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Original Research Article

Variability and Dehulling Effect on Seed Antinutrients and Antioxidant Activity of Cowpea (*Vigna unguiculata* L. Walp.) Genotypes Grown in Two Agroecological Zones of Chad

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Abstract: Major limiting factors of wide consumption of cowpea in day today diet include poor digestibility and the presence of anti-nutritional factors. Whole and dehulled seeds of eight improved cowpea lines grown in N'Djamena and Bebedjia (Chad) were analysed for four antinutritional factors contents (total phenols, tannins, flavonoids, phytates) and antioxidant activity, in order to assess the variability and the effect of decortication. In each locality, the experimental design was a triplicated randomly complete block design. Standard methods were used to evaluate these biochemical traits. The results showed a wide variability among genotypes for these traits in whole and dehulled seeds. In average, the decortication reduced polyphenols, tannins, flavonoids and phytate contents by 72.3%, 64%, 48.6% and 30.1% respectively. The dehulling also reduced the antioxidant activity by 42.25%. Dehulling appeared as a proper processing method to reduce anti-nutritional factors and improve the bioavailability of nutrients, especially when cowpeas are used as food for infants and children.

Keywords: *Vigna unguiculata*, Chad, antinutrients, antioxidant, dehulling.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is an important, high-quality staple food that provides large amounts of protein, calories, vitamins and essential mineral micronutrients to the diets of people in many countries (Hall *et al.*, 2003; Vasconcelos *et al.*, 2010; Goncalvez *et al.*, 2016). In Chad, even if millet and sorghum constitute the staple food of the populations, cowpea is one of the most cultivated crops, and is increasingly becoming a cash crop (Nadjiam, 2021). At the national level, the average cowpea production over 2020-2021 season is estimated at 154 586 t sown on 227 341 ha (FAO, 2021; Nadjiam, 2021). Traditionally, cowpeas are mostly consumed as boiled vegetable using whole or dehulled dry seeds taken as a relish with cereal staples (Olabandji *et al.*, 2018). Cowpeas are also used in the formulation of simple weaning blends, which are relatively cheap for poor rural to afford (Affrifah *et al.*, 2021). The reason for decortication of cowpea is to improve the appearance, texture, aroma and taste, and to reduce the cooking time. Asides, it is important to note that the major limiting factors to the wide utilization of this tropical legume in human diets include poor digestibility, a deficiency of sulphur-containing amino-acids and the presence of anti-nutritional factors as polyphenols, tannins, protease inhibitors and phytates (Bala *et al.*, 2012; Ileke, 2014; Bolade, 2016). Antinutrients are plant's secondary metabolites that antagonize and reduce the nutritional value of food interfering with mineral bioavailability and digestibility of essential nutrients, thereby making them unavailable for the cells when consumed. Cowpeas have been shown to contain high level of polyphenols, which play an important role in the reduction of protein digestibility and starch digestibility (Desphande *et al.*, 1982; Preet and Punia, 2000a; Dalaram, 2015). Phytic acid and oxalic acid, widely distributed in legumes have been reported to reduce mineral bioavailability, leading to various mineral deficiency diseases e.g. anaemia, or form deleterious complexes with metal ions e.g. calcium-oxalate causing renal damage (Ghavidel and Prakash, 2007). Phytic acid can also chelate vitamins and potentially contribute to their deficiency (Adebooye and Singh, 2007). Likewise, condensed tannins have been reported to impair iron availability and also for the hard-to-cook phenomenon

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(Bala *et al.*, 2012; Enyukwu *et al.*, 2020). However, anti-nutrients have been shown to possess pharmacological values and other beneficial effects (Olabandji *et al.*, 2018). Phytates are a storage form of organic phosphorus which is used by the plant in various stages of growth (Vaintraub and Lapteva, 1988). Anti-nutritional factors affect susceptibility of grains to insect attack (Ileke, 2014). Studying the effect of anti-nutrient composition of cowpea on bruchids infestation, Ileke (2014) noted low total phenol contents in infested seeds. They are naturally concentrated in the seed-coat where the play a key role in physical and chemical defence system of the seeds. In particular, they contribute to the antioxidant and antimicrobial activity (Yadav *et al.*, 2014). There is a growing interest in the potential use of antioxidants from natural sources (Singh *et al.*, 2017). Natural antioxidants are good for human health because of decreasing heart diseases risk and possessing anti-carcinogenic properties.

It is necessary to reduce these inhibitors to levels that will render the nutrients readily available for absorption in the body. Processes such as soaking, dehulling, toasting, germination, fermentation, ordinary cooking, autoclaving, and microwave are used to eliminate or reduce these substances (Egounlety and Aworth, 2003; Adebooye and Singh, 2007; Chipurura *et al.*, 2018). Despite of their efficiency, these methods require a supplement of energy from the housewives and cause a reduction of certain nutriments by leaching (Ajeigbe *et al.*, 2008). Some antinutrients are thermostable products and their destruction by processing is difficult (Singh *et al.*, 2017). Therefore, the selection of cowpea cultivars presenting adequate concentration of these elements appears to be the simplest and most effective method to improve nutritional and techno-functional value of this legume (Preet and Punia, 2000b). It was shown that the antinutritional compounds in cereals and dry legumes exhibit a wide variability revealing the possibility of breeding (Preet and Punia, 2000b; Bala *et al.*, 2012; Owolabi *et al.*, 2012). Cowpea seeds are good source of antioxidants and wide variability for this trait has been noted among genotypes (Yadav *et al.*, 2014; Singh *et al.*, 2017; Olabandji *et al.*, 2018; Sombie *et al.*, 2018). In Chad, little effort has been made to ascertain the quality attributes of cowpea genotypes including antinutritional factors. The present study, therefore, seeks to understand the genetic variability and the effect of dehulling on cowpea improved genotypes for polyphenols, tannins, flavonoid and phytates contents, and antioxidant potential in whole grain and decorticated seeds with the aim to developing a strategy for improving the quality of the seeds.

MATERIALS AND METHODS

Testing Environments

After preliminary trial, field experiments were carried out during the year 2020 rainy season at the experimental farm of Chadian Institute of Agronomic Research for Development (ITRAD) in two locations: N'Djamena (12°6'59''N, 15°4'20''E, altitude 298 m) and Bebedjia (08°40'34''N, 16°54'65''E, altitude 382 m). These test locations, selected to sample climatic and edaphic conditions, vary in latitude, rainfall, soil types, temperature and other agro-climatic factors.

Bebedja (Department of Nya, Logone oriental region in Southern Chad) belongs to the savannah Sudano-Guinea belt with an annual average rainfall ranges between 950 to 1300 mm. The climate is tropical semi-humid with a single rainy season that ranges from May to November. The mean annual temperature is between 25 - 30°C, while the annual humidity is about 60%. The soil is sandy- clay with 8.2 $mg.kg^{-1}$ of organic matter and pH of 5.5 (Pias, 1972). The vegetation is a clear forest tree savannah (Nadjiam, 2021).

N'Djamena (capital of Chad) located in the south-west of the country at the confluence of Logone and Chari rivers, belongs to the Sahelian belt with an annual average rainfall ranges between 400 to 700 mm (Vivien, 2006). The climate is tropical hot semi-arid, with a short rainy season ranges from July to September. Based on annual temperatures, N'Djamena is one of the hottest major cities on the planet. The mean annual temperature is about 29°C, while the annual humidity is about 43%. The soils of the experimental site are ferruginous, characterized by a hard sandy-loam texture, and a pH of 6.5 (Pias, 1972).

Plant Material

Eight cowpea homozygous cultivars adapted to the sudano-sahelian zone conditions and originated from Nigeria, Niger, Burkina–Faso and Senegal, were used for the study. Seeds were obtained from the Chadian Institute of Agricultural Research for Development (ITRAD). The tested materials comprised registered genotypes IT81-D994, IT99-K573-1-2 and Vita 5 from the International Institute of Tropical Agriculture (IITA, Nigeria), TN5-78 (Dan Louma), TN-27-80 (Dan Matarawa) and TN-985-61399 selected by the National Institute of Agronomic Research of Niger (INRAN), Melakh obtained by the Senegalese Institute of Agricultural Research (ISRA) and, popular cultivar KVX30- 309-6G (Dan Bobo) from the Institute of Environment and Agronomic Research of Burkina-Faso (INERA). These genotypes are widely cultivated or in extension in Chad.

Field trials

The seeds of eight entries were sown in each of the two locations in a randomized complete block design (RCBD) with three replications. Sowing took place on an experimental area of 185 m^2 (14 m length x 13.2 m broad). Each plot unit consisted of one row of 03 m length x 01 m broad, spaced 01 m apart. Three seeds of each variety mixed with Insector (Imidaclopride 350 $g.kg^{-1}$ + thirame 350 $g.kg^{-1}$), were sown at an intra-row spacing of 30 cm and thinned to

two per hill, 20 days after sowing (DAS). A safety and protection distance of 1.5m surrounded the experimental field. The plots were manually weeded 20, 40 and 60 DAS. At flowering stage, plots were sprayed with a standard insecticide formulation, Cypermethrin + Dimethoate at the rate of 30 g + 250 g a.i/L, to control pod borers and flower midges. At maturity, harvesting was done at five-days intervals and seeds were separated to dry pods.

Biochemical Analysis

The biochemical analyses of cowpea seeds were carried out in the Laboratory of Food Sciences and Nutrition, National School of Agro-industrial Sciences, University of Ngaoundéré, Cameroon. To determine the biochemical content, a random sample of 250 g per genotype was taken from a bulk sample of seeds from each replication for the production of flour. Cowpeas seeds were decorticated manually after soaking in water during five hours. Raw and dehulled seeds were separately ground to a fine powder using a Culatti grinder (Polymix, France) fitted with a 1.5 mm mesh sieve and stored in polyethylene bags at 4°C until analysis.

Total polyphenols were determined using the Folin-Ciocalteu method (Gao *et al.*, 2000; Cai *et al.*, 2003). For the extraction of the phenolic compounds, 0.25 g of flour was extracted by stirring with 15 mL of 70 % methanol at room temperature for 30 min, then mixed and centrifuged at 3000 rpm for 10 min. The residue was re-extracted with 10 mL of extraction solvent. The supernatants were mixed and the volume adjusted to 25 mL to constitute the crude extract of phenolic compounds for the determination of phenolic compounds. Absorbance was read at 725 nm against a blank reagent. Results were expressed as mg Gallic Acid Equivalent (GAE) per 100 g dry weight.

For the determination of total tannins, 100 mg of polyvinyl polypyrrolidone (PVPP) were added to 1.0 mL distilled water and 1.0 mL of the methanolic extract (Makkar *et al.*, 1993). This was then vortexed and the tube kept at 4°C for 15 min, vortexed again and centrifuged at 3000 g for 10 min and the supernatant collected. This supernatant has only simple phenolics other than tannins (the tannins get bound to PVPP). The extracts were immediately used for chemical analysis.

The tannin content of the sample was determined as the difference of total phenols with non-tannin phenols.

Phytic acid was determined according to the method described by Vaintraub and Lapteva (1988). 1.25 g of flour was extracted by stirring with 25 mL of 3.5% HCl for one hour at room temperature and centrifuged at 3000 rpm for 30 min. The supernatant was collected and used for the determination of phytic acid content. The extracts were used immediately after production. 3 mL of the extract was added to 1 mL of Wade reagent (30 mg of $FeCl_3.6H_2O$ and 300 mg of sulfosalicylic acid dissolve in approximately 70 mL distilled water, and the volume completed to 100 mL with distilled water) and the mixture was centrifuged. The absorbance was read at 500 nm against a blank reagent. The phytate concentration was calculated from the difference between the absorbance of the control (3 mL of water+1 mL Wade reagent) and that of sample. Calibration curve was drawn using a solution of sodium phytate diluted to obtain 05 to 40 µg of phytic acid. Results were expressed in mg per 100 g dry weight basis.

Total flavonoid content was determined following Mitic *et al.*, (2014) method based on the flavonoid- aluminium complex with maximum absorption at 510 nm. 0.1 mL of extract was added to 0.4 mL of distilled water. This was followed by 0.1 mL of 5% sodium nitrite. After five minutes of incubation, 0.1 mL of 10% aluminium chloride and 0.2 mL of 1M sodium hydroxide was added and the volume was made up to 2.5 mL with distilled water. The absorbance at 510 nm was measured against the blank. A calibration curve of quercetin was prepared and linearity was obtained in the range of 0.2 – 1 $mg.mL^{-1}$ solution (AOAC, 2002). The total flavonoid content in the samples was expressed as mg of quercetin equivalent per 100g of dry seed weight (mg QE/100g dw).

The antioxidant activity of the flour was evaluated by 2,2-diphenyl-2-picrylhydraxyl hydrate (DPPH) free radical scavenging assay as described by Brand-Williams *et al.*, (1995). Extract (200 µL) was added to 1000 µL of methanolic DPPH solution vortexed and keep for one hour at room temperature. The absorbance of resulting solution was read at 517 nm with lower absorbance representing a higher DPPH scavenging activity. DPPH solution (05 mg / 100 mL) was used as standard and antioxidant activity (AA) was expressed as percentage of inhibition using the following equation:

$$AA\ (\%) = \frac{(DRc - DRs)}{DRc} x100$$

Where, DRc was the degradation rate of the control and DRc was the degradation rate of the sample.

Statistical and Genetic Analysis

All biochemical analyses were done in triplicate. For the genotypic variability, data obtained from the eight pure lines for whole-grain, and dehulled seeds were subjected to analysis of variance (ANOVA) using STATGRAPHICS PLUS version 3.0 (Manugistics 1997). The genotypic means were compared using least significant difference at 5% level of probability (LSD 5%). Environmental means were compared using t-Student test. The relative reduction due to decortication (%R) was calculated as outlined by Desphande *et al.*, (1982) as:

$$\%R = \frac{(WS - DS)}{WS} x100$$

Where, WS is the average value of a biochemical trait in whole seed of a specific genotype and DS the average value of the same trait in it the dehulled seed.

RESULTS AND DISCUSSION

Analysis of variance for polyphenols, tannins, flavonoid and phytic acid contents, and antioxidant potential of whole and decorticated seeds from the eight tested pure lines showed the presence of highly significant differences among the genotype grown in Bebedjia and in N'Djamena ($p < 0.05$).

Total phenolics content of the whole seeds varied from 238.79 for KVX30-30966 grown in N'Djamena to 916.34 mg GAE/100 g for TN-5-78 in N'Djamena (average = 501.28 mg and 511.63 GAE/100 g respectively in Bebedjia and in N'Djamena) with lines TN-5-78 and Melakh showing the highest values while KVX30-30966, IT81-D994 and IT99K-573-1-2 had the lowest rates (Table 1). The environmental means of the two locations do not differ significantly. In decorticated seeds, the phenolics content ranged from 85.51 to 261.75 mg GAE/100 g, showing a reduction rate of 61.37 to 74.77% (average of 70.36% in Bebedjia and 73.71% in N'Djamena) (Table 1).

Tannins content of raw cowpeas ranged from 124.12 (IT81-D994 in Bebedjia) to 602.97 mg /100 g dw (TN-5-78 in N'Djamena). The cowpea lines TN-5-78 and Melakh exhibited highest values while entire seeds from IT81-D994 and KVX30-30966 showed the poorest tannins' concentration (Table 2). Globally, the environmental means of Bebedjia (316.05 mg/100g) and N'Djamena (309.27 mg/100g) do not showed significant difference. In decorticated seeds, the tannin content varied from 44.19 to 209.00 mg/100g in Bebedjia (mean = 112.29), and from 55.91 to 216.48 mg/100g in N'Djamena (mean = 113.28). Dehulling of the seeds decreased the tannin content by 55.72 to 67.76% with average of 64.47% in Bebedjia and 63.37% in N'Djamena (Table 2).

The total flavonoid content of the cowpea whole-seeds (Table 3) varied from 20.88 to 48.30 mg QE/100g dw with Melakh and Vita 5 had highest values compared to KVX30-30966 which showed the poorest values in N'Djamena and Bebedjia. When the seeds are dehulled, the concentration of flavonoid ranged from 10.83 to 22.62 mg QE/100g in Bebedjia (mean = 17.98), and from 12.23 to 24.16 mg QE/100g in N'Djamena (mean = 18.34) (Table 3). The removing of hulls caused a reduction of flavonoid content by 37.16 to 60.42% with an average of 48.42% (Table 3).

In whole seed, the phytic acid composition ranged from 277.59 to 878.61 mg/100 g with TN-5-78 and TN-27-80 showed the highest percentages, compared to the genotype as IT99K-573-1-2 and Vita 5 which had the lowest values (Table 4). In decorticated seed, the reduction rate of phytate ranged from 4.96 to 30.60% (average = 18.26% in Bebedjia and 12.96% in N'Djamena) (Table 4).

The antioxidant activity in raw samples varied from 40.71% inhibition (TN-985-61399) to 91.28% inhibition (TN-5-78) whereas after dehulling a significant decrease (34.51 to 52.49%) in amount of antioxidant activity was found (Table 5). In dehulled samples, the scavenging activity varied from 25.72 to 53.22% inhibition. The difference between the two localities was not significant for the environmental means ($p > 0.05$).

Table 1: Polyphenols content in whole and decorticated seeds of eight cowpea lines and dehulling effect in two agroecological sites of Chad

Genotypes	Total phenol content (mg GAE/100 g dw) and dehulling effect					
	Bebedjia			N'Djamena		
	WS	DS	%R	WS	DS	%R
IT81-D994	307.46^{d}	92.80^{cd}	-69.82	361.23^{e}	91.14^{d}	-74.77
IT99-K573-1-2	330.91^{d}	101.56^{cd}	-69.31	383.03^{e}	123.41^{c}	-67.78
KVX-30-30966	267.28^{d}	85.51^{c}	-68.00	238.79^{f}	92.25^{d}	-61.37
Melakh	806.42^{a}	241.12^{a}	-70.10	773.88^{b}	243.23^{a}	-68.57
TN-5-78	861.33^{a}	250.91^{a}	-70.87	916.34^{a}	261.25^{a}	-71.49
TN-27-80	572.09^{b}	171.00^{b}	-70.11	534.31^{c}	153.77^{b}	-71.22
TN-985-61399	410.75^{c}	121.25^{c}	-70.48	407.77^{de}	115.11^{cd}	-71.77
Vita 5	453.25^{c}	116.80^{cd}	-74.23	477.70^{c}	130.32^{c}	-72.72
Mean	501.28	148.58	70.36	511.63	134.51	-73.71
LSD 5%	65.55	35.40		74.58	33.13	

GAE: Gallic Acid Equivalent; WS: Whole seeds; DS: Dehulled seeds; %R: Percentage of reduction; Means with the same subscript within the same column do not differ significantly ($p > 0.05$); LSD: Least significant difference at 5% level of probability.

Table 2: Tannin content in whole and decorticated seeds of eight cowpea lines and dehulling effect in two agroecological sites of Chad

Genotypes	Tannin content (mg /100 g dw) and dehulling effect					
	Bebedjia			N'Djamena		
	WS	DS	%R	WS	DS	%R
IT81-D994	124.12^{h}	44.19^{f}	-64.39	157.74^{f}	55.91^{f}	-64.55
IT99-K573-1-2	220.85^{f}	73.60^{e}	-66.67	229.75^{e}	74.05^{ef}	-67.76
KVX-30-30966	169.54^{g}	63.61^{ef}	-62.47	146.82^{f}	65.00^{ef}	-55.72
Melakh	480.28^{b}	162.99^{b}	-66.06	492.83^{b}	168.16^{b}	-65.87
TN-5-78	562.86^{a}	209.0^{a}	-62.86	602.97^{a}	216.48^{a}	-64.11
TN-27-80	363.76^{c}	129.36^{c}	-64.43	341.46^{c}	123.83^{c}	-63.73
TN-985-61399	280.66^{e}	102.09^{d}	-63.62	221.37^{e}	85.92^{e}	-61.18
Vita 5	326.32^{d}	113.23^{cd}	-65.30	281.20^{d}	100.95^{d}	-64.10
Mean	316.05	112.29	-64.47	309.27	113.28	-63.37
LSD 5%	38.73	25.30		44.22	23.13	

WS: Whole seeds; DS: Dehulled seeds; %R: Percentage of reduction; Means with the same subscript within the same column do not differ significantly (p>0.05); LSD: Least significant difference at 5% level of probability.

Table 3: Flavonoid content in whole and decorticated seeds of eight cowpea lines and dehulling effect in two agroecological sites of Chad

Genotypes	Flavonoid content (mg QE/100g) and dehulling effect					
	Bebedjia			N'Djamena		
	WS	DS	%R	WS	DS	%R
IT81-D994	26.09^{d}	15.23^{cd}	-41.63	27.39^{d}	17.01^{b}	-37.87
IT99-K573-1-2	41.31^{b}	22.62^{a}	-45.32	42.68^{b}	22.76^{a}	-46.68
KVX-30-30966	20.88^{e}	10.83^{e}	-48.13	22.40^{e}	12.23^{c}	-45.37
Melakh	45.89^{a}	22.91^{a}	-50.07	48.02^{a}	24.16^{a}	-49.68
TN-5-78	34.35^{c}	17.86^{bc}	-47.99	37.31^{c}	17.11^{b}	-54.12
TN-27-80	33.81^{c}	13.46^{d}	-60.17	34.49^{c}	13.65^{c}	-60.42
TN-985-61399	28.55^{d}	17.94^{bc}	-37.16	29.39^{d}	18.05^{b}	-38.58
Vita 5	48.30^{a}	20.55^{ab}	-57.45	44.11^{b}	19.18^{b}	-56.51
Mean	34.91	17.98	-48.49	35.72	18.34	--48.65
LSD 5%	4.18	3.07		3.87	3.15	

WS: Whole seeds; DS: Dehulled seeds; %R: Percentage of reduction; Means with the same subscript within the same column do not differ significantly (p>0.05); LSD: Least significant difference at 5% level of probability.

Table 4: Phytate content in whole and decorticated seeds of eight cowpea lines and dehulling effect in two agroecological sites of Chad

Genotypes	Phytate content (mg /100g dw) and dehulling effect					
	Bebedjia			N'Djamena		
	WS	DS	%R	WS	DS	%R
IT81-D994	350.36^{d}	305.58^{cd}	-12.78	378.53^{e}	321.86^{d}	-14.97
IT99-K573-1-2	277.59^{e}	236.15^{e}	-14.93	295.78^{fg}	233.43^{e}	-21.08
KVX-30-30966	315.02^{de}	289.00^{c}	-08.58	313.77^{fg}	295.91^{d}	-05.69
Melakh	480.28^{c}	385.62^{c}	-19.71	492.83^{c}	420.63^{c}	-14.65
TN-5-78	830.50^{a}	789.31^{a}	-04.96	878.61^{a}	742.16^{a}	-15.53
TN-27-80	717.97^{b}	623.27^{b}	-13.19	676.24^{b}	605.09^{b}	-10.52
TN-985-61399	457.92^{c}	317.78^{cd}	-30.06	431.79^{d}	385.93^{c}	-10.62
Vita 5	345.83^{d}	270.16^{de}	-21.88	287.94^{g}	257.13^{de}	-10.70
Mean	489.69	400.27	-18.26	488.42	425.71	-12.97
LSD 5%	53.77	51.22		49.00	44.13	

WS: Whole seeds; DS: Dehulled seeds; %R: Percentage of reduction; Means with the same subscript within the same column do not differ significantly (p>0.05); LSD: Least significant difference at 5% level of probability.

Table 5: Antioxidant activity in whole and decorticated seeds of eight cowpea lines and dehulling effect in two agroecological sites of Chad

Genotypes	Antioxidant activity (%) and dehulling effect					
	Bebedjia			N'Djamena		
	WS	DS	%R	WS	DS	%R
IT81-D994	51.78^{d}	29.59^{cd}	-42.85	54.74^{d}	31.04^{de}	-46.23
IT99-K573-1-2	60.28^{c}	33.00^{bc}	-45.25	64.73^{c}	37.11^{b}	-42.66
KVX-30-30966	64.86^{c}	33.83^{bc}	-47.84	65.63^{c}	35.42^{b}	-46.03
Melakh	83.26^{a}	53.85^{a}	-35.32	77.38^{b}	49.12^{a}	-36.52
TN-5-78	90.93^{a}	55.78^{a}	-38.64	91.28^{a}	53.22^{a}	-41.69
TN-27-80	73.84^{b}	35.08^{b}	-52.49	54.71^{d}	31.83^{de}	-41.82
TN-985-61399	41.04^{e}	25.72^{d}	-37.32	40.71^{f}	26.66^{f}	-34.51
Vita 5	47.27^{de}	25.77^{d}	-45.48	48.99^{e}	28.82^{ef}	-41.15
Mean	64.16	36.48	-43.14	62.65	36.76	-41.32
LSD 5%	8.08	5.22		9.88	4.04	

WS: Whole seeds; DS: Dehulled seeds; %R: Percentage of reduction; Means with the same subscript within the same column do not differ significantly (p>0.05); LSD: Least significant difference at 5% level of probability.

DISCUSSION

Genotypic Variability

Significant difference observed between the eight pure lines tested for phenolic compounds, tannins, flavonoids, phytates and antioxidant activity in whole and decorticated seed indicate a large genetic variability for these characters. In the present study the mean value of polyphenols content is 506 mg GAE/ 100g DM for whole seeds and 141.55 GAE/ 100g DM for dehulled seeds. Wide variation observed in phenolic content is reported in cowpea elsewhere (Preet and Punia, 2000a; Noubissié *et al.*, 2012; Owolabi *et al.*, 2012; Dalaram, 2015; Singh *et al.*, 2017). Amounts polyphenols close to this study have been reported by Owolabi *et al.*, (2012) for whole grain and by Noubissié *et al.*, (2012) in decorticated seeds. Cowpeas contain phenolic compounds in the three main groups including phenolic acids, tannins and flavonoids like quercetin; myricetin and kaempferol (Preet and Punia, 2000b).

The ranges reported in this study for tannin content of whole grain (124.12 to 602.97 mg/100g DM; average: 312.76 mg/100g) are relatively low than those reported by Preet and Punia (2000b) but high than results of Makinde and Abolarin (2020). In dehulled seeds, the tannin contents ranged between 44.19 to 216.48 mg/100g DM (average: 112.79 mg/100g DM) and, these results are close to those obtained by Nassourou *et al.*, (2020). Many authors also observed large variability for tannins in cowpea (Nasara, 2014; Vasconcelos *et al.*, 2010). In Nigeria, Owolabi *et al.*, (2012) discovered that the antinutrient composition was found to be significantly higher in local varieties of cowpea compared to improved lines.

The total flavonoid content of whole cowpea seeds varied from 20.88 to 48.30 mg QE/100g (average of 35.31 mg QE/100g). In the decorticated seeds the concentrations of flavonoid noted in this study ranged between 10.33 and 24.16 mg QE/100g with an average of 18.16 mg QE/100g. Studying 31 genotypes of cowpea in Burkina Faso, Sombié *et al.*, (2018) observed that the total flavonoid content of seeds varied from 7.46 to 23.95 mg QE/100g. Salawu *et al.*, (2014) reported that the total flavonoid content in cowpea ranged from 36 to 95 mg QE/100g, where the cultivars with darker seed-coat had higher flavonoid content than white cultivars. Most of the non-chlorophyll pigmentation of flowers leaves and seeds can be attributed to flavonoids. The large concentration of flavonoid in the seeds impacts the seed coat colour and could influence the choice of varieties (Sombié *et al.*, 2018).

In whole seed, the phytic acid composition ranged from 277.59 to 878.61 mg/100 g. Amounts of phytates close to this study have been reported Owolabi *et al.*, (2012). The content of phytic acid obtained in this study is different with values reported by Bolade (2016) who found in four cultivars values ranging from 680 to 980 mg/100g DM. Diouf *et al.*, (2020) reported much lower phytate contents in five cowpea genotypes which ranged between 117.79 to 254.64 mg/100g DM.

These different levels in antinutrive factors can be explained by the difference in cultivars used. They can also be attributed to the environmental conditions or the differences in methods of quantification. The results obtained could vary significantly depending on the standards or solvents used. However, most of the cowpea cultivars accessed contained high amounts of antinutritional factors that could inhibit nutrients bioavailability. Much of cowpea cultivars accessed contained high amounts of antinutrients that could inhibits nutrients bioavailability. This suggests that the rate of which these antinutrients affect the availability of nutrients will be relatively high with whole seeds. Several studies (Sombié *et al.*, 2018) suggest that the more pigmented cowpea varieties possess higher total phenolic content, total flavonoid content and antioxidant activities than the colourless ones. Genetic variability allows building *V. unguiculata* genotypes with low levels of antinutritional factors.

The antioxidant activity in raw samples varied from 40.71% (TN-985-61399) to 91.28% inhibition, whereas the scavenging activity varied from 25.72 to 53.22% inhibition in dehulled samples. The results showed great differences among tested varieties in free radical scavenging (DPPH) activity of seeds. Wide variability for antioxidant capacity of cowpea genotypes has been recently reported (Yadav *et al.*, 2014; Singh *et al.*, 2017; Olabandji *et al.*, 2018; Sombie *et al.*, 2018).

Environmental Effect

The comparisons of environmental means of N'Djamena and Bebedjia showed globally non-significant differences for these biochemical traits. Furthermore, the responses of some cultivars change with environments suggesting the effect of genotype x environment interaction. Our results suggested that locality do not affect significantly the antinutritional factors content, and the antioxidant activity but the effects of genotype x environment could be significant. Using 15 local and improved cowpea genotypes grown in three locations of Nigeria, Oluwatosin (1999) noted the importance of genotypes, environments and genotype x environment interaction in the control of some antinutrients contents, and thus suggesting that the variability in the levels of the antinutritional factors in cowpea seeds depends also on the environment where they are grown. Dalaram (2015) pointed out that the environmental conditions had significant effect on phenolic content and antioxidant activity, with severe climatic conditions caused a slight increase in total content of polyphenols and antioxidant capacity. Safe environments for production of cowpea with low levels of antinutritional factors and high antioxidant activity must be identified through multi-locations trials.

Dehulling Effect

Dehulling contributed significantly to reduce the content of the studied antinutrients in all cowpea varieties. The contributory reduction capacity varied with antinutrients: from 4.96 to 30.60% for phytates, 37.16 to 60.42 for flavonoids, 55.72 to 67.76% for tannins, and 61.37 to 74.77% for polyphenols. In average, our results showed that decortication reduced the polyphenols, tannin flavonoids and phytate contents by 72.3%, 64%, 48.6% and 30.1% respectively. Preet and Punia (2000b) noted that removal of seed coat of soaked cowpea reduced the polyphenols by 70- 71%. In two varieties of cowpea cultivated in Zimbabwe, Chipurura *et al.,* (2018) noted that soaking and dehulling decreased the total phenolic content by 79.33 and 89.15%. Decortication of grain cowpeas seeds is an effective method for reducing tannin content that localized predominantly in the seed testa. Chipurura *et al.,* (2018) also found that dehulling after soaking removed the most tannin content of raw cowpeas seeds, with a reduction rate of 65.22 and 63.38%. In cowpea, Nasara (2014) noted that decortication highly reduced tannin content by 85%. In contrast, dehulling decreases moderately the phytic acid content and the flavonoid content of cowpea. Chipurura *et al.*, (2018), also noted a reduction rate of 40.79 and 30.59% for total flavonoid content of two cowpea genotypes when the seeds were soaked and dehulled. Bolade (2016) noted that the contributory reduction capacity of dehulling ranged between 34.0 and 40.4% for phytates, and 39.7 and 47.6% for tannin. A great reduction of phytic acid in dehulled seeds has been reported (Preet and Punia, 2000b). Decortication appeared as a practical way to reduce the level of antinutritional factors as polyphenols, tannins, flavonoid and phytic acid. After decortication, there was little phytic acid, tannin and polyphenols in cotyledons indicating that most of these antinutrients are present in seed coat. Desphande *et al.,* (1982) also reported similar results in decorticated common beans. The reduction of phytates could be attributed also to their leaching capacity or their hydrolysis by phytases during preliminary soaking (Diouf *et al.*, 2020).

The total antioxidant activity was affected by dehulling (contributory reduction capacity of 34.51 to 52.49% with average of 42.25%). Removal of hulls significantly decreased the concentrations of phytochemicals responsible for the antioxidant activity. A highly positive linear correlation was found between antioxidant activity and total phenol (Yadav *et al.*, 2014; Dalaram, 2015; Sombié *et al.*, 2018). According to Yadav *et al.*, (2014), more than 90% of the antioxidant capacities of the seed-coat of cowpea are contributed by phenolic compounds. These results are comparable to the findings of Sombié *et al.*, (2018) in cowpea. In four cowpea genotypes, Yadav *et al.*, (2014) highlighted that seed coat had higher antioxidant activity (33.94 to 86.31%) than the cotyledon fraction (15.93 to 45.75%) confirming that removal of seed coat significantly reduced the antioxidant activity. Although dehulling reduces the antioxidant activity, it appeared as a proper processing method to reduce anti-nutritional factors and improve the bioavailability of nutrients, especially when cowpeas are used as food for infants and children.

CONCLUSION

Cowpea seeds present a large variability for their composition in antinutritional factors and antioxidant activity. Dehulling significantly reduced the antinutrients and the antioxidant capacity. If antinutients are the major concern, dehulling could be recommended in the manufacture of cowpea based foods for special diets. Furthermore, pre- processing steps as dehulling should be discouraged if people are to fully benefit from the phytochemicals with high antioxidant potential found in the seed-coat. Together with industrial processes, breeding could improve the quality of cowpea to meet the needs expressed by different users. The understanding of the genes action for these characters and the genotype x environment interaction effects are the key for best selection

strategies of genotypes presenting adequate contents in these anti-nutritional factors and antioxidant activity.

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