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Phenolic compounds and safety of improved and local peanut varieties grown in Burkina Faso

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Peanuts are a tropical crop widely cultivated throughout the world. The seed is the most important part of the peanut. Burkina Faso is the 16th largest producer of peanuts in the world. Despite its economic and nutritional potential, peanut growers are subject to aflatoxin contamination. This present study aimed to evaluate the phenolic compounds and safety of various improved and local peanut varieties. The aflatoxin contents of the different varieties were determined by ultra-high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). A UV-visible spectrophotometer quantified the phenolic contents. For all samples, results showed that water content varied from 3.85 ± 0.08 to 4.21 ± 0.06%, and pH from 6.11 ± 0.02 to 6.48 ± 0.02. Toxicological results showed total aflatoxin levels ranging from 0.04 to 1.86 µg/kg. Polyphenols had the highest values in peanut extracts, ranging from 5.64 ± 1.35 to 14.94 ± 2.79 mg GAE/g. Flavonoids ranged from 1.23 ± 0.11 to 2.24 ± 0.15 mg QE/g and flavonols from 0.14 ± 0.09 to 0.85 ± 0.36 mg QE/g. Condensed tannin contents range from 0.14 ± 0.02 to 0.26 ± 0.02 mg TAE/100 g and hydrolysable tannins from 0.03 ± 0 a to 0.16 ± 0.09 a mg TAE/g. TC tannins ranged from 0.14 ± 0.02 to 0.26 ± 0.02 mg TAE/100 g and THs from 0.03 ± 0 a to 0.16 ± 0.09 a mg TAE/g. Peanut seeds have interesting levels of phytonutrients. They could therefore be considered foods with therapeutic potential. Low levels of aflatoxins testify to the safety of the seeds.

Keywords *Arachis hypogea*, Seed, Aflatoxin, Phenolic compounds, Peanut, Sanitary quality

Peanut (*Arachis hypogea* L.), is a leguminous member of the Fabaceae family, widely grown in tropical and subtropical climates[1]. It is a tropical plant native to South America and is cultivated in several countries. Its cultivation is widespread throughout the world. Currently, over 100 countries worldwide grow peanuts[2]. It ranks twelfth in the world in terms of crop production. The total area sown for groundnuts in 2022 is estimated at 30,536,263 hectares, for a production of 54,238,560.13 tons[3]. China is the leading producer with an annual output of 18,329,500 tons, followed by India and Nigeria with outputs of 10,134,990 tons and 4,284,000 tons respectively[3]. African groundnut production accounted for 9,84563.04 tons representing 32.01% representing of world production. West Africa is the largest peanut-producing region in Africa, with 98,445,683.04 tons (56.71%)[3]. Burkina Faso, located in the heart of West Africa, is the world's 16th largest peanut producer. Groundnut production has risen considerably over the past decade, from 34,0166 tons to 55,9064.39 tons from 2010 to 2022, for areas of 40,922 hectares and 6,23,769 hectares respectively[3].

Peanut cultivation is of particular economic importance, as the marketing of unshelled peanuts in 2022 generated around U$49,955,941,000[3]. In much of sub-Saharan Africa, the crop is very important for domestic consumption and trade[4,5]. Its economic importance could be further increased if we consider the products derived from peanuts, which include oil, paste, oilcake, cookies, etc. In addition to the financial aspect, peanuts are an important source of nutrients, as peanut seeds are rich in fat and proteins[6]. The fat content of peanut seeds varies from 30 to 60% dry weight, and their protein content from 24 to 36% dry weight. They contain diverse fatty acids, in particular linoleic acid, which is an essential fatty acid for the body[6]. Regular consumption of peanuts has been shown to modulate lipid metabolism, reducing triglyceride and cholesterol levels[7]. Peanut oil

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is generally used in cooking, but also in soap-making. Peanut seeds also contain several amino acids, of which glutamic acid and aspartic acid are the most representative. Peanuts can be consumed in a variety of ways: either as roasted or raw seeds, or in more or less elaborate forms derived from the confectionery peanut market, such as butter, flour, paste, etc.[8]. In addition to primary metabolites, plants also produce so-called "secondary" metabolites. These metabolites confer interesting properties, helping to prevent cardiovascular, degenerative, and metabolic diseases[9]. Properties associated with these polyphenol compounds include antioxidant, anticancer, and anti-inflammatory properties[10,11].

All these data show that peanuts are important in human and animal nutrition. However, despite its economic and nutritional potential, peanut growers face enormous storage difficulties. Peanuts are extremely sensitive to fungi of the genus Aspergillus, which produce toxins notably aflatoxins and fumonisins. Aflatoxins are toxic substances with carcinogenic, mutagenic, and teratogenic effects[12]. Among these mycotoxins, aflatoxin B1 stands out for its high toxicity and carcinogenic potential. It can be found in many food products, particularly peanut derivatives[13,14]. Among oilseeds, peanuts are the most contaminated by toxin-producing molds, most often during cultivation, harvesting, and storage[15]. Numerous studies have documented the presence of aflatoxins in peanuts in severals countries[16–19]. Contamination of food products by the Aspergillus genus is a frequent problem, especially for peanut seeds. It should be noted that the main factors favoring aflatoxin contamination of peanuts are seed and air humidity, and the temperature of the ambient environment during seed storage and marketing. Aflatoxin contamination of peanuts can also occur before harvest, during crop ripening in the field, particularly favored by drought stress and high soil temperature[20]. This is particularly true in hot countries such as Burkina Faso, where temperatures can sometimes reach 48–50 °C, resulting in water stress[21]. This explains the need for special monitoring of Aspergillus contamination of peanuts in Burkina Faso. Studies in Kenya, Ethiopia, and Côte d'Ivoire have shown advanced stages of aflatoxin contamination of peanut seeds[20–24]. Aflatoxins represent a serious health problem affecting the entire food chain, from field production to consumption. Aflatoxins are fungal toxins that possess acute life-threatening toxicity, carcinogenic properties, and other potential chronic adverse effects[5].

Several subsequent studies have shown the presence of secondary metabolites such as flavonoids and polyphenols, mainly in peanut skins[25]. However, very few have quantified their content in whole seeds. Nowadays, several techniques are used to quantify and identify phenolic compounds, including chromatographic and spectrometric methods, depending on the availability of reference standards[26]. Moreover, recent studies have shown that the presence of phenolic compounds in plant matter may limits its contamination by *Aspergilus flavus*[27]. Secondary metabolites such as polyphenols, flavonoids, and tannins have been shown to reduce the multiplication of microorganisms such as *Aspersgillus*[28]. Nowadays, very few studies have examined the link between peanut varieties, their phenolic compound content, and aflatoxin contamination. It was with this in mind that the present study was carried out. Given the importance of groundnuts, new improved varieties have been produced by Burkina Faso's "Institut National de l'Environnement et des Recherches Agricoles (INERA)". The initial aim of introducing these varieties was to increase groundnut productivity and improve its ability to adapt to the country's pedoclimatic conditions, such as drought and soil degradation. Having determined the primary metabolite composition of these new varieties in a previous study, the main aim of the present study was to determine the sanitary quality of these varieties compared with existing varieties and to assess their secondary metabolite composition, including total phenolic compound levels.

Materials and methods

Sampling

Samples were collected from three locations in Burkina Faso: Ouagadougou (12° 21′ 56.4″ N, 1° 32′ 2″ W), Bobo-Dioulasso (11° 10′ 37.7″ N, 4° 17′ 52.4″ W), and Dedougou (12° 27′ 48.2″ N, 3° 27′ 38.7″ W) (Fig. 1). The data used to produce the map comes from the National Topographic Data Base of Burkina Faso. A total of fourteen samples were collected for the study, including twelve varieties supplied by INERA and two local samples collected from fields in the town of Dedougou. The samples were pre-treated by cleaning the peanut pods, which were then shelled. The seeds obtained were ground to serve as a matrix for the various analyses. Sample codes are shown in the table below (Table 1).

Determination of the physicochemical properties of peanut seeds

pH measurement

Seeds were crushed and mixed with distilled water, then the mixture was used to determine the pH using a pH meter (H.I microprocessor pH meter, HANNA INSTRUMENT) previously calibrated with pH 4 and pH 7 buffer solutions[29].

Moisture content

Water content was determined using the AOAC, 2000 method. The fruit samples were weighed and dried in an oven (BINDER, USA Toll Free) at 105 °C for 3 h. The weights of the samples before and after drying were then used to calculate the water content[30].

Aflatoxin assay

The determination of aflatoxins was carried out using a modified QuEChERS triple partitioning method for the analysis of aflatoxins in peanuts. The method involves a simultaneous sample extraction and clean-up step, based on the modified QuEChERS method, using hexane to remove lipids and HPLC–MS/MS (Agilent Varian 1200 GC/MS System, USA) for the determination of target compounds[31].

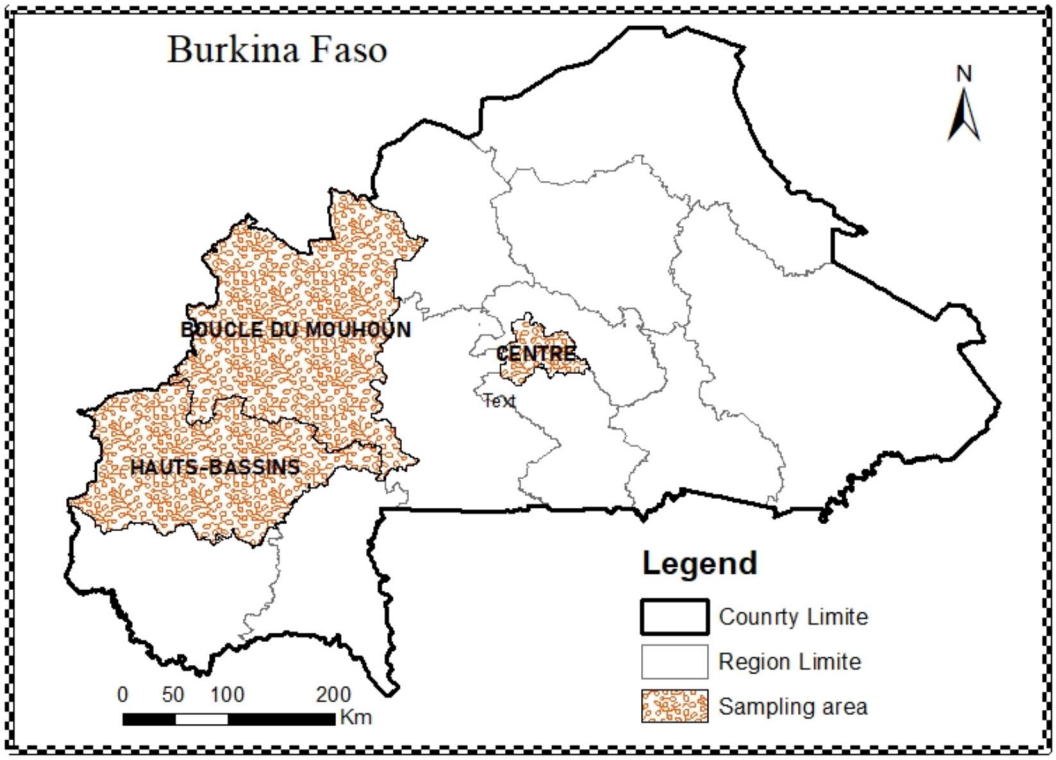

Fig. 1. Map showing sample collection areas. Used software for map: Esri ArcGIS 10.7.1, 2019, Url: https://desktop.arcgis.com/fr/arcmap/latest/get-started/installation-guide/installing-on-your-computer.htm.

Code	Genotypes	Cycles	Variety name	Locality/source
P1	QH423	–	–	INERA Bobo
P2	–	–	–	INERA Bobo
P3	–	L	–	Dedougou
P4	–	C	–	Dedougou
P5-1	ICGV 91328	C	LOKRE	INERA Ouaga
P5-2	–	C	KIEMA 1	INERA Ouaga
P5-3	ICGV 86015	L	YIRWATIGA	INERA Ouaga
P5-4	ICGV 13912	C	SOUKEBA/NAFA2	INERA Ouaga
P6-1	ICGV 13830	C	BEEDA	INERA Ouaga
P6-2	ICGV 93305	C	MIOUPAALE	INERA Ouaga
P6-3	ICGV 01276	L	NAFA1	INERA Ouaga
P6-4	ICGV 13806	C	TOINWARE	INERA Ouaga
QH	QH 243C	C	–	INERA Ouaga
SH	SH 470P	C	–	INERA Ouaga

Table 1. Characteristics of different peanut samples. L: long cycle, C: short cycle.

Preparation of standard solutions

Acetonitrile was used as a solvent for the preparation of individual aflatoxin-containing stock solutions. Stock solution concentrations of 10 µg mL^{-1} were confirmed by UV spectrophotometry. Samples of the stock solutions were then combined to produce an intermediate solution in acetonitrile, with a concentration of 100 ng mL^{-1} for all targeted aflatoxins. In addition, working solutions were made by diluting the intermediate solution in acetonitrile. These solutions were stored at -20°C. To prepare matrix-matched calibration curves, aliquots of the working solution were evaporated to dryness and then resuspended with the same volume of uncontaminated product extracted from samples (blank matrices).

Drying gas temperature	325 °C
Gas flow rate	10L/min
Nebulizer pressure	50 psi
Tension capillary	4000 v
Gas temperature	350 °C

Table 2. Experimental ionization conditions.

	Precursor ion (M/Z)	Quantified product ion (M/Z)	Quantified ion (M/Z)
Aflatoxine B1	313	285,1	241,0
Aflatoxine B2	315	287,1	259,1
Aflatoxine G1	329	233	311
Aflatoxine G2	331	313	245

Table 3. Precursor and product ions for different aflatoxin types. M = mass, Z = valence.

Extraction: deproteinization-delipidation

Collected peanut samples (5 g each) were weighed into 50 mL centrifuge tubes. They were mixed with 10 mL ultrapure water, 10 mL hexane, and 15 mL acetonitrile were added to the tube. The mixture was then stirred for 30 s.

A mixture of 4 g magnesium sulfate and 1.5 g sodium chloride was added to the centrifuge tube. The tube was immediately vortexed vigorously for 1 min and then centrifuged at 3000 rpm for 7 min. An aliquot of 5 mL of the acetonitrile phase was evaporated to dryness under a gentle flow of nitrogen at 45 °C. The resulting solution was filtered through a 0.22 mm polyethylene filter before injection.

HPLC–MS/MS chromatographic analysis

The filtrate obtained after extraction of the various peanut samples was placed in vials for subsequent analysis[32].

- Chromatographic conditions

  The Agilent Technologie 1290 series High-Performance Liquid Chromatography (HPLC) chain used for the separation is composed of the following modules:

  - Injection volume set at 10 ul
  - A constant flow rate of 0.4 ml/min in isocratic mode.
  - Chromatographic separation was performed using a Zorbax Eclipse XDB C18 column (50 × 4.6 mm, 1.8 µm) (Agilent, USA).
  - Mobile phase A: Water/formic acid (99/1 v/v/) + 10 mM ammonium formate.
  - Mobile phase B: methanol/water/formic acid (97/2/1 v/v/v) + 10 mM ammonium formate.

- Mass spectrometry conditions

  Mass analysis was performed using an Agilent Technologies 6430 Triple Quadrupole mass spectrometer with an electrospray ionization source. Experimental ionization conditions are shown in the table (Table 2). To assess linearity, four-point calibration lines were constructed over a 4 levels concentrations range (4 ng/mL, 10 ng/mL, 20 ng/mL and 80 ng/mL for AFB1 and AFG1 and 1 ng/mL, 2.5 ng/mL, 5 ng/mL and 20 ng/mL for AFB2 and AFG2) using the aflatoxin standard (Aflatoxin mix 4 solution). Linearity was determined for each aflatoxin class using linear regression analysis and expressed as the coefficient of determination (R^2).

Multi-Reaction Monitoring (MRM) scanning mode was used. The precursor and product ions for each aflatoxin molecule are presented in the table below (Table 3).

Data acquisition and quantification were performed using Mass Hunter Workstation B.04.01.

Validation and expression of results

For each series of analyses, a calibration-matrix curve was drawn with a correlation coefficient ≥ 0.95. Quantification was performed according to the external standard method using the following formula:

$$C = \frac{c_c \times v_e}{P_e}$$

where:

C: aflatoxin concentration in µg/Kg;
Cc: chromatogram concentration µg/ml;
Pe: Sample size in g;

Relative intensity	Accepted limits in LC–MS/MS
> 50%	± 20%
> 20–50%	± 25%
> 10–20%	± 30%
≤ 10%	± 50%

Table 4. Accepted limits for validation.

Repeatability CV (%)	Validation data of aflatoxins analysis performance			
	AFB1	AFB2	AFG1	AFG2
Intermediate precision CV (%)	5.64	6.54	5.24	5.48
Recovery rate (%)	92.0	90.6	90.2	89.4
LOD	0.005	0.005	0.08	0.4
LOQ	0.01	0.01	0.15	0.7
Linearity (R^2)	0.988	0.997	0.979	0.774

Table 5. Validation of aflatoxins analysis performance.

Ve: sample extraction volume in kg;

The presence of a molecule in a sample is confirmed if and only if the following conditions are simultaneously met:

- Three points of identification by the presence of two fragment ions
- Signal-to-noise ratio > 3
- The relative retention time deviation does not exceed 2.5% of that of the control.
- The deviation of the relative intensities of the identification and quantification ions (fragment ions) expressed as a percentage does not exceed the limits set by Directive 96/23/EC about controls of comparable concentration.

The accepted limits are summarized in the table below (Table 4).

The validation parameters were as follows: Linearity, recovery rate, repeatability, intermediate precision, limit of detection (LOD), and limit of quantification (LOQ). To assess linearity, four-point calibration lines were constructed over a 4 levels concentrations range (4 ng/mL, 10 ng/mL, 20 ng/mL, and 80 ng/mL for AFB1 and AFG1, and 1 ng/mL, 2.5 ng/mL, 5 ng/mL and 20 ng/mL for AFB2 and AFG2) using the aflatoxin standard (Aflatoxin mix 4 solution). Linearity was determined for each aflatoxin class using linear regression analysis and expressed as the coefficient of determination (R^2). The limit of detection (LOD) and the limit of quantification (LOQ) were determined from the signal-to noise ratio as more than 3: 1 and 10: 1, respectively. Repeatability and intermediate precision were determined using samples of peanut flour spiked with 10 µg/kgAFB1 and AFG2 and 2.5 µg/kg AFB2 and AFG2 (Table 5). Each spiked sample was analyzed 6 times on the same day by the same operator with the same equipment and reagents for the repeatability evaluation and 5 times at a rate of once a day for 5 days for the intermediate precision evaluation. The recovery rate was determined by analyzing spiked samples with the aflatoxin standard and its value was calculated by the following equation: Recovery rate (%) = (100 X measured concentration for spiked sample)/added concentration.

Assessment of phenolic compound content

Polyphenols and flavonoids were extracted by maceration with a methanol/water mixture (80/20, v/v).

Total phenolic compounds content

Phenolic compounds determination was carried out using the Folin-Ciocalteu reagent by the method described by Singleton with minor modifications[33]. This method measures the intensity of the color of tungsten oxide, and molybdenum oxide which is proportional to content phenolic compounds. A concentration of 1 mg/mL was used for the spectrophotometric assay. All analyses were performed in triplicate and a calibration curve was obtained using gallic acid as standard. Results are expressed in mg GAE/g (GAE: gallic acid equivalent).

Total flavonoid contents

The total flavonoid contents were determined by a spectrophotometric colorimetric method described by Arvouet-Grand[34]. To 0.5 mL of sample with a mass concentration of 1 mg/mL, a solution of 2% $AlCl_3$ was added. The total flavonoid content was calculated as quercetin equivalent from a calibration curve. Flavonols were determined using the method described by Zhang et al.[35]. Results are expressed in mg QE/g (QE: quercetin equivalent).

Hydrolysable tannin and condensed tannin

Hydrolysable tannin content was determined according to the protocol of Mole et al.[36]. Condensed tannin content was determined using the method of Broadhurst et al.[26].

Peanut seed flours were extracted with ethanol and water (50% v/v; 1 ml) and sonicated for 60 min. The mixtures obtained were centrifuged (15,269 g) for 10 min and the supernatants were used for the above analyses.

Statistical analyses

Figures and calculations were done using GraphPad Prism version 8.4.3, and Excel 2016, respectively. Data were subjected to the analyses of variance (ANOVA) and significant differences between means were revealed via the Tukey test ($p < 0.05$) which was done using XLSTAT (2016) software. Principal component analysis and dendrogram were performed using R, version 4.0.2 (2020) software.

Results and discussion

Moisture and pH

Measuring moisture content and pH in *A. hypogea* seeds is a quality indicator commonly used to assess moisture levels and provide information on the product's ability to resist the risk of deterioration during storage.

The pH of the different peanuts ranged from 6.3 ± 0.06 to 6.48 ± 0.02 for samples P3 and P5-3 respectively. The results show that the pH of the different samples was significantly different ($p^{>}0.05$). Moisture levels varied between 4.21 and 3.88% (Fig. 2). There was no significant difference between moisture levels.

Moisture and pH influence the development of fungi on a substrate. A very high moisture content and a low acid pH favor the growth of fungi, whose presence will lead to the deterioration of peanut seeds[37]. As far as pH is concerned, fungi are much more tolerant than bacteria. Bacteria often require pH levels between 7 and 8, while most fungi can grow between pH 3 and 8. Their optimum growth is generally in the pH range between 5 and 6. Because they tend to be acidic (with a pH < 6), foods such as fruit, vegetables, and meat are more susceptible to spoilage caused by fungi than by bacteria[31]. Our results corroborate previous studies that found respectively on 37 peanut samples a pH ranging from 5.73 ± 0.19 to 6.36 ± 0.03 and in non-germinated peanut seeds a pH of 6.27[37]. The pH of peanut seeds stored before marketing or for later use must be between 3 and 8 respectively[31,38].

Moisture content is an important factor in preservation and deterioration. If it is kept below 10%, this guarantees good preservation of plant products. A water content below 10% ensures good preservation for plant products[1]. Our values are lower than those found in a previous study on *A.hypogaea* seeds, which contained 5.43% moisture[39]. However, moisture favors mold growth and According to UNECE STANDARD, 2022, the moisture content of raw peanut seeds must not exceed 10.0%[31]. This shows that our peanut seeds can be stored for a long time with little risk of microbial contamination[40].

Aflatoxin contamination

The limits of detection and quantification of 0.01 µg/kg and 0.02 µg/kg respectively showed the high sensitivity of the technique used for determination. Calibration curves were made based on standards of different concentrations 5, 10, 20, 40, and 80 (Fig. 3). Aflatoxin G2 levels in seeds of the different peanut varieties were below the quantification threshold (LOQ) for all samples. As for aflatoxin G1, several samples were below the LOQ. The highest aflatoxin content was recorded in sample P5-4, which showed the highest aflatoxin G1 content at 1.08 ± 0.97 µg/Kg (Table 6). Aflatoxins B1 and B2 were quantified in all samples. Aflatoxin B2 levels ranged from 0.02 ± 0.03 (P4) to 0.34 ± 0.19 (P6-2). Aflatoxin B1 concentrations ranged from 0.01 ± 0.01 (P1) to 0.44 ± 0.07 µg/Kg (P5-4). Total aflatoxin content ranged from 0.04 ± 0.04 to 1.86 ± 0.42 µg/Kg in samples P5-3 and P5-4 respectively. For the different types of aflatoxins (AFG1, AFG2, AFB1, AFB2) there was no statistically significant difference (95% confidence interval). However, analysis of variance shows a significant difference for total aflatoxin content. (Table 6). The chromatograms of sample P5-4 were shown as example. An example of the

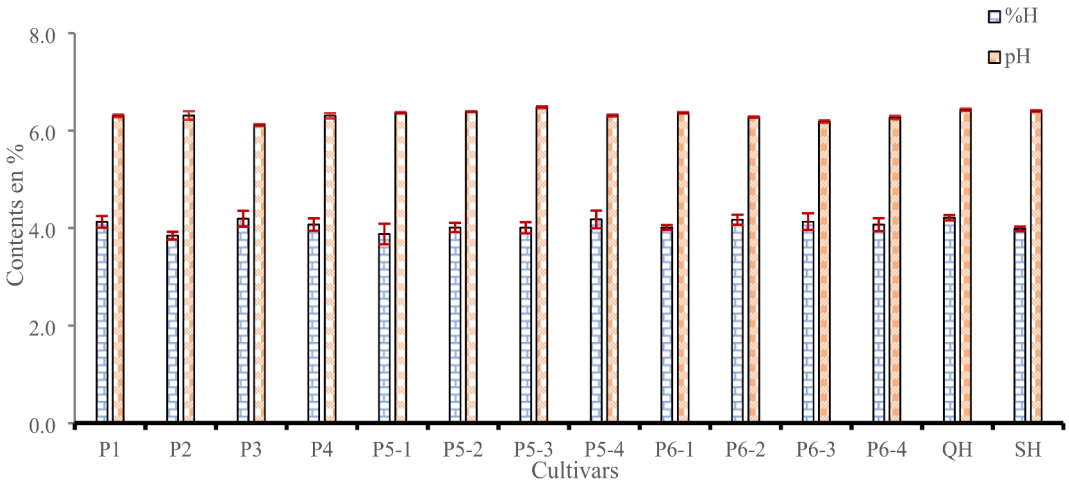

Fig. 2. Moisture content and pH of peanut cultivar seeds. % H: moisture content.

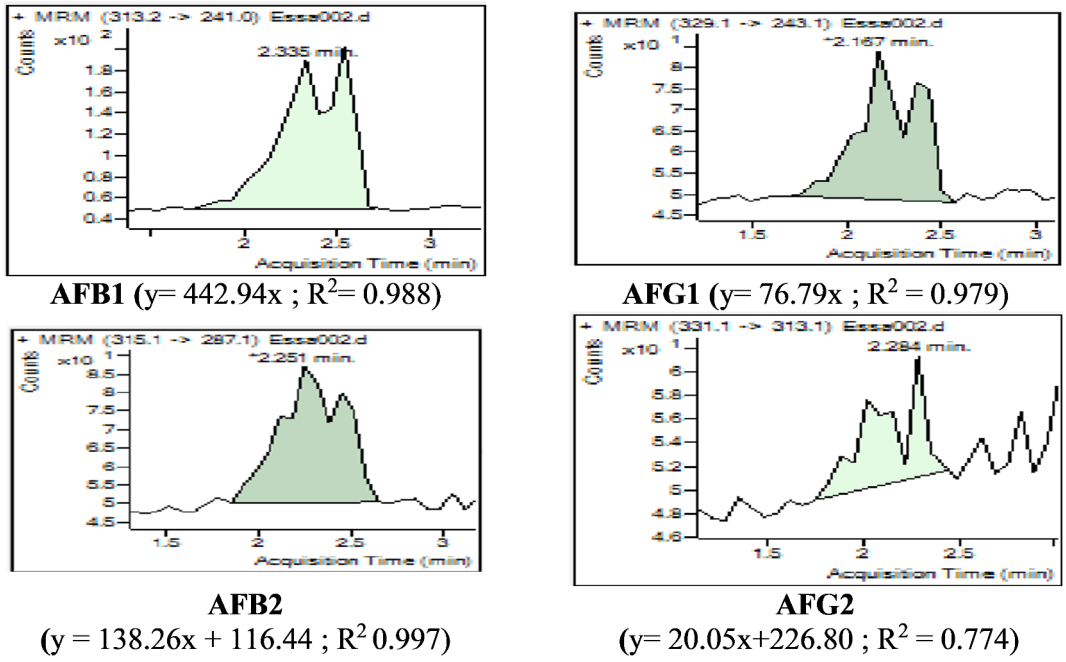

Fig. 3. Chromatogram of a reference at 5 µg/kg of AFB1, AFB2, AFG1 and AFG2. AFG_1: aflatoxin G1; AFG_2: aflatoxin G2; AFB_2: aflatoxinB2; AFB_1: aflatoxin B1; AFT: total aflatoxin.

Sample identity	AFG2	AFG1	AFB2	AFB1	AFT
P1	LOQ	0.72 ± 0.81^{a}	0.12 ± 0.14^{a}	0.01 ± 0.01^{a}	0.86 ± 0.93^{ab}
P2	LOQ	LOQ	0.06 ± 0.07^{a}	0.2 ± 0.28^{a}	0.26 ± 0.23^{b}
P3	LOQ	LOQ	0.06 ± 0.07^{a}	0.01 ± 0.01^{a}	0.06 ± 0.08^{b}
P4	LOQ	LOQ	0.02 ± 0.03^{a}	0.05 ± 0.06^{a}	0.07 ± 0.06^{b}
P5-1	LOQ	LOQ	0.06 ± 0.1^{a}	0.21 ± 0.3^{a}	0.27 ± 0.27^{b}
P5-2	LOQ	LOQ	0.08 ± 0.07^{a}	0.01 ± 0.01^{a}	0.09 ± 0.08^{b}
P5-3	LOQ	LOQ	0.03 ± 0.02^{a}	0.02 ± 0.02^{a}	0.04 ± 0.04^{b}
P5-4	LOQ	1.08 ± 0.97^{a}	0.34 ± 0.09^{a}	0.44 ± 0.07^{a}	1.86 ± 0.42^{a}
P6-1	LOQ	LOQ	0.12 ± 0.11^{a}	0.43 ± 0.7^{a}	0.54 ± 0.6^{ab}
P6-2	LOQ	LOQ	0.34 ± 0.29^{a}	0.16 ± 0.26^{a}	0.5 ± 0.04^{ab}
P6-3	LOQ	LOQ	0.11 ± 0.1^{a}	0.04 ± 0.08^{a}	0.15 ± 0.17^{b}
P6-4	LOQ	LOQ	0.07 ± 0.06^{a}	0.01 ± 0.01^{a}	0.08 ± 0.07^{b}
QH	LOQ	0.45 ± 0.77^{a}	0.17 ± 0.17^{a}	0.27 ± 0.46^{a}	0.88 ± 1.4^{ab}
SH	LOQ	0.47 ± 0.81^{a}	0.11 ± 0.1^{a}	0.07 ± 0.1^{a}	0.65 ± 0.99^{ab}
Pr > F	–	0.096	0.051	0.768	0.029
Significant	–	No	No	No	Yes

Table 6. Aflatoxin B1, B2, G1, G2, and total aflatoxin content in peanut varieties (µg/kg). LOQ: limit of quantification; AFG2: aflatoxin G1, AFG2: aflatoxin G2; AFB1: aflatoxin B1; AFB2: aflatoxin B2; AFT: total aflatoxin; letters a, ab, b mark statistical differences between sample data.

chromatogram obtained for each type of aflatoxin is shown in Fig. 4. Along with the equation used to calculate aflatoxin concentrations and R2 (Fig. 4).

Aflatoxin B1 is the most common aflatoxin contaminant of plant material (USAID, 2012). The data obtained on aflatoxin contamination of peanut seeds are comparable to those found in a study conducted in Côte d'Ivoire, a country bordering Burkina Faso[5]. This study also showed the presence of aflatoxin B in all peanut samples compared with aflatoxin G, which was not detected in all samples. Other previous studies have shown that aflatoxin B1 is predominant in contaminated products and is also the most toxic aflatoxin[23]. Differences in aflatoxin levels between samples could be explained by sample storage conditions, and could also be linked to genetic factors.

Aspergillus species such as *Aspergillus flavus* and *Aspergillus parasiticus* produce aflatoxin B, but aflatoxin type G is produced by *Aspergillus parasiticus*. Indeed, previous work has shown that the double bond present on

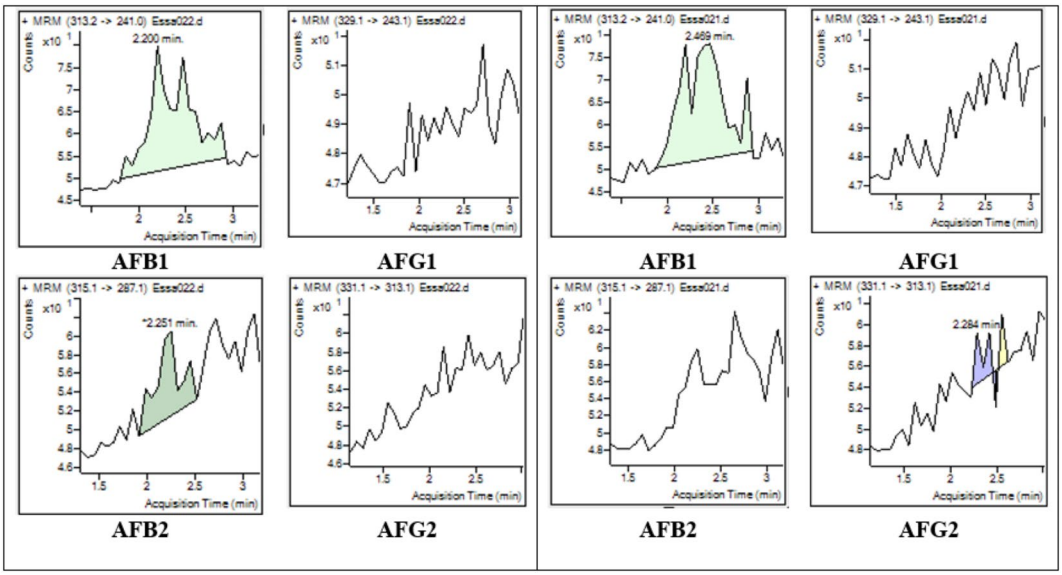

Fig. 4. chromatogram of sample P5-4 1st and 2nd injection. AFG_1: aflatoxin G1; AFG_2: aflatoxin G2; AFB_2: aflatoxinB2 ; AFB_1: aflatoxin B1; AFT: total aflatoxin.

the dihydrofuran ring of AFB1 and AFG1 could be the origin of the difference in toxicity with their respective AFB2 and AFG2 homologs. On the other hand, the difference in toxicity between group B and G aflatoxins is thought to be due to the substitution of the cyclopentane ring by a lactone ring[40]. The toxic effect of aflatoxins is mainly based on aflatoxin B1, considered the main genotoxic metabolite and with the highest carcinogenic potential of all aflatoxins. Aflatoxin B1 stands out among these various mycotoxins for its exceptionally high toxicity and highly carcinogenic potential. It is frequently present in many food products, particularly peanuts and peanut derivatives[13,14,37].

The European Commission has set the limits of acceptable aflatoxin B1 content at 2 µg/kg and 4 µg/kg for total aflatoxin content (AFB1 + AFB2 + AFG1 + AFG2) in nuts, dried fruit, ready-to-sell cereals. Codex Alimentarius sets a maximum limit of 12 µg/kg for total aflatoxins in peanut samples[41]. A comparison of the aflatoxin content of our samples with these international standards shows that the various peanut varieties are of good sanitary quality in terms of aflatoxin contamination. Although there are traces of aflatoxins in peanuts of local varieties collected in the towns of Dedougou, Bobo Dioulasso and Ouagadougou, the state of this contamination does not present a health risk for consumers, as the levels are below the threshold of international standards. It should be noted that the moisture content of around 4% and the slightly acidic pH of the samples help to limit aflatoxin contamination. It should be noted that high levels of aflatoxin ingestion can lead to acute aflatoxicosis, which is often fatal. Recent assessments of past aflatoxicosis outbreaks have estimated that toxic and potentially fatal doses of toxic and potentially fatal doses of AFB1 in humans range from 20 to 120 µg/kg body weight per day when consumed throughout 1 to 3 weeks. In addition, consumption of staple foods containing aflatoxin concentrations of 1 mg/kg or more has also been suspected of causing acute aflatoxicosis[41]. This information is essential from a nutritional point of view, as peanut seeds are widely used in technology to produce oil for consumption, pastes for cooking, tarts for bread, and so on. It should also be noted that no discriminatory differences were observed between samples from INERA selections and those from local crops. Another study conducted in Burkina Faso showed total aflatoxin levels of 14.19 µg/kg, which exceeds the threshold in force in Burkina Faso, which is the same as the Codex Alimentarius threshold[42]. This difference could be explained by post-harvest peanut storage conditions. Indeed, our samples come from a research center and have certainly benefited from better storage conditions, in contrast to the samples in the above-mentioned study, which were collected from market vendors.

Phenolic compound contents of peanut seeds

Total phenolics, flavonoids and flavonols

Quantification of phenolic compounds revealed significant variations from one variety to another. Total phenolics, flavonols, and flavonoids were determined. Total phenolic content ranged from 5.64 ± 1.35 to 14.94 ± 2.79 mg GAE/g for samples P1 and P5-2 respectively. Flavonoid content ranged from 1.23 ± 0.11 to 2.24 ± 0.15 mg QE/g for samples P4 and P3 respectively (Fig. 5). Analysis of the means showed that the values differed significantly. Seeds also contained flavonols in small quantities, with levels ranging from 0.14 ± 0.09 mg QE/g for sample P5-2 to 0.85 ± 0.36 mg QE/g for sample P1. Statistical analysis showed statistically significant differences between samples for quantified compounds. Phenolic compound composition showed no discriminatory statistical differences between varietal selection samples and locally grown varieties.

The phytochemical screening test realized indicates the presence of polyphenols in all extracts. These phenolic compounds are generally concentrated in the skin covering the cotyledons[43]. Flavonoids and phenolic

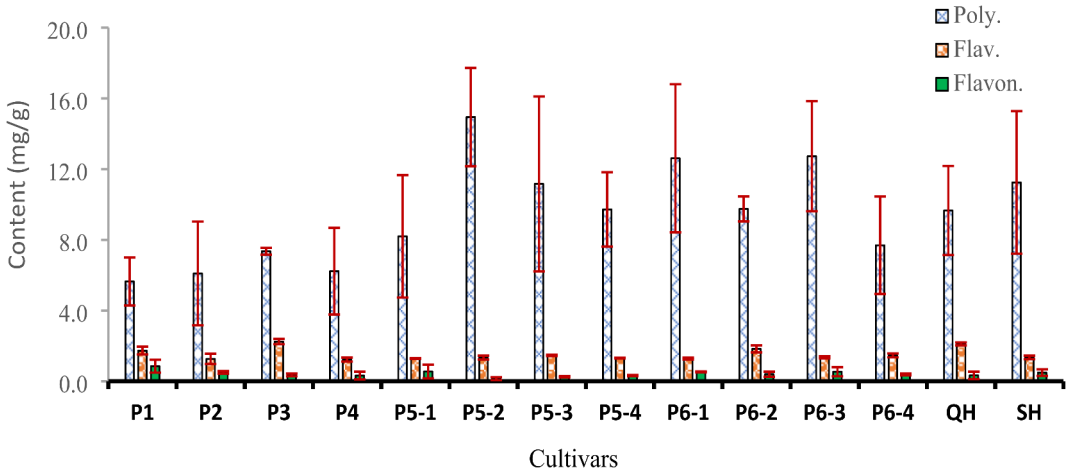

Fig. 5. Total phenolics, flavonoids, and flavonols contents. Poly: polyphenols (mg GAE/g), Flav: flavonoids (mg QE/g), Flavon: flavonols (mg QE/g).

compounds are the main components responsible for the antioxidant activity of extracts. Indeed, previous studies have reported a direct relationship between flavonoid and phenolic content and antioxidant activity[44–46].

The phenolic content is similar to that reported in a previous study on the phenolic profiles and antioxidant activity of sprouted peanuts, which revealed an average phenolic content of 18.21 mg GAE/g in peanuts[47]. Other previous studies have found contents of 129.56 ± 1.61 to 160.17 ± 1.17 mg/g and 85.58 ± 0.59 to 209.18 ± 2.53 mg/g, higher than those recorded in the present study[48,49], while lower average contents of 2.1 mg GAE/g DW and 3.28 mg GAE/g have also been reported[50]. The variation in phenolic compound content could be explained by the influence of environmental factors on the plant. Indeed, phenolic compounds are produced by the plant in response to physical aggression and environmental stress, notably edaphic and climatic factors[50]. This variability may also be linked to genetic factors, i.e. to the variety or cultivar.

Most of the phenolic compounds present in peanut seeds are concentrated in the tegument[51]. Indeed, a previous study reported that the peanut tegument contains phenolic compound contents of 160 mg GAE/g versus 13 mg GAE/g in the cotyledon[43]. Another study conducted in Thailand showed higher levels than those found in this study. These were 21.36 ± 1.90 µg GAE/mg, 8.65 ± 0.16 µg QE/mg, 0.249 ± 0.004 µg QE/mg respectively for total polyphenols, total flavonoïds and flavonols[52]. The authors stated that phenolic compounds confer a functional role on peanuts. The fact that peanut seeds contain phenolic compounds gives them antioxidant, anti-inflammatory, and antimicrobial properties. These compounds can scavenge free radicals, which are generated by the body or formed in reaction to environmental aggressions (tobacco, pollutants, etc.)[11]. They help fight certain metabolic diseases such as cancer, as well as degenerative diseases. Previous studies have demonstrated the beneficial role of polyphenols and flavonoids on human health. Indeed, these compounds play a preventive and protective role against certain chronic diseases, notably cardiovascular diseases, metabolic diseases, and certain cancers[53]. The action of phenolic compounds on human cancer cell lines reduces the number and growth of tumors[54]. These compounds act through several mechanisms, including estrogenic or anti-estrogenic activity, antiproliferative effects, induction of cell cycle arrest or apoptosis, prevention of oxidative stress, anti-inflammatory activity, and modifications of cell signaling[55]. Several studies have demonstrated the positive effect of polyphenol consumption on health and disease prevention, thanks to its antioxidant activity. They also help to prevent lipid peroxidation, an undesirable reaction that can occur in food products, thus ensuring good food preservation[56].

The presence of phenolic compounds in the seeds of different peanut varieties is therefore an important factor to take into account, because of all the properties they confer on the materials containing them.

Hydrolysable tannin and condensed tannin contents

Comparison of means showed statistically significant differences. Peanut seeds had very low concentrations of condensed tannins, ranging from 0.14 ± 0.02 to 0.26 ± 0.02 mg/g (Fig. 6). Hydrolysable tannins are found in trace amounts in the samples. Maximum hydrolyzable tannin levels were 0.03 ± 0.01, while minimum levels were 0.16 ± 0.09 mg/g (Fig. 6).

Tannins are polyphenols with molecular weights ranging from 500 to 3000 D. Tannins belong to the polyphenol family and are produced by certain plants to combat micro-organisms. Previous studies have found average levels of 35.38 ± 0.58 mg TAE/g in raw peanut seed samples[57]. Other studies showed lower tannin contents of 88 ± 0.19 and 4.73 ± 0.43 mg WT/g for cotyledons and 29.7 to 84.7 mg WT/g on 6 peanut varieties[58]. The results of this research clearly show that both tannins and polyphenols are more concentrated in the seed coat than in the seed. Tannins are phenolic compounds found in many plant-based foods such as fruits, vegetables, tea, wine, cereals, and certain herbs. Their nutritional importance is diverse and they can have beneficial effects on health and may also inhibit hydrolytic enzymes[36]. Tannins play an important role in immune system defense for organisms exposed to oxidative damage[47,50]. Tannins stop bleeding and fight infections. Tannin-rich plants are

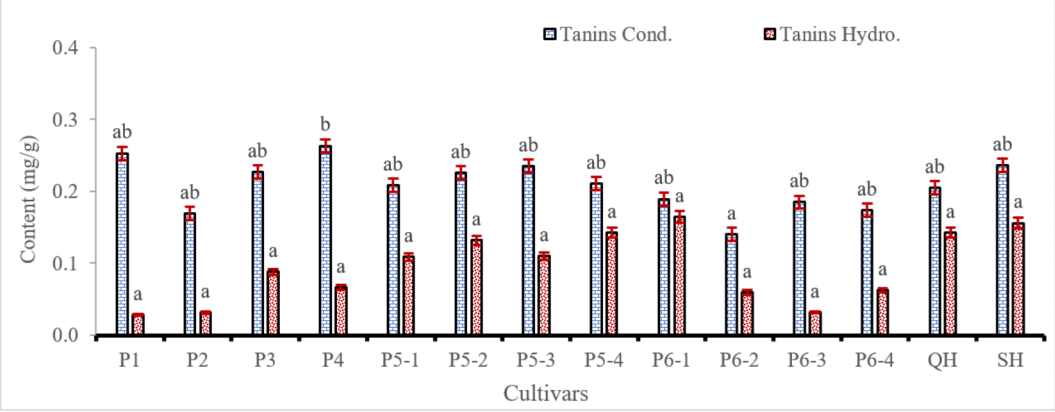

Fig. 6. Content of condensed tannins and hydrolysable tannins.

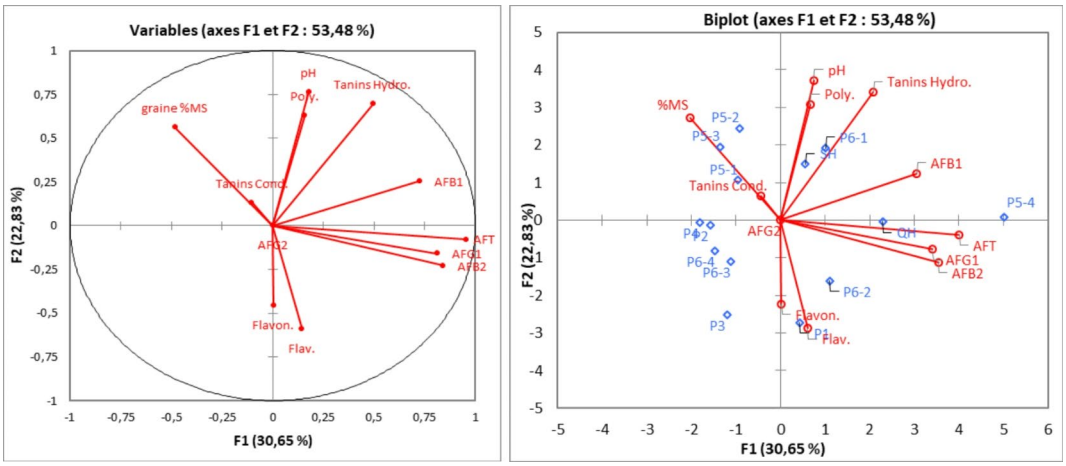

Fig. 7. Principal component analysis of correlations between parameters and varieties.

used to tighten soft tissues as in varicose veins to drain excessive secretions as in diarrhea. and to repair tissues damaged by eczema or burns[57]. It's important to note that the effects of tannins can vary depending on their type concentration and food source. A balanced and varied consumption of tannin-rich foods as part of an overall healthy diet can help to benefit from their properties while minimizing any adverse effects. However high tannin levels can be detrimental to human health. For this reason, the low tannin content of our peanut samples may be an advantage given their nutritional importance.

Correlation between water content, aflatoxin, and phenolic contents

Principal component analysis revealed the links between physico-chemical parameters, aflatoxin levels and secondary metabolites in the different peanut varieties studied. Aflatoxin levels AFB1, B2, G1, and G2 are carried by the F1 axis (30.65%), while pH and metabolites are also carried by the F2 axis (22.83%) (Fig. 7). Furthermore, samples with high levels of phytochemicals show a negative correlation with aflatoxins levels (i.e. low contamination). The dendrogram shows two clusters and six groups (Fig. 8). The cluster C1 regroups {P1, P2, P4, P5-1, P6-4}; and the cluster C2 regroups{P5-4, P6-2, QH, P5-2, P6-1, P6-3, P5-3, SH}. The varieties included in each cluster show a similar profile in terms of phenolic compound composition and total aflatoxin content. There was also a positive correlation between polyphenol, hydrolyzable tannin, flavonoid and flavonol content, and Aflatoxin B1, B2, G1, and G2 levels, and between condensed tannin and dry matter content (Table 7).

The sum of F1 and F2 gives 53.48%. The correlation graph shows that polyphenols, hydrolysable tannins and pH are strongly positively correlated. On the other hand, with the exception of aflatoxin G2, the other forms of aflatoxin showed positive correlations. This shows that there are no major statistical differences in aflatoxin content between the different samples. It should also be noted that there was little correlation between phenolic compound levels and aflatoxin levels. These compounds are thought to be factors limiting the proliferation of the Aspergillus genus. their antimicrobial properties mean that they create a more or less hostile environment for microorganisms. It seems logical that samples containing high levels of phytochemicals showed a negative correlation with aflatoxin content because phenolic compounds are produced by plants to fight against their

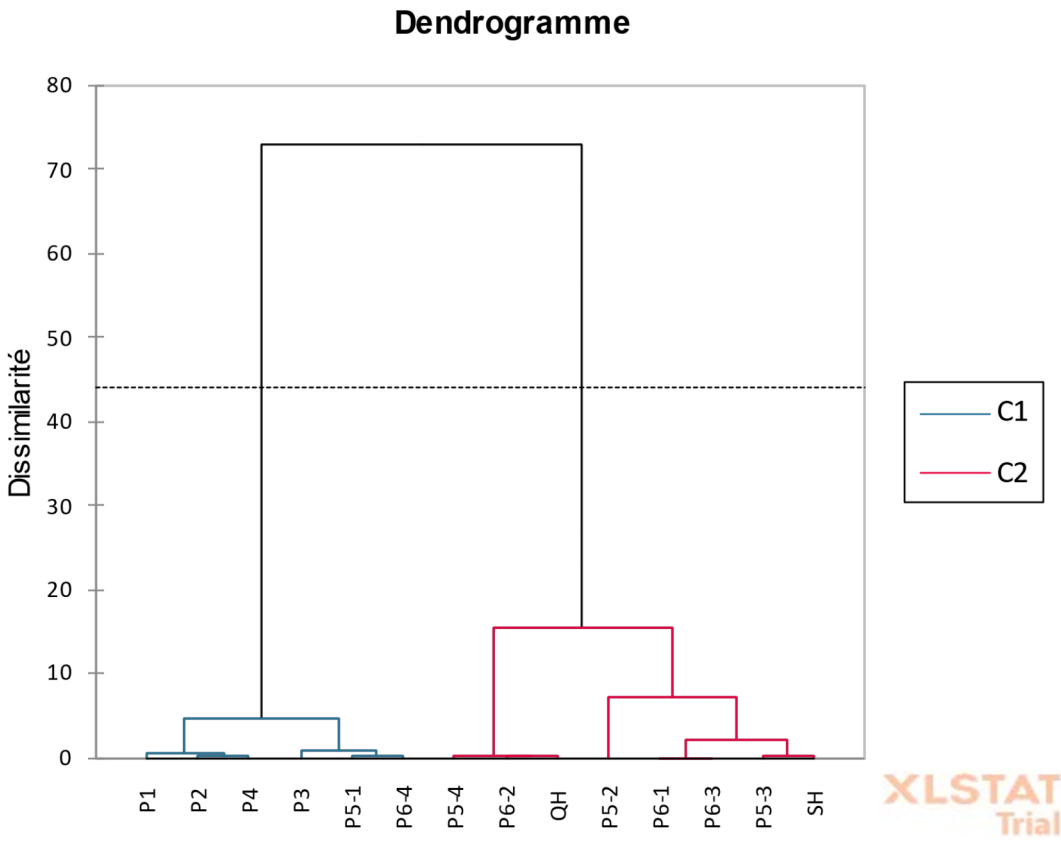

Fig. 8. Dendrogram showing the genetic similarities of varieties according to the parameters studied.

Variable	P	F	Fl	CT	HT	%DM	pH	AFG_1	AFB_2	AFB_1	AFT
P	1	-0,22	-0,41	-0,13	0,55	-0,01	0,32	-0,13	0,13	0,11	-0,03
F	-0,22	1	-0,01	-0,04	-0,06	-0,67	-0,30	0,08	0,20	-0,18	0,04
Fl	-0,41	-0,01	1	0,00	-0,41	0,11	-0,22	0,23	0,00	0,03	0,17
CT	-0,13	-0,04	0,00	1	0,20	-0,02	0,22	0,29	-0,45	-0,33	0,01
HT	0,55	-0,06	-0,41	0,20	1	0,01	0,56	0,25	0,15	0,49	0,35
%DM	-0,01	-0,67	0,11	-0,02	0,01	1	0,39	-0,38	-0,53	0,00	-0,38
pH	0,32	-0,30	-0,22	0,22	0,56	0,39	1	0,14	-0,07	0,21	0,15
AFG1	-0,13	0,08	0,23	0,29	0,25	-0,38	0,14	1	0,56	0,38	0,92
AFB2	0,13	0,20	0,00	-0,45	0,15	-0,53	-0,07	0,56	1	0,55	0,76
AFB1	0,11	-0,18	0,03	-0,33	0,49	0,00	0,21	0,38	0,55	1	0,68
AFT	-0,03	0,04	0,17	0,01	0,35	-0,38	0,15	0,92	0,76	0,68	1

Table 7. Correlation matrix (Pearson (n)). P: polyphenol; F: flavonoids; Fl: Flavonols; CT: condensed tannin; HT: hydrolysable tannin; %DM: dry matter; AFG_1: aflatoxin G1; AFB_2: aflatoxinB2 ; AFB_1: aflatoxin B1; AFT: total aflatoxin.

physical or chemical attackers. Phytochemicals have been proven to have antifungal power[24]. The presence of several groupings on the dendrogram indicates a genetic similarity between the varieties making up the different groups. The varieties have not been grown on the same soil types, nor have they been subjected to the same climate. It has been shown that genetic factors are largely responsible for the traits expressed by plants[58]. Even if these genetic factors are often influenced by the environment[59]. This information can be very important if you want to use different varieties to fortify foods for particular groups, especially to prevent certain metabolic diseases. The fact that peanuts are not produced under the same environmental conditions (soil types, fertilizers, same locations) may be a limitation for this study and would partly explain some of the differences observed.

Conclusion

The peanut, *A. hypogea*, is a legume whose seeds are its most important part. These seeds contain bioactive compounds, but are also subject to aflatoxin contamination. Peanut seeds contain polyphenols, flavonoids and flavonols. Determination of aflatoxin levels revealed that contamination levels were below the toxicity threshold recommended by the Codex alimentarius and the European Commission. It also appeared that the moisture content and pH of our peanuts could limit their contamination by the Aspergillus genus. As a result, peanut seeds produced in Burkina Faso are of satisfactory sanitary quality and can contribute to the prevention of certain pathologies, as they are rich in phenolic compounds with antioxidant properties.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

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Author contributions

The study protocol was drafted by S.Z., S.Z., F.A.K., P.R.B., E.N.R., and R.D.. The manuscript was written by S.Z., S.Z., and C.S.C., and performed statistical analyses by D.B., R.D., and M.D. Scientific supervision of the study was provided by K.K. and M.H.D. All authors contributed to the article and approved the submitted version.

Declarations

Competing interests
The authors declare no competing interests.

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