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Antinutrients and metabolomic compounds of Bambara groundnut (*Vigna subterranean*) as affected by traditional processing by smallholder farmers

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Abstract: Bambara groundnut (BG) (*Vigna subterranean*) is an underutilized, indigenous crop in South Africa that has nutritional and associated health benefits. Decreasing the antinutrients in food sources can potentially increase the digestibility of proteins and mineral absorption. To determine the effect of traditional processing (cooking) on the antinutrient content and metabolome of this crop, BG was sampled from 12 rural farms in three districts of the Mpumalanga province, South Africa. The four main colors that were identified (cream, orange, brown, and purple) were pooled together according to the district they were obtained from. One-half of each color sample obtained from each of the three districts was dehulled, color sorted, milled, and subjected to subsequent antinutrient and metabolome analyses, while the other half was cooked, air-dried, and milled prior to analyses. Samples were analyzed for phytate and tannins (antinutrients) by hydrochloric acid extraction methods as well as metabolome constituents by ultraperformance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC–QTOF-MS). Phytate, tannins, as well as other metabolomic constituents, namely, catechin, epicatechin, procyanidin, as well as citric acid, were identified in all raw and cooked BG samples. The cooking process resulted in a significant decrease ($p < 0.05$) in the phytate and tannin content as well as an increase in the health-associated phenolic compounds.

KEYWORDS
antinutrient, Bambara groundnut, cooking process, phenolic compounds, phytates, *Vigna subterranean*

1 | INTRODUCTION

Bambara groundnut (BG) (*Vigna subterranean*) is an underutilized indigenous crop that is gaining popularity in South Africa. Its continued maintenance by local populations is due to its drought resistance and reasonable yield (Adzawla et al., 2016). The promotion of adapted and resistant crops could be a way to alleviate the impacts of

climate change and successive droughts that have been and are currently being experienced in South Africa (Abbas et al., 2019; Odeny, 2007). Even though it is gaining popularity among rural growers and smallholder farmers, not many consumers are familiar with its production and preparation, subsequently posing a challenge to its uptake (Chibarabada et al., 2017).

BG has been described as a complete food due to its high carbohydrate (65%) and relatively high protein content (18%) (Mazahib et al., 2013). In addition to macro- and micronutrients, the seed also contains secondary plant metabolites including phenolic compounds that have a wide range of beneficial health properties. For example, catechin, epicatechin, and procyanidin have diverse biological properties, of which the major biological properties they possess are anti-inflammatory, anticancer, cardioprotective, and neuroprotective activity. The first biological property of epicatechin to be reported was its antidiabetic activity in the early 20th century (Fechtner et al., 2017; Ide et al., 2018; Le Bourvellec et al., 2019; Ou & Gu, 2014; Sari, 2019; Zhang et al., 2019). Citric acid is a weak organic acid that is commonly found in citrus fruit. Similar to phenolic compounds, it also has antioxidant activity that has associated health benefits (Foley et al., 2020). A recent study analyzed the metabolome of BG seeds in South Africa, Swaziland, and Mozambique using ultrahigh performance liquid chromatography–quadrupole time-of-flight mass spectrometry (UPLC–QTOF-MS) and found that the seeds are a source of natural antioxidants for human consumption (Tsamo et al., 2018).

In addition to beneficial nutrients, BG also contains antinutrients. These are substances that reduce the nutritional value of foods by binding to and lowering the mineral bioavailability of essential nutrients. Examples of antinutrients found in BG include phytic acid phosphate and condensed tannins (Unigwe et al., 2018). These antinutrients limit the uptake of minerals and proteins, respectively, resulting in poor nutritional quality and limiting the utilization of BG (Oyeyinka et al., 2019).

Processing is an important factor that can impact the content and availability of macro- and micronutrients, phytonutrients, and antinutrients of BG (Nyau et al., 2017; Unigwe et al., 2018). Legumes are traditionally processed in different ways according to recipes and culinary preferences of the various regions (Oyeyinka et al., 2017). Among the rural community in the Mpumalanga province in South Africa, the hulls of dried Bambara pods are removed after which the seeds are usually cooked for 90 min prior to consumption (Gqaleni, 2014; Nyau et al., 2017). A consumer's food choices and processing methods may be affected by the knowledge of the effects of these methods on antioxidant activity and antinutrients (Gqaleni, 2014). The aim of this study was to determine the effect of traditional processing used by rural households in the Mpumalanga province of South Africa on the antinutrient and metabolomic content of BG.

TABLE 1 List of areas in the Ehlanzeni District Municipality, Mpumalanga, South Africa, from where Bambara groundnut seed samples were collected.

Region	Farm name	Sample code
Langeloop	Phezukomkhono	S1
Gomoro, Boschfontein	Sabo	S2
	Hlowe	S3
	Mahwya	S4
	Salaphi Masilela	S5
	Makamo	S6
	Moyane	S7
	Masilela	S8
	Mknan	S9
Mbombela	Tingwenyana	S10
	Ka-khankela	S11
	Phalatrust Thushanang	S12

2 | MATERIALS AND METHODS

2.1 | Sampling and sample preparation

A total of 12 freshly harvested BG samples weighing approximately 500 g each were collected from 12 farms (Table 1) in three areas in the Ehlanzeni District Municipality of the Mpumalanga province, South Africa. The farms were identified with the help of officials and extension officers from the Mpumalanga Department of Agriculture, Rural Development, Land and Environmental Affairs. Farmers were given rice in exchange for groundnut samples. BG pods were harvested directly from the soil, placed into labeled brown paper sampling bags, transported in cooler boxes to the laboratory, and stored at 4°C until analyses within 8 days.

The BG from each area was dehulled and sorted into their respective colors to a minimum weight of 500 g by using a visual technique to describe seed color at the Agricultural Research Council-Vegetable and Ornamental Plant Institute (ARC-VOPI) at Roodeplaat, South Africa (Mohammed et al., 2016). The main colors found were cream, orange, brown, and purple. The seeds with the same respective colors from the 12 farms were pooled together according to the area they were obtained from. The respectively colored seeds were then divided into two portions (~250 g each).

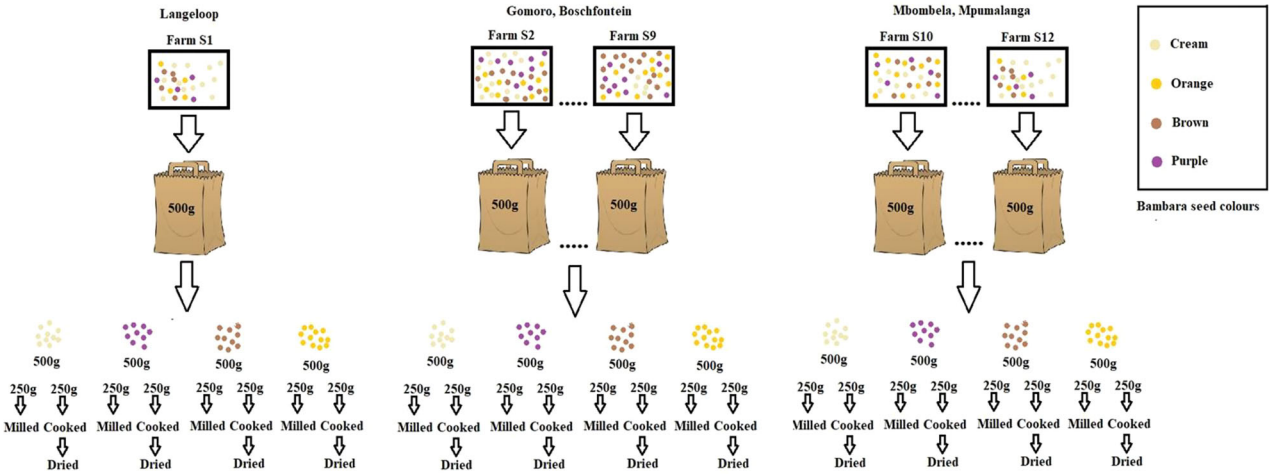

FIGURE 1 Sampling and pooling of Bambara groundnut samples obtained from 12 farms located in three districts of Mpumalanga, South Africa.

Figure 1 shows the sampling and pooling of the BG samples. One portion was directly milled using a mechanical grinder (Peanut almond grinder LGJMS 240; Longer, Zhengzhou, China) prior to subsequent chemical analyses, while the other half was cooked at 100°C for 90 min in a seed-to-water ratio of 1:10 (w/v). This is the established cooking time among rural people in the Mpumalanga area. After cooking, the water was drained using a strainer. This was followed by air drying for 2 days, milling using a mechanical grinder (Peanut almond grinder LGJMS 240; Longer), and subsequent chemical analyses.

2.2 | Identification of metabolites (catechin, epicatechin, procyanidin, and citric acid) using UPLC–QTOF-MS

The samples were analyzed by the Central Analytical Facility (CAF) at Stellenbosch University, South Africa, using their optimized standardized operating protocol (Stander et al., 2019). A total of 2 g of each dried sample was extracted with 15 mL of extraction solvent (15 mL 50% MeOH in 0.1% formic acid), with ultrasonication and shaking overnight to break the cell walls. Briefly, 2 mL of the extracted sample was then withdrawn and centrifuged (Centrifuge 5910R, Thomas Scientific, Swedesboro, NJ, USA) at 14,000 rpm before being transferred into a glass vial for analysis.

A Waters Synapt G2 Quadrupole time-of-flight (QTOF) mass spectrometer (MS) connected to a Waters Acquity ultraperformance liquid chromatograph (UPLC) (Waters, Milford, MA, USA) was used for high-resolution UPLC–MS analysis. Electrospray ionization was used in negative mode with a cone voltage of 15 V, desolvation temperature of 275°C, desolvation gas at 650 L/h, and the rest of the MS settings optimized for best resolution and sensitivity. Data were acquired by scanning from *m/z* 150 to 1500 *m/z* in resolution mode as well as in MSE (mass spectrometry where E represents collision energy) mode. In MSE mode, two channels of MS data were acquired, one at a low collision energy (4 V) and the second using a collision energy ramp (40–100 V) to obtain fragmentation data as well. The instrument was calibrated with sodium formate, and leucine enkephalin ($C_{28}H_{37}N_5O_7$) (100 mg/L) was used as reference mass. It was infused into the mass spectrometer at a rate of 5 μL/min and sampled every 30 s for accurate mass determination. Separation was achieved on a Waters HSS T3, 2.1 × 100 mm, 1.7 μm column. An injection volume of 2 μL was used, and the mobile phase consisted of 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid as solvent B. The gradient started at 100% solvent A for 1 min and changed to 28% B over 22 min in a linear way. It then went to 40% B over 50 s and a wash step of 1.5 min at 100% B, followed by re-equilibration to initial conditions for 4 min. The flow rate was 0.3 mL/min and the column temperature was maintained at 55°C. Accurate mass elemental composition together with fragmentation and UV spectra were used to identify some of the compounds present. These compounds were semiquantitatively determined against catechin as a pseudo-standard. Preferably, the metabolomics should have been quantified using an external calibration method. However, most compounds occurring in plant samples are commercially not readily available or are very expensive to acquire for analytical purposes. Therefore, well-known and abundantly available standards such as catechin/rutin were used.

MarkerLynx (Waters) was used to conduct an unsupervised analysis of the data to identify RT_*m/z* pairs and then to integrate the peak areas of these features

across all samples. This gave a *.csv file that was uploaded to MetaboAnalyst (www.metaboanalyst.ca) for metabolite identification (Xia et al., 2009).

2.3 | Total Tannins

A modified vanillin–hydrochloric acid (HCl) assay (1% concentrated HCl in methanol extraction) (Price et al., 1978) was used for the quantitative measurement of condensed tannins. For the extraction, 0.5 g of milled flour from each sample was weighed in triplicate into a 50-mL centrifuge tube. This was followed by the addition of 25 mL of 0.1% concentrated HCl in methanol that was shaken vigorously for 5 s. The extraction was performed for 20 min at room temperature by vigorous shaking at a 5-min interval. The tube was then centrifuged at 1200 × *g* for 10 min at room temperature. After centrifugation, 1 mL of extract or catechin standard (1.5 mg/mL = 75 mg in 100 mL of absolute methanol) were respectively transferred to test tubes containing 5 mL of vanillin–HCl reagent (which consisted of an equal proportion of 8% HCl in methanol and 1% vanillin in methanol). Each sample as well as the standard was then vortexed and incubated at room temperature for 20 min. For the color blank determination, 1 mL of extract or catechin was transferred into test tubes containing 5 mL of 4% HCl in methanol. These were then vortexed and incubated. Vanillin hydrochloric acid reagent was used as blank. The absorbance was read at 500 nm in a spectrophotometer (Unico SQ2800E Scanning Spectrophotometer; Unico, Dayton, NJ, USA). The tannin content of the sample in milligrams of catechin equivalent per 100 mg of sample for both the extract determination and the color blank determination was calculated using the following equation:

$$\text{Catechin equivalent (mg) per 100 mg sample} = \frac{(\text{Abs Sample} - \text{Abs Std})}{(\text{Abs Std} - \text{Abs Sample})} \times 1.5 \times 5,$$

where 1.5 is the catechin concentration; 5 is the dilution factor; and Abs Sample = Abs Sample – reagent blank.

2.4 | Phytates

Phytic acid was determined as described by Frühbeck et al. (1995). For the extraction, 20 mL of 2.4% HCl (0.66 N) was added to a 1-g freeze-dried sample to a final volume of 30 mL in tubes with caps. The tubes were left to stand for 2 h at room temperature on an electric shaker at 300 rpm. The creamy mixture was then centrifuged at 14,020 × *g* for 30 min at 15°C. A 5 g/mL sample of the supernatant was collected thereafter and diluted to 1:10–40 (depending on the content of the sample) with distilled water. The pH of the diluted sample was then adjusted to 6 using 0.5 N NaOH after which the weight of each sample was recorded.

A glass barreled Econo-column (0.7 × 15 cm) (Bio-Rad Industries, Hercules, CA, USA) was vertically clamped and washed with 15 mL of 0.7 M NaCl followed by 3 mL of bidistilled water to ensure chloride saturation. Then, 0.5 g of AG 1 × 4 anion-exchange resin (Bio-Rad) was added to the column. The aliquot of the obtained supernatants was diluted with bidistilled water to decrease the total anion concentration. This had to be done for all BG samples as it is known that BG contains 1% or more phytate (Unigwe et al., 2018). To maximize recovery, the pH of the diluted aliquot was adjusted to 6.00 with 1 N NaOH to bring the pH above the isoelectric point of proteins (Ellis & Morris, 1983). After dilution, 5 mL of the sample was slowly poured into the column. The inorganic phosphate was then eluted with 7.5 mL of 0.7 M NaCl and discarded. This was followed by eluting the phytate with 7.5 M NaCl and collecting it in 15-mL plastic centrifuge tubes, while the column was left to dry.

For spectrophotometrical determination, 3 mL of water (0 µL phytate/mL distilled water), sodium phytate standard solutions (5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 µg phytate/distilled water), and purified samples were pipetted into 15-mL conical centrifuge tubes. Thereafter, 1 mL of Wade reagent (0.03% $FeCl_3$ $6H_2O$ + 0.3% sulfosalicylic acid) was added to each of the tubes after which they were vortexed for 5 s and left to stand for 30 min. The absorbance of the supernatant was then measured at 500 nm using water as a blank.

2.5 | Statistical analyses

Three-factor analysis of variance was used to test for differences between the two cooking processes (Raw [R] and Cooked [C]), three areas, and four cultivars (Cream [C], Orange [O], Brown [B], and Purple [P]), as well as all their interaction effects (Freund et al., 2010). The standardized residuals after analysis were acceptably normally distributed (Shapiro & Wilk, 1965), but the treatment variances were not homogeneous (O'Neill & Mathews, 2002); therefore, testing was done at the 1% level. Means were compared with Fisher's protected least significant difference test. Data were analyzed using the statistical program GenStat® (VSN International, 2017).

3 | RESULTS AND DISCUSSION

The most common method of processing BG is domestic cooking and involves boiling the seed legumes until soft using firewood or electricity as heating sources. The nuts

are often soaked overnight to shorten the cooking time, cooked until tender, strained, and consumed after roasting or boiling as a snack food or added to, for example, maize porridge (Mubaiwa et al., 2018; Nyau et al., 2017).

This study investigated the effect of traditional processing on the antinutrient and metabolomic compounds in the nuts as consumed. Therefore, the drained water was not analyzed as it is not consumed in this scenario.

3.1 | Identification of metabolites using UPLC–QTOF-MS

The following compounds, namely, catechin, epicatechin, procyanidin, as well as citric acid, were detected in both the raw and the cooked BG. Of these compounds, catechin, epicatechin, and procyanidin are phenolic compounds that belong to the flavonoids (Rothwell et al., 2013). Procyanidin is further classified as a proanthocyanidin (condensed) tannin, a class of flavonoids, and is a polymer consisting of catechins and epicatechins monomers (Ou & Gu, 2014; Rothwell et al., 2013).

For catechin, the values for the BG seeds on average ranged between 7.0 and 204.2 mg/kg and between 9.1 and 205.6 mg/kg for raw and cooked, respectively. There was a significant difference ($p \leq 0.01$) in catechin content between the raw and cooked BG seeds in the overall average among the various BG colors (Table 2). The raw orange-colored BG (204.2 ± 15.4 mg/kg) was found to have the highest values on average, followed by the brown-colored (124.2 ± 1.5 mg/kg), purple-colored (123.9 ± 10.7 mg/kg), and cream-colored BG (7.0 ± 2.5 mg/kg). The cooked orange-colored BG (205.6 ± 11.4 mg/kg) was found to have the highest values on average, followed by the purple-colored (132.7 ± 6.6 mg/kg), brown-colored (119.6 ± 9.3 mg/kg), and cream-colored BG (9.1 ± 1.9 mg/kg).

The average epicatechin content for each color of the BG seeds ranged between 2.5 and 24.3 mg/kg and between 3.7 and 23.8 mg/kg for raw and cooked, respectively. There was a significant difference ($p < 0.01$) in epicatechin content among the various BG colors (Table 2). The highest values on average were found in the raw orange-colored BG (24.3 ± 2.9 mg/kg), followed by the brown-colored (14.9 ± 4.0 mg/kg), purple-colored (11.9 ± 1.5 mg/kg), and cream-colored BG (2.5 ± 2.5 mg/kg). The cooked orange-colored BG (23.8 ± 2.9 mg/kg) was found to have the highest content on average followed by the purple-colored (14.3 ± 1.5 mg/kg), brown-colored (13.7 ± 1.8 mg/kg), and cream-colored BG (3.7 ± 1.1 mg/kg).

The cooking process did not have a significant effect on the catechin or the epicatechin values, but the cooked version of each sample had a higher average value than its

TABLE 2 Quantification of metabolite content (mg/kg) utilizing UPLC–QTOF-MS in raw and cooked Bambara groundnut seeds from three different regions in Mpumalanga, South Africa.

	Raw				Cooked				Color	Cooking	Color × processing
Metabolites	Cream	Orange	Brown	Purple	Cream	Orange	Brown	Purple	*p*-value	*p*-value	*p*-value
Catechin	$7.0^{a} \pm 2.5$	$204.2^{c} \pm 15.4$	$124.2^{b} \pm 1.5$	$123.9^{b} \pm 10.7$	$9.1^{a} \pm 1.9$	$205.6^{c} \pm 11.4$	$119.6^{b} \pm 9.3$	$132.7^{b} \pm 6.6$	<0.001	0.519	0.486
Epicatechin	$2.5^{a} \pm 2.5$	$24.3^{c} \pm 2.9$	$14.9^{b} \pm 4.0$	$11.9^{b} \pm 1.5$	$3.7^{a} \pm 1.1$	$23.8^{c} \pm 2.9$	$13.7^{b} \pm 1.8$	$14.3^{b} \pm 1.5$	<0.001	0.503	0.276
Procyanidin	$5.1^{a} \pm 1.0$	$221.3^{d} \pm 15.1$	$117.0^{b} \pm 25.2$	$131.5^{b} \pm 18.0$	$4.0^{a} \pm 0.8$	$198.6^{d} \pm 22.9$	$125.5^{b} \pm 11.8$	$157.7^{c} \pm 11.2$	<0.001	0.552	0.005
Citric acid	$2726^{b} \pm 130.6$	$3054^{c} \pm 129.8$	$2190^{a} \pm 133.7$	$2616^{b} \pm 256.2$	$2862^{bc} \pm 120.7$	$3405^{d} \pm 372.1$	$2041^{a} \pm 55.0$	$3044^{c} \pm 120.4$	<0.001	0.001	0.003

Different superscripts in a row indicates significant differences at 0.01 level of probability.
Abbreviation: UPLC–QTOF-MS, ultraperformance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry.

raw counterpart. This contrasts with Nyau et al. (2017) who found the raw counterpart to have a significantly higher value for these two respective compounds. It should, however, be noted that there was a substantial difference in cooking time (7 h 10 min) between Nyau et al. (2017) and the current study (90 min). A reason for the increase in catechin and epicatechin during the cooking process could be that bound phenolics are released during the heating (cooking) process (Cheynier, 2005).

The average procyanidin content of each color of the raw and cooked seeds ranged between 5.1 and 221.3 mg/kg and between 4.0 and 198.6 mg/kg, respectively. There was a significant difference ($p < 0.01$) in the procyanidin content among the various colors as well as the interaction between the cooking process and color (Table 2). The raw orange-colored BG (221.3 mg/kg) was found to have the highest values on average followed by the purple-colored (131.5 mg/kg), brown-colored (117.0 mg/kg), and cream-colored BG (5.1 mg/kg). The cooked orange-colored BG (198.6 mg/kg) was found to have the highest values on average followed by the purple-colored (157.7 mg/kg), brown-colored (125.5 mg/kg), and cream-colored BG (4.0 mg/kg). The cooking process has a significant effect ($p < 0.01$) on the procyanidin content of the BG. Procyanidin in orange- and cream-colored BG decreased by 10.3% and 21.6%, respectively. However, procyanidin in the brown- and purple-colored BG increased by 7% and 20%, respectively. The decrease in procyanidin content during the cooking process could be explained by the fact that these polymers disintegrated into their monomer building blocks (catechin and epicatechin) as the latter phenomenon has been demonstrated before (Shadkami et al., 2009). This hypothesis could also be an explanation for the increase in catechin and epicatechin content after cooking, although this was not significant.

It is observed that orange BG has the highest catechin, epicatechin, and procyanidin content and cream BG has the lowest content in both raw and cooked samples. Catechin/epicatechin can be easily oxidized and undergo a nonenzymatic browning reaction (Yuann et al., 2021). It is also reported that the color of purple rice darkens due to oxidization (Hayashi et al., 2018). As the BG samples were dehulled and immediately sorted into the respective colors, it can be concluded that oxidization in this instance does not have a pronounced effect on the color of the seeds.

The detection of citric acid in the BG seed is interesting as it has not, according to the authors' knowledge, been reported previously in BG seed. It has, however, been found in other types of legumes including mung bean (70.8 mg/100 g), reddish brown kidney bean (205.2 mg/100 g) (Towo & Kamala, 2003), and choco perla (*Lupinus mutabilis* Sweet) (1.24 g/100 g fresh weight) (Pérez-Balladares et al., 2019). The citric acid content for all colors of the raw and cooked seed on average ranged between 2190 and 3054 mg/kg and between 2041 and 3405 mg/kg, respectively. There was a significant difference ($p < 0.01$) in the citric acid content among the various colors on average as well as the interaction between the cooking process and color (Table 2). The raw orange-colored BG (3054 mg/kg) was found to have the highest values on average followed by the cream-colored (2726 mg/kg), purple-colored (2616 mg/kg), and brown-colored BG (2190 mg/kg). The cooked orange-colored BG (3405 mg/kg) was found to have the highest values on average followed by the purple-colored (3044 mg/kg), cream-colored (2862 mg/kg), and brown-colored BG (2041 mg/kg). The cooking process significantly increased the citric acid content for the orange-, cream-, and purple-colored BG by 11.5%, 5%, and 16.3%, respectively. The cooking reduced the citric acid content in the brown-colored BG; however, the reduction was not significant. The effect of cooking on citric acid content has had varying effects as both increases (Gonçalves et al., 2010) and losses (Li et al., 2016) have been documented in the literature. It is hypothesized that the observed increases in citric acid in BG are due to possible heat-induced reactions between nitrogen-free carboxylic acids and sugars as Gonçalves et al. (2010) mentioned.

3.2 | Antinutrients (phytates and tannins)

Plants store excess phytate as phosphorus. It is found in high concentrations in pulses, seeds, and grains. Phytate cannot be metabolized by humans upon consumption as it has double-charged phosphate groups that have a strong mineral-binding capacity that binds to cations and hinders their absorption and digestion (Gupta et al., 2015). The phytate content of all colors of the BG seeds ranged on average between 1.2 and 2.3 mg/g for raw seeds and between 0.6 and 1.6 mg/g for cooked seeds (Table 3). In the raw seeds, the purple-colored BG (2.3 mg/g) was found to have the highest phytate content on average, followed by the brown-colored (2.2 mg/g), orange-colored (1.8 mg/g), and cream-colored BG (1.2 mg/g). In the cooked seeds, the brown-colored BG (1.6 mg/g) was found to have the highest phytate content on average, followed by the purple-colored (1.3 mg/g), orange-colored (0.9 mg/g), and cream-colored BG (0.6 mg/g). The variability for phytic acid content observed for differently colored raw BG seed corresponds to Unigwe et al. (2018). The cooking process significantly ($p < 0.01$) reduced the phytate content of each differently colored raw BG sample and its cooked counterpart. A significant reduction in phytate content upon cooking has also been demonstrated by other studies despite

TABLE 3 Quantification of antinutrients (phytate and tannin) (g/kg) in different colored (cream, orange, brown, and purple) raw and cooked Bambara groundnut seeds from three different regions in Mpumalanga, South Africa.

	Raw				Cooked				Color	Processing	Color × processing
	Cream	Orange	Brown	Purple	Cream	Orange	Brown	Purple	*p*-value	*p*-value	*p*-value
Phytates	$1.2^{c} \pm 0.1$	$1.8^{e} \pm 0.2$	$2.2^{f} \pm 0.2$	$2.3^{f} \pm 0.2$	$0.6^{a} \pm 0.1$	$0.9^{b} \pm 0.2$	$1.6^{d} \pm 0.2$	$1.3^{c} \pm 0.3$	<0.001	<0.001	0.005
Tannins	$2.2^{b} \pm 0.07$	$2.3^{b} \pm 0.6$	$4.1^{d} \pm 0.4$	$4.0^{d} \pm 0.1$	$1.6^{a} \pm 0.2$	$1.5^{a} \pm 0.3$	$3.2^{c} \pm 0.2$	$3.1^{c} \pm 0.1$	<0.001	<0.001	0.263

Note: Different superscripts in a row indicate significant differences at 0.01 level of probability.

variations in cooking practices. In the current study, seeds were cooked for 90 min as opposed to other studies where seeds were soaked and cooked for longer periods of time (Mazahib et al., 2013; Ndidi et al., 2014). The loss in phytates during cooking of Bambara seeds may also be due to leaching of phytate ions into the cooking water that was drained and not analyzed (Mazahib et al., 2013).

Tannins are antinutrients that can form complexes with metal ions such as zinc and iron as well as some macromolecules such as proteins. This hinders the absorption and digestion of these minerals and proteins, which subsequently affects the nutritional value of food (Olawoye & Gbadamosi, 2017). The tannin values of all colors of the raw and cooked seeds on average ranged between 2.2 and 4.1 g/kg and between 1.5 and 3.2 g/kg (Table 3), respectively. The raw brown-colored BG (4.1 g/kg) was found to have the highest tannin values on average, followed by the purple-colored (4.0 g/kg), orange-colored (2.3 mg/kg), and cream-colored BG (2.2 g/kg). The average cooked brown-colored BG (3.2 g/kg) was found to have the highest tannin values, followed by the purple-colored (3.1 g/kg), cream-colored (1.6 g/kg), and orange-colored BG (1.5 g/kg). The variability for tannin content observed for differently colored raw BG seed corresponds to Unigwe et al. (2018). A reduction in the tannin content after cooking was observed. The cooked version of each sample had a significantly lower ($p < 0.01$) tannin content than its raw counterpart. Like phytate, a significant reduction in tannin content upon cooking has also been demonstrated by other studies (Khan et al., 2021; Mazahib et al., 2013; Ndidi et al., 2014). It is, however, important to note that the vanillin–HCl assay method used to analyze tannins in this study has some limitations. It is based on the principle that certain parts of the condensed tannin molecule react with the vanillin–HCl reagent to provide a color that is then read by a spectrophotometer. A limitation of this method is thus that phenolic compounds other than tannins can also react with the vanillin–HCl reagent giving a false-positive result (Herald et al., 2014; Price et al., 1978).

4 | CONCLUSION

This study has shown that there are differences in the quantitative content of antinutrients and metabolomic compounds of differently colored BGs. The processing method currently used by rural smallholder farmers in the Mpumalanga area is beneficial as it causes a significant reduction ($p < 0.01$) in phytate and tannin content among the main colors obtained. The raw and cooked orange-colored BG had the highest content of the metabolomics (catechin, epicatechin, procyanidin, and citric acid). In addition, it had the second lowest phytate and tannin

content. Among various colors of BG analyzed, it is therefore the most beneficial line to further cultivate. This also highlights the importance of future research to explore more colors in terms of health-beneficial components and antinutrients to identify lines with even higher contents of health-beneficial components and a low antinutrient content.

In conclusion, optimization could potentially be done by adjusting additional variables that have been shown to have an influence on nutritional and health-beneficial compounds during processing, including soaking, cooking time, and the use of salt or a combination of processing methods. A reduction in the content of antinutrients is reported after soaking prior to cooking. As soaking can also cause a reduction of mineral content, soaking time needs to be optimized (Mazahib et al., 2013). It is reported that both cooking and roasting reduced the antinutritional properties of BGs. Although cooking needs a longer processing time than roasting, moist heating causes an even greater reduction in antinutrients than dry heat. This is most probably due to leaching (Ndidi et al., 2014). The addition of salt to the water during soaking and cooking can further reduce the tannin content (Abbas & Ahmad et al., 2021). These processing methods need to be optimized for more effectiveness in decreasing the antinutrient content without also decreasing the nutrient content.

As research on the classification of BG species in South Africa is fragmented, the formal classification of BG species can lead to targeted breeding, potentially lowering the antinutrient content and optimizing the nutrient contribution to the diet of consumers.

Nomenclature

BG	Bambara groundnut
HCl	hydrochloric acid
UPLC–QTOF-MS	ultra performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry

AUTHOR CONTRIBUTIONS

Beulah Pretorius: Supervision; writing—review and editing; conceptualization. **Margot Otto**: Methodology; formal analysis; investigation; writing—original draft. **Hettie C. Schönfeldt**: Conceptualization; supervision; project administration; funding acquisition.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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