SHORT COMMUNICATION

Seed coat metabolite profiling of cowpea (*Vigna unguiculata* L. Walp.) accessions from Ghana using UPLC-PDA-QTOF-MS and chemometrics

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ABSTRACT

Cowpea (*Vigna unguiculata* L. Walp.) is an important grain legume in Africa exhibiting high morpho-genetic diversity. However, not much information exists on the phytochemical profiles of its hulls. This study explored the metabolite profiles of seed-coats from thirteen cowpea accessions of varying phenotypes using UPLC-QTOF-MS and chemometric analysis. A total of 34 secondary metabolites were identified, which comprised phenolic acids, flavonoids, anthocyanins, sphingolipids and fatty acids. Quantification of selected phenolic compounds revealed marked variations among the cowpea accessions. The chemical profiles of the test accessions were distinguished by multivariate analysis, and the results revealed a marked influence of seed-coat pigmentation on the observed differences in their metabolite profiles. Moreover, delphinidin (traces to 2257.6 μg/g), catechin glucoside (traces to 2840.6 μg/g), catechin (traces to 2089.2 μg/g) and epicatechin (26.3 to 3222.7 μg/g) contributed to the segregation amongst the studied samples. The concentrations of the discriminant metabolites were greater in the dark seeded cowpeas compared to their lighter seeded counterparts. The findings represent a useful contribution to the literature on cowpea seed coat metabolites, and also reveal their potential for use in the development of food and pharmaceutical products.

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1. Introduction

Legumes played fundamental roles in the development of civilizations by supplying human food, animal feed and traditional medicines (Oomah et al. 2011). In sub-Saharan Africa, legumes for human consumption include peanuts, common beans, Bambara groundnuts, Kersting’s groundnuts, soybeans, chickpea, African yam bean and cowpea. Unlike other grain legumes, cowpea (*Vigna unguiculata* L. Walp.) can be consumed at different stages of its development: fresh green leaves, dry leaves, green pods, green beans, or dry grain (Pule-Meulenberg et al. 2010). There are several varieties, breeding lines, landraces and cultivars of cowpea exhibiting variations in seed coat pigmentation. The bioactive phenolics present in seeds of legumes are mostly accumulated in the seed coats, where they play important roles as protectants during seed development, and against detrimental agents from the environment (Singh et al. 2017). Despite its rich phenolic content and antioxidant potential (Oomah et al. 2011), the hulls of cowpea are often considered as waste since they contribute to undesirable texture in cooking. As an important crop in Africa, cowpea hulls could be a potential source of bioactive compounds and can be used as food supplements and natural antioxidants.

Given the large variations in seed coat pigmentation among the cultivated cowpea germplasm, there is a need to explore their phytochemical profiles to understand genotypic differences in their chemical compositions. An earlier study has detailed the fatty acids composition of cowpea seeds, highlighting their potential for utilization as functional foods (Lo Turco et al. 2016). Whereas some studies have reported on the phenolic contents of whole cowpea seeds in relation to their antioxidant capacity (Gutiérrez-Uribe et al. 2011; Ha et al. 2010; Ojwang et al. 2012; Ojwang et al. 2013), there is not much information regarding the hulls of cowpea.

The present work, therefore, evaluated the phytochemical contents, identified and characterized the metabolites in the hulls of 13 cowpea accessions using non-targeted UPLC-ESI-QTOF-MS coupled with chemometric analysis. This information may contribute to a more integral utilization of cowpea as functional foods and industrial crops.

2. Results and discussion

The local names, seed phenotypes, number of seed/100g and origin of cowpea accessions from Ghana used in this study are presented in Table 1S and Figure 1S.

2.1. Phytochemical content (TPC, TFC, TAC) of cowpea hulls

The total phenols (TPC), total flavonoids (TFC) and total anthocyanins (TAC) contents in seed coats of the test cowpea accessions are shown in Table 2S. In this study, there were significant differences in the TPC in seed coats of the thirteen cowpea accessions, with values ranging from $401.9 \pm 95.97$ to $4116.7 \pm 95.97 \mu g$ GAE/g dry weight (Table 2S). The accessions T15 and T1 with brown and mottled seed coats, respectively, exhibited the highest TPC ($4116.7 \pm 95.97$ and $3340.9 \pm 53.52 \mu g$ GAE/g dry weight, respectively) as opposed to much lower values recorded in the white seeded T28 and T41 ($401.9 \pm 5.34$ and $438.94 \pm 5.34 \mu g$ GAE/g dry weight, respectively). There
were marked differences in the TFC of the test cowpeas, with values ranging from 187.40 ± 21.85 to 54.98 ± 1.12 μg QUE/g dry weight for all the analysed samples. The highest total flavonoid content (187.40 ± 21 mg QUE/g dry weight) was recorded in hulls of the cream seeded accession T12 followed by T4 (brown) and T41 (white), with the values of 175.05 ± 10.82 and 170.56 ± 10.70 QUE/g, respectively. The TAC showed marked variations due to differences in seed coat pigmentation and ranged from 939.00 ± 25.40 to 12.23 ± 0.73 mg CAE/g dry weight. The TAC was markedly higher in the black (T15), brown (T5, T31 and T36) and mottled (T1 and T16) accessions as opposed to much lower values observed in hulls of the white (T17, T28 and T41) and cream (T4, T12, T38 and T50) seeded accessions (Table 2S). The results indicate that, the dark coloured cowpeas accumulated more anthocyanins such as delphinidin-3-O-glucoside, cyanidin-3-O-glucoside and petunidin-3-O-glucoside compared to their counterparts with lighter seed coats (Table 4S). The findings also suggest that the biosynthesis of anthocyanins is more pronounced in darker seeds than in lighter seeded cowpeas.

2.2. Metabolite profiling of seed coats from different cowpea phenotypes

From the phytochemical analysis (Figure 2S), a total of 35 metabolites (6 phenolic acids, 12 flavonoids, 4 sphingolipids, 6 fatty acids and 7 anthocyanins) were identified (Table 3S). Of the compounds identified, flavonoids were the predominant class of metabolites (7 flavonols and 5 flavanols). The identities of compounds 1, 2, 6, 8, 11 and 16 were confirmed by comparing their retention times with those of their respective pure standards. The 29 other metabolites were tentatively identified by comparing their UV/vis spectra and MS data with those of compounds reported in the literature.

2.3. Quantification of major (poly)phenolic compounds in cowpea hulls

A total of 35 metabolites were tentatively identified based on MS/MS data. Of the compounds identified, eight were quantified (Table 4S). The findings revealed a marked effect of seed coat pigmentation on the concentrations of (poly)phenolic compounds in hulls of the test cowpeas. Of the eight metabolites quantified, six of them (caffeic acid, catechin, epicatechin, catechin glucoside and gallocatechin) were detected in the hulls of all the tested accessions. Quercetin was not present in samples T12 and T38. Although delphinidin-3-O-glucoside was not detected in samples T38, T41 and T50, large amounts of the compound were accumulated in the darker coloured phenotypes (T1, T15, T16, T31 and T36). The highest percentage of the quantified phenolics was recorded in the seed coats of dark coloured genotypes, with epicatechin being the most abundant (3222.7 ± 958.75 μg/g) followed by catechin glucoside (2840.6 ± 105.83 μg/g), catechin (2089.2 ± 209.54 μg/g) and delphinidin-3-O-glucoside (2257.6 ± 115.70 μg/g). In all the studied accessions, catechin and its derivatives were the most abundant, suggesting a pronounced biosynthesis of secondary metabolites belonging to the flavanol class in cowpea seeds. These findings are consistent with those reported by earlier studies (Ojwang et al. 2013; Tsamo et al. 2018). The greater abundance of catechin derivatives makes cowpea hulls an
important candidate for investigating the bioactive properties attributed to catechins and other flavan-3-ols.

2.4. Principal component analysis (PCA) reveals differences in phenolic profiles of cowpea hulls

PCA was carried out using the relative amounts of metabolites identified in each sample. The first two principal components (PCs) explained 91% of the total data variance and was therefore robust to discriminate between the samples. The separation of the samples into two groups in the PC1/PC2 scores plot (Figure 3S-A) is a clear indication of the differences in the phytochemical composition of the cowpea seed coats. The two clusters formed were distributed over two opposite regions, revealing the differences in the composition of the two groups. An examination of the loadings plot (Figure 3S-B) showed that the variables refer to the MS signals of catechin, epicatechin, catechin glucoside and delphinidin-3-O-glucoside which contributed most to the discrimination among the test cowpea samples and were also among samples in cluster 1.

2.5. Hierarchical cluster analysis (HCA)

The HCA revealed two clear clusters of 6 and 7 phenotypes, each referred to as groups 3A and 3B, respectively (Figure 4S). An inspection of group 3A showed that samples T1, T5, T15, T16, T31 and T36 were more closely related in terms of their chemical composition when compared to the samples in group 3B. The plots from PCA and HCA corroborated and clearly suggested that the differences in metabolites profiles found in our study correlated with seed coat color. Darker seed coats could accumulate more catechin derivatives and delphinidin compared to seeds with lighter seed coats. These findings could indicate that the genes involved in the biosynthesis of flavanols and anthocyanins are highly expressed in the dark seeded cowpeas than in those with lighter coats. The differences in seed coat pigmentation may therefore be responsible for the observed variations in catechin conjugate and delphinidin contents, including the increased amounts of other phenolic compounds. The findings of this study clearly show similar chemical profiles among cowpea accessions with varying phenotypes, although with marked variations in the concentrations of individual compounds.

3. Conclusion

This study assessed the metabolites diversity in the seed coats of thirteen cowpea accessions exhibiting variable seed coat pigmentation. All the studied samples elicited similar chemical profiles, although the accumulation of metabolites differed among the test accessions. Catechin glucoside, catechin, epicatechin and delphinidin were the most predominant compounds in samples T1, T5, T15, T16, T31 and T36; however, cultivar T15 appeared to be the richest in secondary metabolites. These compounds were also the metabolites responsible for the discrimination among the cowpea accessions.
These results have contributed to our understanding of the roles of metabolites accumulation in the determination of seed coat variations among cowpea accessions.

**Disclosure statement**

There is no conflict of interest.

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