds and deterioration of the grain [15]. CGFs are flavonoids that are potentially goitrogenic.

MATERIALS AND METHODS

The different seed material used in this work was obtained in African local markets or provided from different institutions listed in Tab. 1.

Preparation of flours

One gram of seed flour, finely powdered, was obtained using a Retsch Mixer Mill M 301 (Retsch-Allee, Haan, Germany) 2 times for 30 s at the vibrational frequency of 30 $Hz \cdot s^{-1}$). Subsequently, each sample was subjected to extraction using several solvents described in following paragraphs, according to the analysis to perform. All the analyses of each crop variety were performed in triplicate.

Total polyphenols

The total content of phenolics was measured according to the method described by Medoua et al. [16]. For each sample, 0.3 g of seed flour was homogenized with 1.5 ml of 50% methanol solution (pH 3.0), employing mechanical agitation in an orbital shaker (Eppendorf, Hamburg, Germany) at 6.5 Hz for 30 min at 4 °C and then centrifuged at 3 000 ×*g* for 10 min. The supernatants were collected and the residue pellets were further washed with 1.5 ml of 70% acetone employing mechanical agitation (6.5 Hz, 30 min, 4 °C) and then centrifuged. The resulting supernatants were assayed using the Folin-Ciocalteu reagent (Sigma-Aldrich, Saint Louis, Missouri, USA). Absorbance was measured at 725 nm using a UV-Vis spectrophotometer (Jasco, Easton, Maryland, USA) and results were expressed in gallic acid equivalents using a gallic acid standard curve.

Condensed tannins

Proanthocyanidins were analysed according to the method described by Doria et al. [17]. One hundred milligrams of each seed flour were incubated in extraction buffer (70% acetone, 1% HCl in methanol) at 60 °C for one hour, shaking at 6.5 Hz. After 15 min of sonication and subsequent centrifugation at 25 Hz, supernatants were collected and the remaining pellets were homogenized with 500 μl of ethylacetate-diethylether 1:1 (v/v) with shaking at 60 °C for 30 min. The combined seed extracts were subsequently incubated at 90 °C with a butanol-HCl (95:5, v/v) solution. Upon development of red coloration, the absorbance of

Tab. 1. List of the examined cereal lines and their origin.

Species	Variety	Origin
Fonio (*Digitaria exilis*)	Fini de Djouna Fini de Mane CVF477 Fini de Tongo	Cinzana Agricultural Research Station (Segou, Mali)
Teff (*Eragrostis tef*)	Unknown (black) Unknown (white) Eritrean	Grain market in Addis Abeba (Ethiopia)
Sorghum (*Sorghum bicolor*)	Unknown (black) Unknown (white)	Grain market in Addis Abeba (Ethiopia)
	Subsp. *bicolor*	National Agronomy Institute (Tunis, Tunisia)
African rice (*Oryza glaberrima*)	White C614 Black 56-81	Institute of Agricultural Research of Senegal (Dakar, Senegal)
Finger millet (*Eleusine coracana*)	Unknown (black)	Debre Zeyit Agricultural Research Centre (Debre Zeyit, Ethiopia)
Pearl millet (*Pennisetum glaucum*)	B02 (53-56) B08 (65-68) B09 (53-56)	International Crops Research Institute for the Semi-Arid Tropics (experimental station of Niamey, Niger)

samples was read at 550 nm using a UV-Vis spectrophotometer (Jasco) and compared with a delphinidin standard curve similarly prepared.

Phytic acid

Phytic acid content of the examined plant seeds was determined by using the ferric precipitation method followed by the colorimetric assay based on Chen's reactant described by Pilu et al. [18] and Doria et al. [19].

C-glycosyl flavones

For the goitrogenic CGFs analysis, the contents of three compounds frequently detected in cereal seeds were determined, namely, of vitexin, C-glycosyl vitexin and orientin. Seed flours were subjected to extraction by 2.5 ml of methanol (100%) at 50 °C for 6 h, shaking at 2.5 Hz. After centrifugation at 15 000 ×*g* for 10 min, the supernatant was filtered using nylon membrane syringe filters (diameter 25 mm, pore size 0.45 μm; Phenomenex, Torrance, California, USA) and high-performance liquid chromatography (HPLC) analysis was carried out using a Jasco HPLC system (Jasco), equipped with an auto-sampler. The chromatographic separation was performed at room temperature, by isocratic elution with a flow rate of 0.4 ml·min^{-1}, using a Kinetex PFP 100 Å column (250 mm × 4.6 mm, particle size 5 μm), equipped with a corresponding pre-column, both from Phenomenex. The injection volume was 10 μl and the signal was monitored at 330 nm. The mobile phase consisted of acetonitrile 21% in a water solution of acetic acid (1%). The run time was 18 min, followed by 5 min elution by pure acetonitrile to wash the column, and 10 min back to the initial condition in order to re-equilibrate the column. Four different concentrations of the standards (G-vitexin, vitexin and orientin) ranging between 10 μg·ml^{-1} and 50 μg·ml^{-1} were prepared in methanol for the construction of calibration curves.

Mass spectrometric analysis

Reverse-phase liquid chromatography with photodiode array-electrospray ionization mass spectrometer (RP-HPLC-PDA-ESI-MS^n) analysis was performed using a Thermo Finnigan Surveyor Plus HPLC (Conquer Scientific, San Diego, California, USA), equipped with a quaternary pump, Surveyor UV-Vis diode array detector and a liquid chromatography quadrupole advantage max ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA), connected through an electrospray ionization source. The HPLC column was the same as described above, but the mobile phase consisted of water acidified with 1% formic acid (eluent A) and acetonitrile (eluent B), and a gradient was applied as follows: 0–2 min 5 % B, from 5 % to 21 % B in 1 min, a 23 min isocratic run of 21 % B, from 21 % to 95 % B in 1 min, followed by a 10 min isocratic run of 95 % B. Total run time was 45 min, including column re-conditioning. The flow rate was maintained at 0.4 ml·min^{-1}, the autosampler and column temperatures were both maintained at 25 °C. The samples were analysed at different concentrations and 5 μl of the solution was injected into the chromatographic system. Chromatograms were spectrophotometrically registered at 254 nm, 280 nm and 330 nm, spectral data being collected in the range of 200–800 nm for all peaks.

HPLC-ESI-MS^n data were acquired under positive ionization mode, using Xcalibur software (Thermo Fisher Scientific). To achieve this, the ion trap operated in full scan (200–1 500 *m/z*), data-dependent scan and sequential mass spectrometry mode (MS^n) modes, which provide further useful data on the molecular structure, enabling discrimination between isomeric compounds not showing significant differences. To obtain MS^2 data, 35% collision energy and an isolation width of 2 *m/z* were applied. To optimize MS operating conditions, a preliminary experiment was performed, in which 5 $\mu g \cdot ml^{-1}$ caffeine (0.1% formic acid and methanol, 50 : 50, v/v) solution was directly infused through the ESI interface at a flow rate of 25 $\mu l \cdot min^{-1}$ into the mass spectrometer. The optimized conditions were as follows: sheath gas 50 (arbitrary units), auxiliary gas 20 (arbitrary units), capillary temperature 220 °C, capillary voltage 3 V and spray voltage 5 kV.

Statistics analyses

Statistics analyses were carried out using Excel program (Microsoft, Redmond, Washington, USA). T-student and ANOVA analysis were used to compare means of data on different cereal varieties.

RESULTS AND DISCUSSION

Total polyphenols

Polyphenol levels were found to be variable among all the analysed cereals (Fig. 1).

Black sorghum samples presented the highest content of total polyphenols and also showed twice the content compared to the other two lines, white and bicolor ($P < 0.05$). The content of phenolic compounds found in this black variety (around 0.8 $g \cdot kg^{-1}$) was relevant, but in line with those presented in a review published by Dykes and Rooney [20], which compared varieties of sorghum of different colours of pericarp.

African rice presented the second (behind black sorghum) highest value of polyphenols (around 0.55 $g \cdot kg^{-1}$), without any significant difference between the white and the black line ($P > 0.05$). Several phenolic acids were usually identified in rice where the total value ranged from 73 $g \cdot kg^{-1}$ to 87 $g \cdot kg^{-1}$ in the endosperm, 1 776 $g \cdot kg^{-1}$ to 3 198 $g \cdot kg^{-1}$ in the bran, 208 $g \cdot kg^{-1}$ to 783 $g \cdot kg^{-1}$ in the whole grain, and 4 776 $g \cdot kg^{-1}$ in the husk, depending on the rice colour [21].

In fonio lines, total polyphenol level in whole seed flours was rather high, close to that of the other orphan cereals analysed in this work, but a 30–50% reduction could be noticed after decortication. Polyphenols in cereals are mainly located in the pericarp, so the removal of this external layer can lead to a substantial reduction in the level of these metabolites.

In teff, a clear difference was found between hulled and dehulled seed. The value found in this study is in line with that obtained by Girma et al. [22], around 0.35 $g \cdot kg^{-1}$. The Eritrean teff (dark coloured) was found to have a still higher content of polyphenols (0.43 $g \cdot kg^{-1}$), while fermentation increased the content of these compounds by around 35 % with respect to the black one.

In the finger millet, the total polyphenol content was around 0.5 $g \cdot kg^{-1}$, falling within the average range of the other studied cereals. However, in this case it is not possible to directly compare the data obtained in this work with those published in literature because of the remarkable variability of cultivars. In a paper published by Siwela et al. [23], several African finger millet varieties were analysed for the content of polyphenols and the range of these compounds was found between 0.5 $g \cdot kg^{-1}$ and 0.7 $g \cdot kg^{-1}$, similar to the values presented in this work.

Considerable phenolic content was registered in all three pearl millet lines (B2, B8 and B9). For this millet variety, the average content of polyphenols (around 0.5 $g \cdot kg^{-1}$) was found to be lower than that found by Nambiar et al. [24], showing the phenolic content to range from 2.68 $g \cdot kg^{-1}$ to 4.20 $g \cdot kg^{-1}$ of dry weight.

Condensed tannins

Most sorghum varieties do not contain condensed tannins but there are some species showing a prominent pigmented testa that contain these components, due to the presence of the nucleotide polymorphisms in the *Tan1* gene, as demonstrated by Wu et al. [25]. The black sorghum studied in this work showed a very high level of tannins, almost 0.7 $g \cdot kg^{-1}$ (Fig. 2). In general, type II and III sorghums have tannin levels of 0.2–1.9 $g \cdot kg^{-1}$ and 4.0–35.0 $g \cdot kg^{-1}$ of catechin equivalents, respectively [26]. Recent data on tannin content of Type II and III sorghum varieties were summarized by Dykes and Rooney [20] who showed a huge variability in the content of these metabolites (from 0.1 $g \cdot kg^{-1}$ to 50 $g \cdot kg^{-1}$).

Condensed tannins are also abundant in African rice flour, which contains the second highest level, behind black sorghum. As mentioned previously, this property appears to be associated with the bran layer of the crop seed. In fact, the black line showed a content of tannins by almost 30% higher than that measured in the white one. The black line presented almost twice the content of condensed tannins than the white and Eritrean lines, while fermentation seemed to drastically decrease the content of these compounds.

In fonio lines, the level of tannins was found to be very low in wholemeal flour and almost absent in flour obtained from dehulled seeds.

The content of condensed tannins extracted from the varieties of finger millet, analysed in the study conducted by SIWELA et al. [23] was between 2- and 100-fold lower, once more demonstrating that tannin occurrence in finger millet grain is a varietal property, similar to the situation in sorghum.

C-glycosyl flavones

The contents of CGFs vitexin, G-vitexin and orientin is reported in Tab. 2, while the total level of glycosyl flavones, for each crop, is summarized in Fig. 3. The contents of these flavones was found to be very variable, from few milligrams per kilogram in African rice to more than 200 mg·kg^{-1} in pearl millet. In fact, the highest total contents of these metabolites were found in all the three pearl millet samples, where the average value was 145 mg·kg^{-1}, which was about three-fold higher than the level found in teff, the second richest cereal (around 70 mg·kg^{-1} in the case of white teff) and about 10-fold higher than the average level of the rest of the examined cereals. In particular, the B8 line of pearl millet reached the value of 370 mg·kg^{-1}, more than thrice as high as the

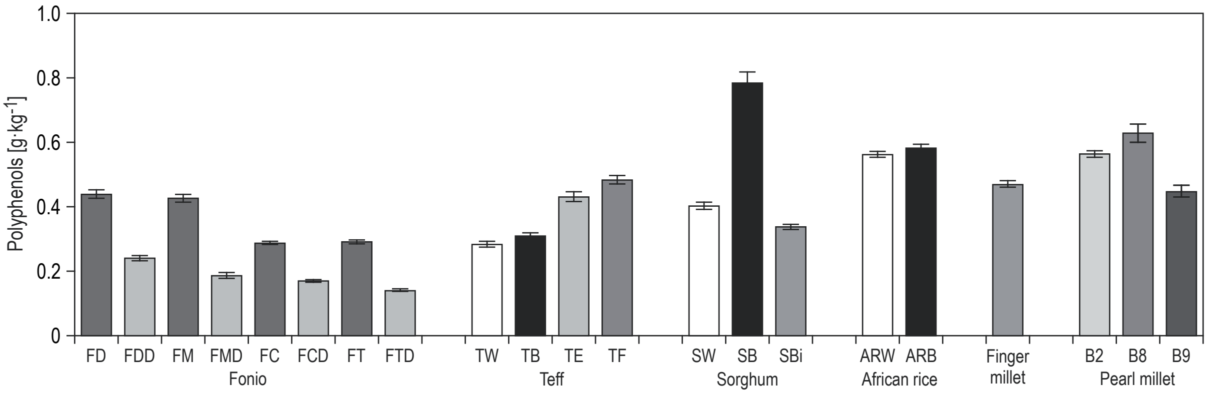

Fig. 1. Polyphenol contents in the examined cereal samples.

FD – Fonio Djouna, FDD – Fonio Djouna dehulled, FM – Fonio Mane, FMD – Fonio Mane dehulled, FC – Fonio CVF477, FCD – Fonio CVF477 dehulled, FT – Fonio Tongo, FTD – Fonio Tongo dehulled, TW – White teff, TB – Black teff, TE – Eritrean teff, TF – Fermented teff, SW – White sorghum, SB – Black sorghum, SBi – Bicolor sorghum, ARW – White African rice C614, ARB – Black African rice 56-81, B2 – Pearl millet B2, B8 – Pearl millet B8, B9 – Pearl millet B9.

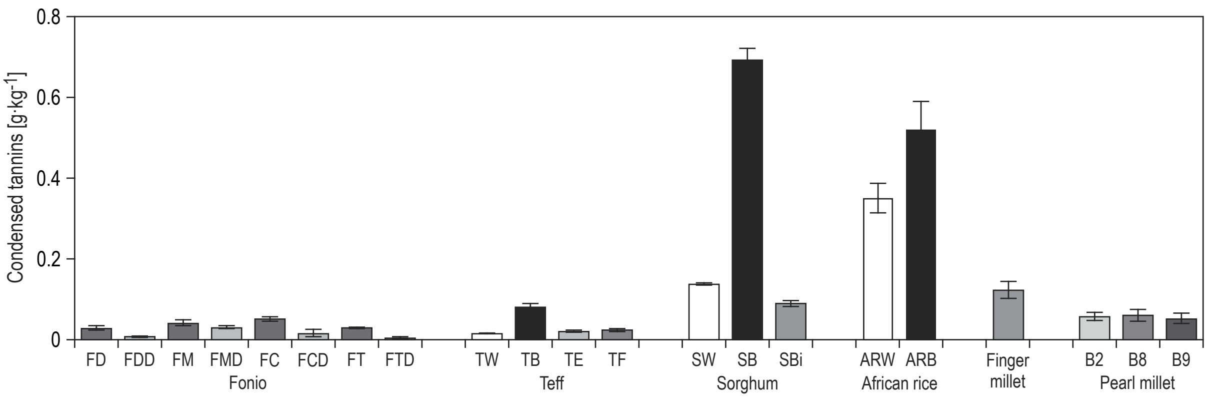

Fig. 2. Proanthocyanidin contents determined in the seed cereal flours.

FD – Fonio Djouna, FDD – Fonio Djouna dehulled, FM – Fonio Mane, FMD – Fonio Mane dehulled, FC – Fonio CVF477, FCD – Fonio CVF477 dehulled, FT – Fonio Tongo, FTD – Fonio Tongo dehulled, TW – White teff, TB – Black teff, TE – Eritrean teff, TF – Fermented teff, SW – White sorghum, SB – Black sorghum, SBi – Bicolor sorghum, ARW – White African rice C614, ARB – Black African rice 56-81, B2 – Pearl millet B2, B8 – Pearl millet B8, B9 – Pearl millet B9.

average value registered for the other two lines of millet. Presence of G-vitexin was registered in all the analysed crops except for white teff and fermented teff, while oreintin and vitexin were detected only in 9 and 11 varieties, respectively (out of 21 studied). In particular, only some cultivars of fonio (CVF477 and Djouna) and pearl millet were found to contain relevant amounts of all three examined flavones.

Besides the content of vitexin and glucosyl vitexin, whose presence had already been pointed out in pearl millet, the HPLC chromatograms

Tab. 2. Contents of three C-glycosyl flavones in the crops.

Cereal	G-Vitexin [mg·kg^{-1}]	Orientin [mg·kg^{-1}]	Vitexin [mg·kg^{-1}]
Fonio Djouna	6.32 ± 0.06	15.15 ± 0.38	8.29 ± 0.35
Fonio Djouna dehulled	5.19 ± 0.33	7.01 ± 1.04	4.54 ± 0.77
Fonio Mane	8.15 ± 0.35	nd	nd
Fonio Mane dehulled	5.35 ± 0.44	nd	nd
Fonio CVF477	8.08 ± 0.07	14.08 ± 0.95	6.82 ± 1.13
Fonio CVF477 dehulled	8.50 ± 0.61	4.88 ± 1.02	5.06 ± 0.05
Fonio Tongo	8.52 ± 0,09	nd	nd
Fonio Tongo dehulled	4.68 ± 0.57	nd	nd
White teff	nd	nd	67.11 ± 0.82
Black teff	20.65 ± 0.56	40.84 ± 1.26	nd
Eritrean teff	17.13 ± 0.80	44.04 ± 2.01	nd
Fermented teff	nd	36.57 ± 0.93	nd
White sorghum	12.64 ± 0.19	nd	12.66 ± 0.26
Black sorghum	16.68 ± 0.05	nd	16.57 ± 0.04
Bicolor sorghum	25.52 ± 0.90	nd	nd
White African rice C614	11.35 ± 1.18	nd	nd
Black African rice 56-81	3.80 ± 0.47	nd	nd
Finger millet	5.26 ± 1.21	nd	2.64 ± 0.15
Pearl millet B2	78.64 ± 3.21	25.10 ± 1.35	32.67 ± 1.63
Pearl millet B8	226.76 ± 4.15	47.11 ± 2.46	97.68 ± 3.45
Pearl millet B9	14.97 ± 0.23	31.14 ± 1.19	45.41 ± 2.29

Data are expressed as mean ± standard deviation (n = 3). nd – not detectable.

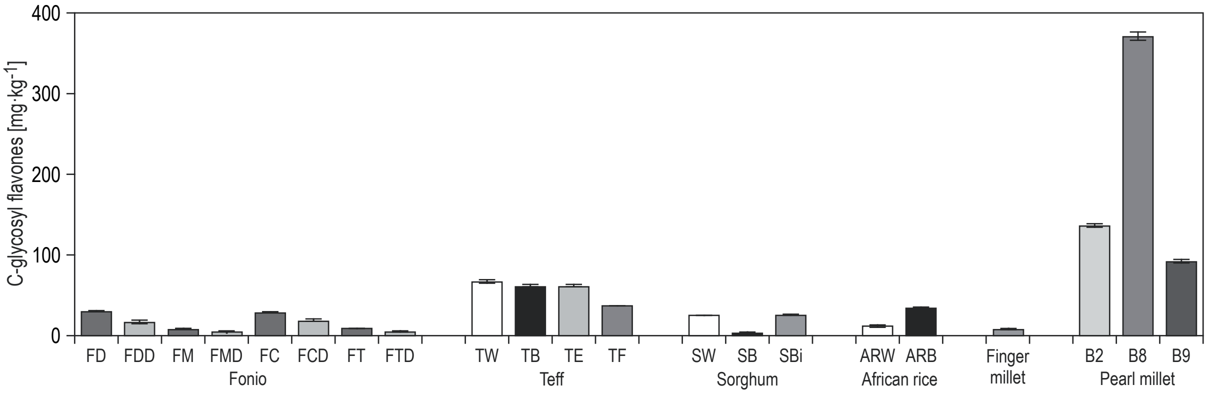

Fig. 3. Total content of C-glycosyl flavones in the examined cereal samples.

FD – Fonio Djouna, FDD – Fonio Djouna dehulled, FM – Fonio Mane, FMD – Fonio Mane dehulled, FC – Fonio CVF477, FCD – Fonio CVF477 dehulled, FT – Fonio Tongo, FTD – Fonio Tongo dehulled, TW – White teff, TB – Black teff, TE – Eritrean teff, TF – Fermented teff, SW – White sorghum, SB – Black sorghum, SBi – Bicolor sorghum, ARW – White African rice C614, ARB – Black African rice 56-81, B2 – Pearl millet B2, B8 – Pearl millet B8, B9 – Pearl millet B9.

of pearl millet extracts showed also the relevant presence of a peak corresponding to orientin (which was precisely quantified in the three analysed lines thanks to the commercial availability of the standard) and of additional peaks that we hypothesized to correspond to other compounds belonging to the same chemical family. Therefore, pearl millet samples were submitted to RP-HPLC-PDA-ESI-MS^n analysis to determine CGFs occurring in each of them. The chromatograms of the three samples (B2, B8, B9), analysed at different concentrations in order to better reveal the compounds of interest, were acquired at a wavelength of 330 nm (Fig. 4).

Tab. 3 shows the chromatographic behaviour, UV-Vis, MS and MS-MS data of the identified compounds. In detail, five compounds were determined, by comparison of the experimental fragmentation patterns with those already available in the literature.

Peaks 1 and 2 were identified as the glucosyl and rhamnosyl derivatives of orientin. In both cases, the fragmentation pattern revealed the presence of an MS-MS ion corresponding to luteolin (m/z = 287) and one corresponding to glucosil-luteolin, also known as orientin (m/z = 449). The identification was possible due to loss of a glucosyl $[M\text{-}162]^+$ and a rhamnosyl $[M\text{-}146]^+$ moiety, respectively, from the molecular ions, which leads to the aglycone orientin. Peaks 3 and 4 were vitexin-derivatives when, according to the data published by Bai et al. [27], peak 3 was identified as glucosyl-vitexin, the only compound detected in all the tested samples, and peak 4 was identified as rhamnosyl-vitexin. Finally, the aglycone vitexin was detected as peak 5.

Rhamnosyl derivatives of orientin and vitexin had never been previously reported in millet grains and represent a very important fraction of CGFs accumulated in pearl millet seeds. The goitrogenic potential of the above cited six CGFs was verified and proven only in the case of glucosyl-orientin, vitexin and glucosyl vitexin [12], while orientin and the rhamnosyl compounds have not yet been investigated for this property. It must anyhow be taken into account that, when adsorbed on intestinal level, CGFs undergo de-glycosylation that prompts the goitrogenic action, i.e. orientin and orientin-derived CGFs are synthesized from luteolin, while apigenin acts as a precursor of vitexin and vitexin-derived CGFs [12]. In order to estimate the goitrogenicity of any pearl millet genotype, the contents of glucosyl-orientin, vitexin and glucosyl-vitexin, but also of orientin, rhamnosyl-vitexin and rhamnosyl-orientin, must be accurately determined in the seeds.

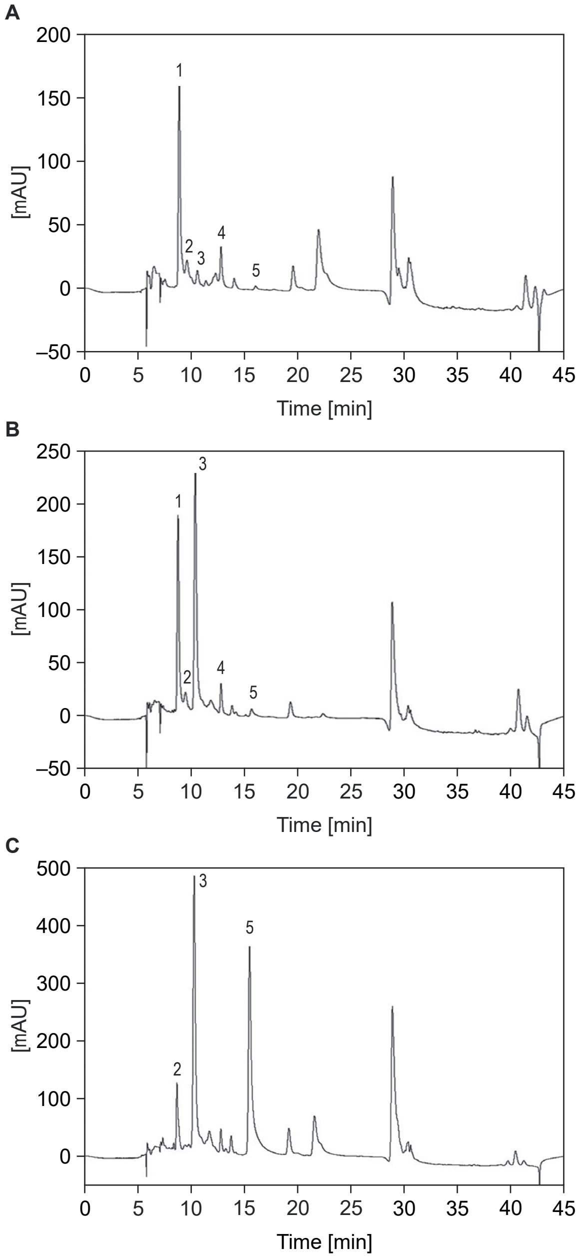

Fig. 4. Mass spectrometry chromatograms with detection at 330 nm of the pearl millet samples.

A – pearl millet B2, B – pearl millet B8, C – pearl millet B9. Peaks: 1 – glucosyl-orientin, 2 – rhamnosyl-orientin, 3 – glucosyl-vitexin, 4 – rhamnosyl-vitexin, 5 – aglycone vitexin.

Phytic acid

Data on phytic acid contents in the analysed cereals are presented in Tab. 4.

Seeds of pearl millet lines were found to contain the highest phytic acid amounts, on average 3.1 $g\cdot kg^{-1}$. These results are in line with El Hag et al. [28], who analysed two varieties of pearl mil-

Tab. 3. Mass spectrometry analysis data of the three pearl millet lines.

Peak	*RT* [min]	λ [nm]	*m/z* [M+H]+	MS2	Proposed structure
1	8.9	226, 257, 346	611	449 (100 %), 431 (20 %), 329(20 %), 491(20 %), 287(2 %)	Glucosyl-orientin ac
2	9.4	226, 271, 336	595	449(100 %), 431(5 %), 329(20 %), 287(2 %), 491(5 %)	Rhamnosyl-orientin bc
3	10.9	226, 269, 336	595	433(100 %), 271(5 %), 313(10 %), 415(2 %), 475(10 %)	Glucosyl-vitexin abc
4	12.3	226, 270, 333	579	433(100 %), 313(20 %)	Rhamnosyl-vitexin ac
5	16.4	226, 323	433	343(70 %), 387(100 %), 313(40 %)	Vitexin or isovitexin bc

RT – retention time, λ – wavelength, *m/z* – mass/charge ratio, [M+H]+ – positive ionization modality, MS2 – fragmentation of the main ion, values in brackets represent the percentage of each fragment.
a – compounds occurring in pearl millet B2 line, b – compounds occurring in pearl millet B8 line, c – compounds occurring in pearl millet B9 line.

let from Uganda and found an average level of 10 g·kg-1.

A rather high level of phytate was determined in all the lines of fonio without any decrease after dehulling.

The content of phytic acid was found very low in teff, especially in white teff, while dark teff varieties showed a content of phytate of around 7 g·kg-1, in line with data presented in literature and summarized in the report of Baye [6] concerning the Ethiopia Strategy Support Program (from 6 g·kg-1 to 13 g·kg-1 of dry material) [6]. Fermentation finally promoted a 50% reduction in the content of phytate, appearing to be an effective strategy in decreasing this anti-nutritional factor.

The content of phytic acid determined in finger millet (6.63 g·kg-1) was close to the average value determined in other cereals and in line with values presented in literature. Mbithi-Mwikya et al. [29], analysing a finger millet variety from Kenya, found a lower content of phytates (3.6 g·kg-1), while some other studies reported that the level of phytic acid in some African variety of finger millet ranged from 8.5 g·kg-1 to 15 g·kg-1.

Finally, African rice showed the second lowest phytic acid content behind white teff. As reported by different researchers, the phytic acid content of rice shows less variation than that of other antioxidant compounds. Moreover, the value reported in this work (average of 5.5 g·kg-1) was in the range of those found for the Asian varieties (between 4 g·kg-1 and 7 g·kg-1).

Tab. 4. Phytic acid content and the corresponding phytic acid phosphorus measured in the cereal flours.

Cereal	Phytic acid phosphorus [g·kg-1]	Phytic acid [g·kg-1]
Fonio Djouna	2.33 ± 0.08	8.26 ± 0.72
Fonio Djouna dehulled	2.70 ± 0.09	9.58 ± 0.58
Fonio Mane	2.77 ± 0.06	9.85 ± 0.72
Fonio Mane dehulled	3.06 ± 0.01	10.87 ± 0.90
Fonio CVF477	3.22 ± 0.08	11.45 ± 0.89
Fonio CVF477 dehulled	2.78 ± 0.05	9.86 ± 0.58
Fonio Tongo	2.75 ± 0.01	9.76 ± 0.70
Fonio Tongo dehulled	2.74 ± 0.01	9.74 ± 0.71
White teff	1.13 ± 0.05	4.03 ± 0.25
Black teff	2.03 ± 0.01	7.19 ± 0.82
Eritrean teff	1.64 ± 0.01	5.83 ± 0.77
Fermented teff	0.99 ± 0.03	3.53 ± 0.44
White sorghum	2.11 ± 0.04	7.50 ± 0.63
Black sorghum	2.35 ± 0.01	8.34 ± 0.53
Bicolor sorghum	2.36 ± 0.04	8.38 ± 0.69
White African rice C614	1.38 ± 0.04	4.9 ± 0.45
Black African rice 56-81	1.71 ± 0.04	6.0 ± 0.45
Finger millet	1.87 ± 0.02	6.63 ± 0.47
Pearl millet B2	3.89 ± 0.03	13.82 ± 0.40
Pearl millet B8	3.22 ± 0.02	11.43 ± 0.53
Pearl millet B9	3.93 ± 0.02	13.97 ± 0.45

CONCLUSIONS

In conclusion, each of the six examined cereals showed the presence of variable amounts of anti-nutritional or potentially noxious compounds. A synthetic summary may be drawn as follows:

Fonio showed a relevant content of phytate and polyphenols, compounds hindering the bioavailability of phosphate and mineral cations. However, polyphenols level, but not phytic acid level, markedly decreased in dehulled seeds.

Teff flours, in particular those from black teff samples, possessed a remarkable level of polyphenol compounds. Moreover, phytic acid content appeared to be moderate. However, the level of goitrogenic flavones was rather high and it should be reduced in order to exclude any goitrogenic effects in consumers using teff as staple food.

Sorghum did not contain high amounts of phytic acid or goitrogenic compounds. Thus, perhaps due to the fact that it is one of the most studied among the six analysed cereals and due to the previous published literature about the nutritional properties, confirmed in this work, it represents one of the major candidates to be employed in breeding programs.

African rice flours appeared very interesting, even if still to be improved, as regards the paucity of anti-nutritional factors like goitrogenic flavones and phytic acid.

Finger millet was shown to be of interest mainly due to the absence of goitrogenic CFGs and, in general, due to the low content of anti-nutritional factors.

Pearl millet was found to be endowed with negative features such as contents of phytic acid and, most of all, of goitrogenic CGFs. In this work, besides glycosyl-orientin, vitexin and glycosyl-vitexin, the three CGFs already observed and reported previously in pearl millet grain, three additional CGFs, namely, orientin, rhamnosyl-vitexin and rhamnosyl-orientin, were detected and identified, together with glucosyl-orientin, by HPLC-ESI-MS^n. The significant area relative to these peaks leads us to argue that they, together with orientin, may contribute to the overall goitrogenic potential of this cereal.

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