Journal of Food Measurement and Characterization (2019) 13:2310–2317
https://doi.org/10.1007/s11694-019-00150-3

ORIGINAL PAPER

Identification and quantification of anthocyanins in seeds of Kersting's groundnut [*Macrotyloma geocarpum* (Harms) Marechal & Baudet] landraces of varying seed coat pigmentation

Armelle T. Tsamo[1,2,4] · Mustapha Mohammed[3] · Peter P. Ndibewu[1] · Felix D. Dakora[1,4]

Received: 13 August 2018 / Accepted: 2 May 2019 / Published online: 15 May 2019

Abstract

The present study identified and quantified the anthocyanin pigments in seed of eight Kersting's groundnut [*Macrotyloma geocarpum* (Harms) Marechal & Baudet] landraces of variable seed coat pigmentation. The analysis was carried out using ultra-performance liquid chromatography coupled with a photodiode array detector and mass spectrometry. Although the findings revealed similar anthocyanin profiles in seeds of the eight landraces tested, the relative concentrations of the individual anthocyanin compounds showed marked variations. Delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside and peonidin-3-*O*-glucoside were the most abundant anthocyanins, with concentrations ranging between 21.4–239.0, 23.6–130.6 and 21.4–135.4 µg/g of dry seed, respectively in seeds of the test landraces, while total anthocyanins ranged between 95 and 505 µg/g of dry seed. The preponderance of delphinidin 3-*O*-glucoside was observed in the black and brown mottled seeds. This study is the first report regarding the profile and concentrations of anthocyanins in Kersting's groundnut and suggests that the seeds of this underutilized grain legume can potentially be exploited as a natural source of anthocyanins for the development of cosmetic, food and pharmaceutical products.

Keywords Kersting's groundnut · UPLC–DAD–qTOF-MS · Anthocyanins · Natural antioxidants · Colorants

Abbreviations

KG	Kersting's groundnuts
ESI	Electrospray ionization
LC	Liquid chromatography
MS	Mass spectrometry
PDA	Photodiode array detector
SD	Standard deviation
TAC	Total anthocyanin content
UV	Ultraviolet
Vis	Visible

✉ Armelle T. Tsamo
armelletsamo@yahoo.fr; TsamoTontsaA@tut.ac.za

✉ Felix D. Dakora
DakoraFD@tut.ac.za

[1] Department of Chemistry, Tshwane University of Technology, Pretoria 0001, South Africa

[2] Department of Organic Chemistry, University of Yaoundé I, BP. 812, Yaounde, Cameroon

[3] Department of Crop Sciences, Tshwane University of Technology, Pretoria 0001, South Africa

[4] Tshwane University of Technology, 175 Mandela Drive Street, Arcadia Campus, Building 3, Room 608B, Pretoria, South Africa

Introduction

Legume crops are widely consumed around the world owing to the nutritional attributes of their grains and leaves. Despite their nutritional and medicinal values however, many indigenous African crops are neglected and under-utilized due to limited research regarding their genetic and phytochemical potentials as well as their agronomic requirements [1]. Kersting's groundnut [*Macrotyloma geocarpum* (Harms) Marechal & Baudet] is one of the rare and under-exploited indigenous African legumes which is gradually disappearing from its cultivation areas due to lack of research on its improvement and potential utilization [2, 3]. Nevertheless, the crop is adapted to growth under drought conditions and marginal soils, and also possesses health and nutritional values which can be tapped for improved food/nutritional security [4–6]. In West Africa where Kersting's groundnut originates, it is cultivated by small holder farmers mainly for home consumption [7]. However, because of the low yields

often obtained on farmers' fields and the lack of improved seeds or varieties, commercial farmers have shown very little interest in cultivating the crop on a large scale [8].

Currently, the available landraces of Kersting's groundnut differ in traits such as seed size, seed coat colour and texture [9]. However, the colour and texture variations are more prominent, with black, brown, and white seeds being common [9]. These traits play fundamental roles in consumers' acceptance and sensory impression of the food [10]. In grain legumes, black and red seed coat pigmentation are attractive sources of anthocyanins, which are natural food colourants and antioxidants [11, 12]. Anthocyanins are a group of reddish or purple water-soluble pigments mostly found in the angiosperms or flowering plants, which represent a major food source [13]. They are widely used as natural colourants in food, pharmaceutical and cosmetic industries [14]. The strikingly high anthocyanin levels in legumes gives them their characteristic colouring properties, and also play vital roles in recruiting pollinators, aiding in seed dispersal and protection of plants from photo-oxidative damage and oxidative stress [15]. In addition to their role in plants, anthocyanins are known to possess health benefits such as vision improvement [16], reduced risk of inflammatory [17], cardiovascular [18], and age-related degenerative diseases [19, 20].

Although extensive information exists regarding the anthocyanin composition in fruits, vegetables, cereals and some grain legumes [12, 21–24], there is currently no information on the anthocyanin profiles and concentration in Kersting's groundnut. This study was partly motivated by the need to explore for cheaper sources of anthocyanin pigments to meet the increasing demand for natural colourants and antioxidants. Moreover, the phytochemical characterization of plant matrices is key to understanding their biological and nutritional properties [25]. The use of liquid chromatography coupled with mass spectrometry is known to be a reliable technique for unbiased screening of compounds in plant materials and food products [25–28]. The aim of this work was therefore to characterize the anthocyanin contents and composition in the seeds of eight Kersting's groundnut landraces exhibiting variable seed coat pigmentation. This was done to identify anthocyanin-rich landraces that can be used in breeding programs aimed at improving varieties for future industrial utilizations in the preparation of food supplements, natural antioxidants and colourants.

Materials and methods

Plant materials

In this study, eight Kersting's groundnut (KG) landraces exhibiting different seed coat pigmentation/colourations [namely, Puffeun (KG1 = black), Boli (KG2 = white), Funsi (KG3 = brown mottled), Sigiri (KG4 = brown mottled), Nakori (KG5 = brown mottled), Heng Milk Mottled (KG6 = brown mottled), Dowie (KG7 = brown mottled) and Belane Mottled (KG8 = brown mottled)] were used (Table 1). The landraces were sourced from the University for Development Studies at Nyankpala, Ghana. The plants were identified by examining the morphological characteristic of the flowers, leaves and seed structures. Voucher specimens (TUTMG1401–TUTMG1408, corresponding to KG1–KG8, respectively) were deposited at the Herbarium of the Crop Sciences Department, Faculty of Science, Tshwane University of Technology, Pretoria, South Africa.

Table 1 Comparison of anthocyanin contents in seeds of 8 Kersting's groundnut landraces of varying seed coat pigmentation

Sample code	Local name	Colour	Anthocyanin content			
			D3G (µg/g)	C3G	P3G	Total
KG1	Puffeun	Black	239.0 ± 0.84[a]	130.6 ± 2.47[a]	135.8 ± 3.03[a]	505
KG2	Boli	White	24.3 ± 0.44[f]	24.8 ± 0.66[f]	35.3 ± 0.56[b]	84
KG3	Funsi	Brown mottled	23.2 ± 0.13[f]	23.6 ± 0.37[f]	33.8 ± 0.13[b]	80
KG4	Sigiri	Brown mottled	49.9 ± 3.46[c]	26.9 ± 0.88[c]	23.6 ± 0.44 c[d]	100
KG5	Nakori	Brown mottled	69.8 ± 0.32[b]	34.8 ± 0.71[b]	26.2 ± 0.18[c]	131
KG6	Heng MM	brown mottled	32.7 ± 0.52[e]	24.4 ± 0.14[e]	22.8 ± 0.03 [cd]	80
KG7	Dowie	Brown mottled	21.4 ± 0.00[f]	26.0 ± 0.61[f]	21.4 ± 0.00[d]	69
KG8	Belane M	Brown mottled	43.8 ± 0.82[d]	27.6 ± 0.22[d]	23.9 ± 0.08 [cd]	95
F statistics	NA	NA	3055.8***	3055.8***	1241.9***	NA

Data (mean ± se) in the same column marked with different lowercase letters are significantly different at $p < 0.001$ (***)

D3G delphinidin-3-*O*-glucoside, *C3G* cyanidin-3-*O*-glucoside, *P3G* petunidin-3-*O*-glucoside, *NA* not applicable

[a]Average of three determinations

The landraces were grown in experimental fields at three locations (Nyankpala, Savelugu and Gbalahi) in the Northern region of Ghana, in 2014. At maturity, (120 days after sowing), plants were harvested, and the pods collected. Pods were sun-dried and threshed to obtain the seeds, which were then dried to 13% moisture content and stored at 4 °C until further pretreatment (extraction) and analysis.

Chemicals and reagents

Reagent grade methanol (CH_3OH, ≥ 99.8%), acetonitrile (MeCN, ≥ 99.5%), hydrochloric acid (HCl, ≥ 37%), trifluoroacetic acid (CF_3COOH, ~ 98%) were purchased from Merck Chemicals (Pty) Ltd (Johannesburg, South Africa). Pure delphinidin standard was obtained from Sigma Chemicals Co. (Germany). A 0.22 µm membrane filter was purchased from Chemical Co. (Johannesburg, South Africa). Only ultrapure water (UPW) (H_2O, conductivity: ~ 18.2 Mega Ohm at 25 °C) was used in this study.

Anthocyanin extraction

To extract anthocyanins, seeds of each Kersting's groundnut landrace was separately ground to powder using a coffee grinder (Cuisinart, model DCG-20N series). Anthocyanins were then extracted using the procedure described by [29] with slight modifications. One gram of each ground sample was extracted in 10 mL of methanol acidified with 1.0 N HCl (80:20, v/v). The mixture was vortexed for 1 min and the extraction was carried out under agitation in the dark at 4 °C for 24 h. The extract was centrifuged for 20 min at 4000 rpm and the supernatant was collected. The residue was extracted two or more times, and the supernatants were combined and evaporated to one-third (1/3) of the total volume with a rotary evaporator (Buchi Rotavapor R-100, Germany) at 40 °C. The resulting crude extracts were then filtered with Whatmann No. 1 paper and stored at − 4 °C until liquid chromatographic analysis.

UPLC–MS analysis

The anthocyanins present in seeds of the test Kersting's groundnuts were characterized by UPLC–qTOF-MS analysis. The experiment was done using a Waters Acquity Ultra Performance Liquid Chromatographic system with a PDA detector (Waters, Milford, MA, USA). Separation was achieved using an Acquity UPLC BEH C18 column (150 mm × 2.1 mm, i.d., 1.7 µm particle size, Waters) maintained at 40 °C. To shorten the analysis time and to obtain better resolutions for chromatograms, preliminary tests were done before setting the chromatographic conditions. The mobile phase consisted of 0.1% trifluoroacetic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 0.3 mL/min. The gradient elution was executed as follows: initial ratio was 95% A:5% B, keeping for 1 min, changed to 20% A:80% B in 10 min, to 5% A:95% B in 5 min, keeping for 1 min and back to initial ratio in 0.5 min, with the equilibration of the system for 1.5 min. The total running time was 18 min and the injection volume was 2.0 µL (full-loop injection). The positive ion mode was used for anthocyanins detection. Thus, the mass spectrometry was operating in a negative ion electrospray mode and nitrogen (N_2) was used as the desolvation gas. Data were acquired between 50 and 1200 *m/z*. The following parameters were then set: capillary voltages of 3000 V; sampling cone voltages of 45 V; extraction cone of 4; source temperature of 100 °C; desolvation temperature of 350 °C; desolvation gas flow was 400 L/h. The chromatographic software MassLynx 4.1 was used to process and generate all the chromatographic data.

Quantification by UPLC–DAD

To quantify the anthocyanins in seed extracts, chromatographic analysis was carried out on a Shimadzu® LC-20AD (Kyoto, Japan) UPLC system equipped with a LC-20AD UPLC pump, a SIL-20AD autosampler, a CTO-20AD thermostatted column compartment and a SPD-20AD photodiode array (PDA) detector. The analytical column was an ODS-Ultra Aqua C18 column (100 mm × 2.1 mm i.d., 3 µm particle size, Restek®, Bellefonte, PA, USA) protected with a Guard Cartridge (Restek®, Bellefonte, PA, USA). The column was maintained at 25 °C. The mobile phase consisted of water solution of 0.1% trifluoroacetic (solvent A) and acetonitrile containing 0.1% trifluoroacetic (solvent B). A linear gradient program at a flow rate of 0.200 mL min^{-1} flow was used: 0–1 min, 5% B; 1–22 min, 5–32% B; 22.5–23 min, 45–100% B; 23–24.5 min, 100% B; 24.5–26 min, 100–5% B; 26–27 min, 5% B. The PDA detection wavelength was set at 200–700 nm and the injection volume at 20 µL. Prior to analysis, all samples were filtered through a 0.22 µm membrane filter. The absolute quantification of individual anthocyanins was done using external standard. Concentrations of the compounds were determined from the UPLC diode array detection (DAD) signal peak area by interpolation based on calibration curves using pure standards and expressed as mg g^{-1} of seed dry weight. Standard calibration curves for each standard with linearity range from 0 to 1000 µg mL^{-1} ($R^2 = 0.99$) were constructed using five concentrations. The assumption made for the quantification of anthocyanin glucosides was that, according to the literature, their molar absorptivities were similar those of their aglycone.

Statistical analysis

The data were reported as means of three replicates with standard errors (SE). The differences in quantities of

anthocyanins between the landraces were analysed by one-way ANOVA statistical model using Statistica software version 10. Where means showed significant differences, they were separated using Duncan's multiple range test at $p \leq 0.05$.

Results and discussion

Identification of anthocyanins

The seeds of each Kersting's groundout landrace was separately extracted with acidic MeOH (1% HCl) followed by analytical reverse phase column chromatography. The anthocyanins were separated by reverse phase C-18 column chromatography as previously described. The anthocyanin profiles of the acidic methanolic crude extracts (measured at 520 nm) of the test samples are illustrated in Fig. 1. Anthocyanins are usually abundant in the pigmented seed coats of legumes [30]. In this study, three major anthocyanins were detected by LC-DAD/HR-MS analysis, and their mass spectra were revealed by fragmentation patterns arising from MS created by electrospray ionization (ESI) in the positive mode (Figs. 1 2; Table 1). The UV–Vis absorption spectra of the compounds identified had peaks that appeared at (277, 525 nm), (277, 526 nm) and (279, 529 nm) for compounds 1, 2 and 3, respectively, a finding characteristic of anthocyanins [31, 32]. Their mass spectra revealed the peaks of molecular ions $[M]^+$ and fragment ion $[M\text{-Glucose}]^+$ resulting from the loss of the sugar moiety. Peak 1, with a molecular ion at *m/z* 465 was predominant in the anthocyanin profile, and also yielded a fragment ion at *m/z* 303 which corresponded to delphinidin aglycon probably arising from the loss of a hexose unit (162 U). Peaks 2 and 3 produced molecular ions at *m/z* 449 and 479, respectively. The presence of the aglycons cyanidin (*m/z* 287) and petunidin (*m/z* 317) were detected in peaks 2 and 3 (Fig. 2). Previous studies have reported that the glucosides of delphinidin, cyanidin and petunidin are the common anthocyanins in seed and seed coats of grain legumes, with values ranging from 0.25 to 35.11 mg GAE/g DW in Kidney beans and from 1.46 to 57.98 mg GAE/g DW in seed coat of beans (Folin–Ciocalteu, HPLC) [33]. Similarly, an earlier study reported the dominance of cyanidin-3-*O*-glucoside relative to other anthocyanins in black-seeded common beans [34], black and red peanuts [30], kidney beans [35], and adzuki beans [36].

Quantification of anthocyanins

The concentrations of various anthocyanins in the test samples were determined via UPLC–DAD. Although anthocyanins analysis in plant samples usually span between 40 and 60 min [37], the separation of anthocyanins in Kersting's groundnuts was achieved within 18 min via the complete UPLC–DAD system (Fig. 1). Varying concentrations of pure delphinidin were used as standards to construct a curve for extrapolating the concentrations of individual anthocyanins in the test samples. The total anthocyanins in samples was then computed as the sum of individual anthocyanin compounds in the samples. The UPLC chromatograms obtained at 520 nm revealed three major peaks in all test landraces (Fig. 1 and Table 2). There were marked differences in the anthocyanin contents of the landraces, with higher ($p < 0.001$) concentrations being found in the black seeded puffeun when compared to the white or brown seeded landraces. For example, the seeds of puffeun had the highest ($p < 0.001$) concentration of delphinidin-3-*O*-glucoside (239.0 µg/g) followed by the brown mottle seeded nakori (69.8 µg/g), sigiri (49.9 µg/g), belane M (43.8 µg/g) and then heng MM (32.7 µg/g), each accounting for 47, 53, 50, 46 and 41% of the total anthocyanins in those landraces, respectively (Table 1). On the other hand, the white seeded landrace boli, together with the brown mottled funsi and dowie recorded the least but similar ($p > 0.05$) delphinidin-3-*O*-glucoside contents (21.4–24.3 µg/g). As with delphinidin-3-*O*-glucoside, the concentrations of cyanidin-3-*O*-glucoside and petunidin-3-*O*-glucoside were also higher (130.6 µg/g and 135.8, respectively) in the black seeded puffeun when compared to their concentrations in the other landraces (Table 1). As shown in Table 1, the concentrations of both delphinidin-3-*O*-glucoside and petunidin-3-*O*-glucoside respectively varied between 23.6–34.8 µg/g and 21.4–34.3 µg/g in the landraces KG2–KG8 as opposed to the observed higher concentrations in seeds of puffeun. Further, petunidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside were each the second major anthocyanins in puffeun and boli, respectively, (Table 1). As expected, the concentration of total anthocyanins was much higher in the black seeded Kersting's groundnut phenotype when compared to the other test landraces. The similarities in the anthocyanin profiles of the different Kersting's groundnut landraces indicate that this trait may be conserved within the species. Although there is currently no report on the anthocyanins composition of Kersting's groundnut, the dominance of delphinidin-3-*O*-glucoside was earlier reported in black seeded common bean [11]. Moreover, previous studies have reported up to 2094 µg/g of anthocyanins in seed coats of cowpea [38], 1.5–20.18 mg/g in those of soybeans and 0–2.61 mg/g of delphinidin glucoside in those of kidney beans [12]. The anthocyanin concentrations in seeds of the test Kersting's groundnut landraces are within the range of values observed in several other grain legumes [33]. The fact that anthocyanins were detected in high amounts in the black seeded Kersting's groundnut (puffeun) as opposed to very low concentrations in the other seed phenotypes indicates a strong genetic influence on the synthesis and accumulation of anthocyanin compounds in

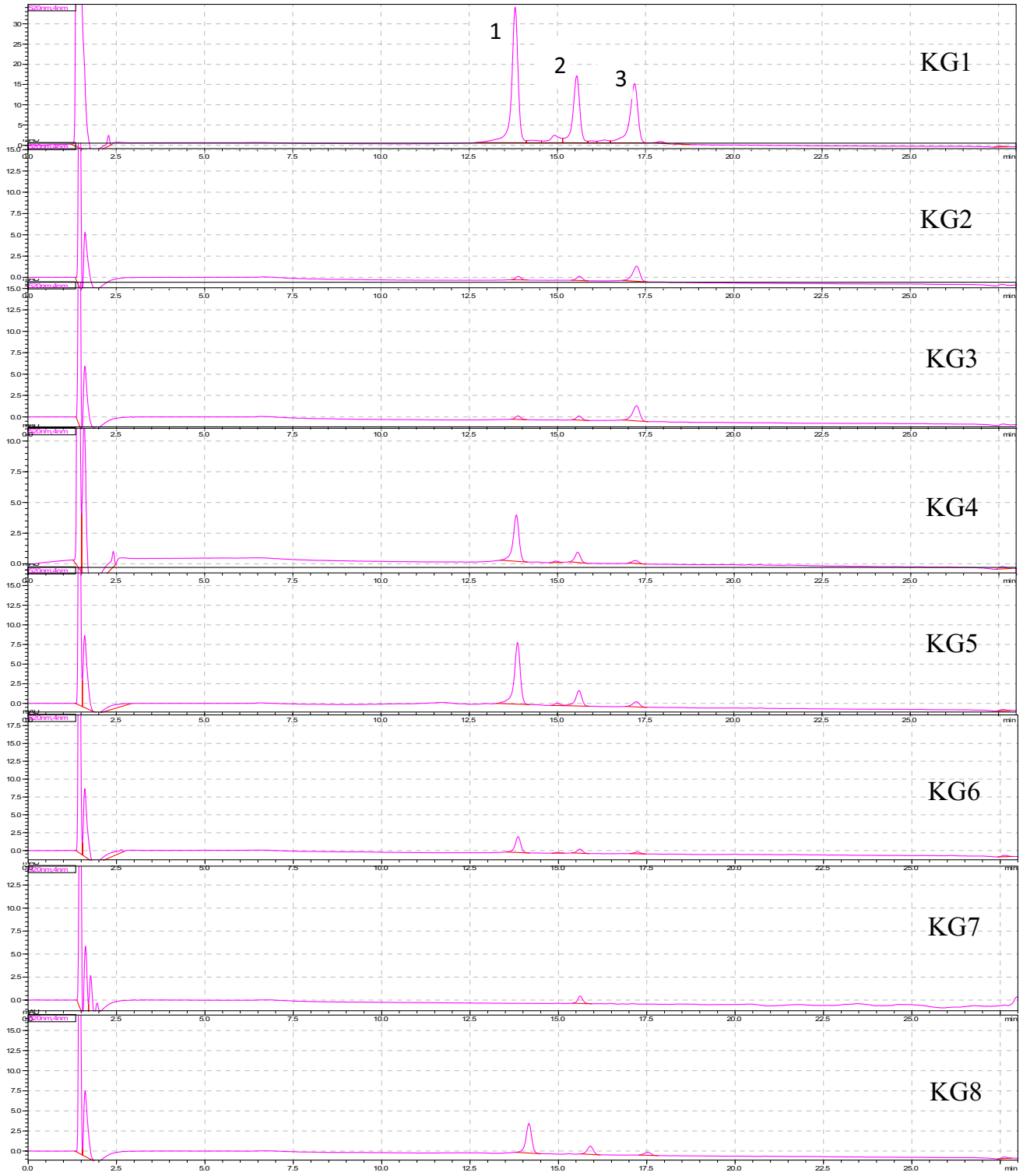
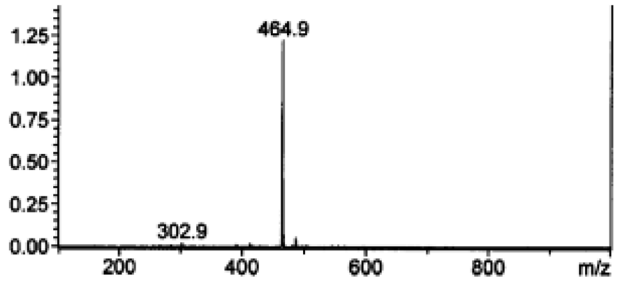
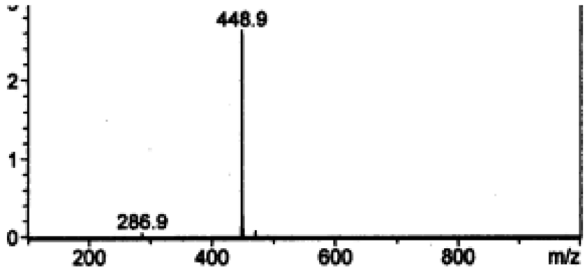


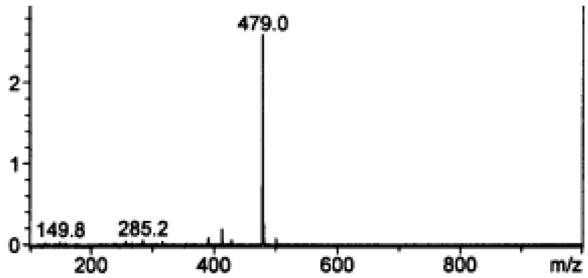
Fig. 1 Anthocyanin profile of eight (8) Kersting's groundnut landraces obtained by UPLC–DAD–(ESI)-HR-MS in positive mode and recorded at 520 nm: (1) delphinidin-3-*O*-glucoside, (2) cyanidin-3-*O*-glucoside, (3) peonidin-3-*O*-glucoside. For sample codes (KG1–KG8) refer to Table 1



Peak 1: Delphinidin-3- O-glucoside



Peak 2: Cyanidin-3-O-glucoside



Peak 3: Peonidin-3-O-glucoside

Fig. 2 Mass fragmentation patterns of identified anthocyanins

Table 2 Chromatographic and spectral characteristics of anthocyanins in seeds of Kersting's groundnut landraces detected by HPLC–ESI/HR-MS

Peaks	Retention time (min)	Molecular ion (*m/z*)	Formula	Fragment ions (*m/z*)	Absorbance maxima (nm)	Assigned compound name
1	13.30	465.1018	$C_{21}H_{21}O_{12}$	303	277, 525	Delphinidin-3-*O*-glucoside
2	15.54	449.1066	$C_{21}H_{21}O_{11}$	287	277, 526	Cyanidin-3-*O*-glucoside
3	17.18	479.1172	$C_{22}H_{23}O_{12}$	301	276, 529	Petunidin-3-*O*-glucoside

the species. In this study, all the anthocyanins identified in seeds of the test Kersting's groundnut have previously been found in the seeds and seed coats of other grain legumes such as cowpeas [38], Bambara groundnut [39], soybean [40], kidney beans [12] and common beans [41].

Conclusion

The present study revealed marked variations in the concentrations of anthocyanin in seeds of Kersting's groundnut landrace exhibiting differences in seed coat pigmentation. Of the landraces evaluated, the black seeded Kersting's groundnut landraces had had the highest concentration of anthocyanins when compared to the white or brown mottled landraces. The observed wide variations in the anthocyanin composition of the test landraces represents a useful genetic pool that offers opportunity for selection in the development of anthocyanin-rich seeds through breeding programs. Moreover, the black seeded Kersting's groundnut (KG1 or Puffeun) can potentially be used in the preparation of seed-based foods, supplements and as a source of natural colorants due to its higher anthocyanin content. The findings of this study is a useful contribution to the literature regarding the phytochemical composition and potential utilization of important but underutilized African legumes such as Kersting's groundnut.

Acknowledgements We are grateful to the South African Research Chair in Agrochemurgy and Plant Symbioses, the National Research Foundation and the Tshwane University of Technology for financial support. ATT is postdoctoral fellow under the South African Research Chair in Agrochemurgy and Plant Symbioses.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

1. P. Chivenge, T. Mabhaudhi, A.T. Modi, P. Mafongoya, The potential role of neglected and underutilised crop species as future crops under water scarce conditions in Sub-Saharan Africa. Int. J. Environ. Res. Public Health. **12**, 5685–5711 (2015)
2. R. Adu-Gyamfi, J. Fearon, T. Bayorbor, I. Dzomeku, V. Avornyo, The status of Kersting's groundnut (*Macrotyloma Geocarpum* [Harms] Marechal and Baudet) An underexploited legume in Northern Ghana. Outlook Agric. **40**, 259–262 (2011)
3. M. Mohammed, S.K. Jaiswal, E.N. Sowley, B.D. Ahiabor, F.D. Dakora, Symbiotic N_2 fixation and grain yield of endangered Kersting's groundnut landraces in response to soil and plant associated *Bradyrhizobium* inoculation to promote ecological resource-use efficiency. Front. Microbiol. (2018). https://doi.org/10.3389/fmicb.2018.02105
4. O. Ajayi, F. Oyetayo, Potentials of *Kerstingiella geocarpa* as a health food. J. Med. Food **12**, 184–187 (2009)
5. F.D. Dakora, Nodule function in symbiotic Bambara groundnut (*Vigna subterranea* L.) and Kersting's bean (*Macrotyloma geocarpum* L.) is tolerant of nitrate in the root medium. Ann. Bot. **82**, 687–690 (1998)
6. P. Assogba, E.B. Ewedje, A. Dansi, Y. Loko, A. Adjatin, M. Dansi, A. Sanni, Indigenous knowledge and agro-morphological evaluation of the minor crop Kersting's groundnut (*Macrotyloma geocarpum* (Harms) Maréchal et Baudet) cultivars of Benin. Genet. Resour. Crop Evol. **63**, 513–529 (2016)
7. M.A.T. Ayenan, V.A. Ezin, Potential of Kersting's groundnut [*Macrotyloma geocarpum* (Harms) Maréchal & Baudet] and prospects for its promotion. Agric. Food Secur. **5**, 10 (2016)
8. A.H. Bampuori, Effect of traditional farming practices on the yield of indigenous Kersting's Groundnut (*Macrotyloma geocarpum* Harms) crop in the Upper West Region of Ghana. J. Dev. Sustain. Agric. **2**, 128–144 (2007)
9. T. Bayorbor, I. Dzomeku, V. Avornyo, M. Opoku-Agyeman, Morphological variation in Kersting's groundnut (*Kerstigiella geocarpa* Harms) landraces from northern Ghana. Agric. Biol. JN Am. **1**, 290–295 (2010)
10. M.A. Chauvin, M. Whiting, C.F. Ross, The influence of harvest time on sensory properties and consumer acceptance of sweet cherries. HortTechnology **19**, 748–754 (2009)
11. G.R. Takeoka, L.T. Dao, G.H. Full, R.Y. Wong, L.A. Harden, R.H. Edwards, J.D.J. Berrios, Characterization of black bean (*Phaseolus vulgaris* L.) anthocyanins. J. Agric. Food Chem. **45**, 3395–3400 (1997)
12. M.-G. Choung, B.-R. Choi, Y.-N. An, Y.-H. Chu, Y.-S. Cho, Anthocyanin profile of Korean cultivated kidney bean (*Phaseolus vulgaris* L.). J. Agric. Food Chem. **51**, 7040–7043 (2003)
13. A. Castaneda-Ovando, M. de Lourdes Pacheco-Hernández, M.E. Páez-Hernández, J.A. Rodríguez, C.A. Galán-Vidal, Chemical studies of anthocyanins: a review. Food Chem. **113**, 859–871 (2009)
14. P. Hoffmann, Natural edible dye preparation from bean husks giving red shades. Google Patents (1983)
15. L. Chalker-Scott, Environmental significance of anthocyanins in plant stress responses. Photochem. Photobiol. **70**, 1–9 (1999)
16. H. Matsumoto, Y. Nakamura, S. Tachibanaki, S. Kawamura, M. Hirayama, Stimulatory effect of cyanidin 3-glycosides on the regeneration of rhodopsin. J. Agric. Food Chem. **51**, 3560–3563 (2003)
17. W.-H. Shin, S.-J. Park, E.-J. Kim, Protective effect of anthocyanins in middle cerebral artery occlusion and reperfusion model of cerebral ischemia in rats. Life Sci. **79**, 130–137 (2006)
18. T.C. Wallace, Anthocyanins in cardiovascular disease. Adv. Nutr. **2**, 1–7 (2011)
19. J.A. Joseph, B. Shukitt-Hale, N.A. Denisova, D. Bielinski, A. Martin, J.J. McEwen, P.C. Bickford, Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. J. Neurosci. **19**, 8114–8121 (1999)
20. S. Zafra-Stone, T. Yasmin, M. Bagchi, A. Chatterjee, J.A. Vinson, D. Bagchi, Berry anthocyanins as novel antioxidants in human health and disease prevention. Mol. Nutr. Food Res. **51**, 675–683 (2007)
21. K. Koh, J.E. Youn, H.-S. Kim, Identification of anthocyanins in black soybean (*Glycine max* (L.) Merr.) varieties. J. Food Sci. Technol. **51**, 377–381 (2014)
22. Y. Salinas Moreno, G.S. Sánchez, D.R. Hernández, N.R. Lobato, Characterization of anthocyanin extracts from maize kernels. J. Chromatogr. Sci. **43**, 483–487 (2005)
23. M.-K. Kim, H.-A. Kim, K. Koh, H.-S. Kim, Y.S. Lee, Y.H. Kim, Identification and quantification of anthocyanin pigments in colored rice. Nutr. Res. Pract. **2**, 46–49 (2008)
24. D.-O. Kim, O.K. Chun, Y.J. Kim, H.-Y. Moon, C.Y. Lee, Quantification of polyphenolics and their antioxidant capacity in fresh plums. J. Agric. Food Chem. **51**, 6509–6515 (2003)
25. P. Mena, E.M. Sánchez-Salcedo, M. Tassotti, J.J. Martínez, F. Hernández, D. Del Rio, Phytochemical evaluation of eight white (*Morus alba* L.) and black (*Morus nigra* L.) mulberry clones grown in Spain based on UHPLC-ESI-MSn metabolomic profiles. Food Res. Int. **89**, 1116–1122 (2016)
26. M.A. Farag, H.A. Gad, A.G. Heiss, L.A. Wessjohann, Metabolomics driven analysis of six *Nigella* species seeds via UPLC-qTOF-MS and GC–MS coupled to chemometrics. Food Chem. **151**, 333–342 (2014)
27. A.T. Tsamo, P.P. Ndibewu, F.D. Dakora, Phytochemical profile of seeds from 21 Bambara groundnut landraces via UPLC-qTOF-MS. Food Res. Int. **112**, 160–168 (2018)
28. A.T. Tsamo, H. Mohammed, M. Mohammed, P.P. Ndibewu, F.D. Dakora, Seed coat metabolite profiling of cowpea (*Vigna unguiculata* L.) accessions from Ghana using UPLC-PDA-QTOF-MS and chemometrics. Nat. Prod. Res. (2018). https://doi.org/10.1080/14786419.2018.1548463
29. E.-S.M. Abdel-Aal, J.C. Young, I. Rabalski, Anthocyanin composition in black, blue, pink, purple, and red cereal grains. J. Agric. Food Chem. **54**, 4696–4704 (2006)
30. R. Attree, B. Du, B. Xu, Distribution of phenolic compounds in seed coat and cotyledon, and their contribution to antioxidant capacities of red and black seed coat peanuts (*Arachis hypogaea* L.). Ind. Crops Prod. **67**, 448–456 (2015)
31. T.N.H. Lai, M.-F. Herent, J. Quetin-Leclercq, T.B.T. Nguyen, H. Rogez, Y. Larondelle, C.M. André, Piceatannol, a potent bioactive stilbene, as major phenolic component in *Rhodomyrtus tomentosa*. Food Chem. **138**, 1421–1430 (2013)
32. F. Shahidi, M. Naczk, *Phenolics in Food and Nutraceuticals* (CRC Press, Boca Raton, 2003)
33. B. Singh, J.P. Singh, A. Kaur, N. Singh, Phenolic composition and antioxidant potential of grain legume seeds: a review. Food Res. Int. **101**, 1–16 (2017)
34. A. López, T. El-Naggar, M. Dueñas, T. Ortega, I. Estrella, T. Hernández, M.P. Gómez-Serranillos, O.M. Palomino, M.E. Carretero, Effect of cooking and germination on phenolic composition and biological properties of dark beans (*Phaseolus vulgaris* L.). Food Chem. **138**, 547–555 (2013)
35. M. Dueñas, C. Martínez-Villaluenga, R.I. Limón, E. Peñas, J. Frias, Effect of germination and elicitation on phenolic composition and bioactivity of kidney beans. Food Res. Int. **70**, 55–63 (2015)
36. K.-H. Han, T. Kitano-Okada, J.-M. Seo, S.-J. Kim, K. Sasaki, K.-I. Shimada, M. Fukushima, Characterisation of anthocyanins

and proanthocyanidins of adzuki bean extracts and their antioxidant activity. J Funct. Foods. **14**, 692–701 (2015)
37. X. Wu, R.L. Prior, Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United States: fruits and berries. J. Agric. Food Chem. **53**, 2589–2599 (2005)
38. L.O. Ojwang, L. Dykes, J.M. Awika, Ultra performance liquid chromatography–tandem quadrupole mass spectrometry profiling of anthocyanins and flavonols in cowpea (*Vigna unguiculata*) of varying genotypes. J. Agric. Food Chem. **60**, 3735–3744 (2012)
39. E. Pale, M. Nacro, M. Vanhaelen, R. Vanhaelen-Fastré, Anthocyanins from bambara groundnut (*Vigna subterranea*). J. Agric. Food Chem. **45**, 3359–3361 (1997)
40. M.-G. Choung, I.-Y. Baek, S.-T. Kang, W.-Y. Han, D.-C. Shin, H.-P. Moon, K.-H. Kang, Isolation and determination of anthocyanins in seed coats of black soybean (*Glycine max* (L.) Merr.). J. Agric. Food Chem. **49**, 5848–5851 (2001)
41. A.M. Díaz, G.V. Caldas, M.W. Blair, Concentrations of condensed tannins and anthocyanins in common bean seed coats. Food Res. Int. **43**, 595–601 (2010)