Title: Comparison of the phenolic contents, antioxidant activity and volatile compounds of different sorghum varieties during tea processing

Running title: Sorghum grain tea processing and quality

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ABSTRACT:

BACKGROUND: Sorghum grain is rich in phenolic compounds and has the potential to be developed into functional beverages such as sorghum grain tea, in which the health benefits and flavour are the important quality attributes to be considered in the tea product development. Therefore, this study investigated the effect of grain tea processing steps on the phenolic contents, antioxidant activity, and aroma profile (volatile compounds) of MR-Buster (red-coloured) and Shawaya Short Black 1 (black-coloured) sorghum and compared with our previously reported Liberty (white-coloured) sorghum.

RESULTS: Tea processing had significant impacts on sorghum polyphenols and volatile compounds, but the effect and level varied among sorghum varieties. The phenolic contents and antioxidant activity in these three sorghum varieties were consistent in both raw grain and grain tea samples and in the order of Shawaya Short Black 1 > MR-Buster > Liberty. However, the volatile profiles (both individual and grouped volatiles) were significantly different between sorghum varieties, and the abundance and diversity of the volatile compounds of the tea samples were in the order of Liberty > MR-Buster > Shawaya Short Black 1.

CONCLUSION: Black-coloured sorghum with high phenolic content and antioxidant activity is more suitable for making sorghum tea considering the health benefit. In terms of the aroma intensity and diversity, white-coloured sorghum could be the ideal material. However, future study is needed to determine the key volatile compounds that positively contribute to the aroma. This work provides important insights into the selection of grain materials for sorghum grain tea production.

Keywords: Sorghum grain tea; grain tea processing; phenolic compounds; volatile compounds.

Abbreviation:

- Raw-L, Raw-M, Raw-S = raw Liberty, raw MR-Buster and raw Shawaya Short Black 1 sorghum respectively
- Soaked-L, Soaked-M, Soaked-S = soaked Liberty, soaked MR-Buster and soaked Shawaya Short Black 1 sorghum respectively
- Steamed-L, Steamed-M, Steamed-S = steamed Liberty, steamed MR-Buster and steamed Shawaya Short Black 1 sorghum respectively
- Roasted-L, Roasted-M, Roasted-S = roasted Liberty, roasted MR-Buster and roasted Shawaya Short Black 1 sorghum respectively

1 Introduction

Sorghum (*Sorghum bicolor* L. Moench) is a cereal crop originating in north-eastern Africa¹, and is the fifth most produced cereal crop in the world². Depending on the variety, it can contain high levels of various phenolic compounds such as phenolic acids, flavonoids and condensed tannins³. The phenolic compounds found in sorghum grain are more abundant and diverse compared to other main cereals such as wheat, barley, maize and rice⁴. Studies have shown that sorghum has high antioxidant activity, cholesterol-lowering ability, anti-inflammatory and anti-carcinogenic properties³. Epidemiological results suggested that regular consumption of sorghum has the potential to reduce the risk of developing certain diseases, such as cancers, cardiovascular diseases and type II diabetes⁵. Therefore, there is an increased interest in using sorghum to develop health-promoting functional foods. Recently, there is a trend of using cereal grains such as Tartary buckwheat and barley to make grain tea beverages due to their pleasant flavour and health-promoting properties^{6,7}.

Processing is the prerequisite for the production of sorghum grain tea. Wu *et al.*⁶ made sorghum grain tea using a red sorghum variety and found that soaking, steaming and roasting significantly affected the phenolic contents, antioxidant activities, and α -glucosidase and α -amylase inhibitory activities of the sorghum grains. We have also conducted a study on a white sorghum variety (Liberty) and investigated the effect of processing on the aroma profile⁸. Aroma is a key indicator of tea quality to be considered in new product development, and a total of 63 volatile compounds including alcohols, alkanes, aldehydes, carboxylic acids, esters, ketones, pyrazines and phenylenediamines were identified⁸. However, this white sorghum had relatively low phenolic contents and antioxidant activities and may not be the ideal raw material for the production of sorghum grain tea. Dykes *et al.*⁹ showed that the phenolic contents and antioxidant activity in sorghum are correlated with the pericarp

thickness and colour, and sorghum with darker and thick pericarp had high levels of phenolic contents and antioxidant activity, thus potentially greater health benefits. Therefore, the present study was to investigate the effect of processing on the phenolic contents, antioxidant activity and aroma profile of a red and a black grained sorghum varieties, and compare them with the previously studied white sorghum variety⁸. This research may promote the development and potential commercialisation of sorghum grain tea products.

2 Materials and Methods

2.1 Sorghum grain samples

Two sorghum genotypes of different pericarp colours were used in this study. Red (MR-Buster) sorghum was supplied by Pacific Seeds (Toowoomba, QLD, Australia) and black (Shawaya Short Black 1) sorghum was sourced from Curtin University (Bentley, WA, Australia).

2.2 Processing of sorghum grain tea

The sorghum grain tea was produced as described previously⁸. Briefly, raw sorghum grains were soaked, steamed, and then roasted to obtain sorghum grain tea. The steaming and roasting processes were done on a commercial oven (Convotherm 4 easy Dial 10.10 multifunction oven, Wolfratshausen, Germany). Raw sorghum was soaked in water (1:4 w/v) for 17 h at room temperature (21 °C, in dark), then washed and drained. The soaked sorghum grain was steamed at 100 °C for 50 min to give the steamed sorghum. Finally, the steamed sorghum grain was roasted at 150 °C for 60 min and cooled to room temperature, which was the sorghum whole grain tea product ready for infusion. The grain tea processing was conducted in triplicate, and samples were collected at each processing point and stored in the dark at -20 °C until analysis.

2.3 Chemicals and materials

Folin & Ciocalteu phenol reagent, gallic acid, aluminium chloride, (+)-catechin hydrate, vanillin, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ($\pm$)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), SPME fibre assembly polydimethylsiloxane/divinylbenzene (df 65 μ m, Fused Silica/SS) (PDMS/DVB), potassium persulphate, n-alkanes (C8–C22), 4-octanol, 500 μ m fine test sieve, and 9.00 cm Whatman filter paper (No. 4) were all purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). Methanol, sodium carbonate anhydrous, sodium nitrate, and sodium hydroxide pellets were from Chem-Supply (Gillman, SA, Australia). Hydrochloric acid 32% was obtained from Science Supply (Mitcham, VIC, Australia). Headspace screwtop clear vials (20 ml) and magnetic PTFE/sil hdsp cap were purchased from Agilent Technologies (Singapore). All chemicals were of analytical reagent or HPLC grade.

2.4 Analysis of phenolic contents and antioxidant activity

2.4.1 Sample preparation and phenolic compounds extraction

All sorghum samples from each processing stage were freeze-dried at -20°C for 6 days, and each dried sorghum sample was ground and sieved (100% through 500 μ m sieve) to obtain a powder. The phenolic compounds were extracted as described by Xiong *et al.*⁸. Briefly, 1g of each dried sorghum powder was extracted twice with 45 ml of 80% methanol solution (v/v) for 1 h at 50 °C. After centrifugation, the supernatants were collected, pooled and concentrated at 50 °C to dryness using a rotary evaporator, and then redissolved in 10 ml methanol to give the phenolic extract solution. The extracts were stored at -20 °C in the dark until use.

2.4.2 Determination of the phenolic contents

The total phenolic contents (TPC) was determined using the Folin-Ciocalteu method described by de la Rosa, Alvarez-Parrilla and Shahidi¹⁰. The absorbance was measured by a UV-Vis spectrophotometer (M501 Single Beam Scanning, Camspec, Leeds, UK) at 760 nm. Gallic acid was used as the standard while methanol was the blank, and the results were expressed as mg gallic acid equivalents (GAE)/g dry basis (db).

2.4.3 Determination of total flavonoid content

The total flavonoid content (TFC) was measured by the aluminium chloride colourimetric method as described by de la Rosa *et al.*¹⁰. The absorbance was measured on the M501 UV-Vis spectrophotometer at 415 nm. (+)-Catechin hydrate was used as the standard and methanol was the blank. Results were expressed as mg catechin equivalents (CAE)/g db.

2.4.4 Determination of condensed tannin content

Condensed tannin content (CTC) was determined by the vanillin-HCl assay using the method of Wu *et al.*¹¹. The absorbance was measured at 500 nm on the M501 UV-Vis spectrophotometer. (+)-Catechin hydrate was used as the standard while methanol was the blank, and the results were expressed as mg catechin equivalents (CAE)/g db.

2.4.5 Determination of DPPH radical scavenging activity

The DPPH radical scavenging activity (DPPH) was conducted following the method of Wu *et al.*¹². The absorbance was measured at 515 nm by the M501 UV-Vis spectrophotometer. Trolox was used

as the standard and methanol was the blank. Results were expressed as mg Trolox equivalents (TE)/g db.

2.4.6 Determination of ABTS radical scavenging activity

The ABTS radical scavenging activity (ABTS) was measured by the method of Wu *et al.*¹². The absorbance was measured at 734 nm by the M501 UV-Vis spectrophotometer. Trolox was used as the standard and methanol as the blank. Results were expressed as mg Trolox equivalents (TE)/g db.

2.5 Analysis of volatile compounds

The volatile compounds were extracted by the headspace solid-phase microextraction (HS-SPME) method, and a 65 μ m PDMS/DVB fibre was used and equipped with an SPME PAL 3 multi-purpose automated sampler (Agilent, Santa Clara, CA, USA).

The sample preparation and HS-SPME procedure were followed by our previous method⁸. Briefly, the fibre was pre-conditioned at 250 °C for 30 min in the GC injection port before analysis. Sorghum sample was ground into powder with liquid nitrogen, and 2 g of the sorghum powder was immediately transferred into a 20-ml headspace vial with an addition of 20 μ L of internal standard 4-octanol (0.01 g/100 ml). The vial was tightly sealed with a magnetic PTFE/sil cap, and then shaken and incubated at 80 °C for 20 min for equilibrium; the SPME fibre was then exposed to the headspace at 80 °C for 35 min for adsorption.

The volatile compounds were analysed by gas chromatography (Agilent Technologies 6850 series II gas chromatograph, USA) coupled with a mass spectrometer (Agilent Technologies 5973 mass

selective detector, USA), using a J & W DB-5ms capillary column (30 m x 250 μ m x 0.25 μ m). The GC-MS analysis conditions were the same as our previous method⁸. Each compound was identified by comparing the mass spectra and linear retention indices (RI), using the NIST reference database (NIST 11.0) and NIST Chemistry Webbook¹³, where available. The linear retention index (RI) of each compound was calculated using a series of n-alkanes (C8–C22). All compounds were semi-quantified as internal standard (I.S.) equivalents according to our previous method⁸.

2.6 Statistical analysis

The sorghum tea processing was repeated three times, and the analysis of phenolic content and antioxidant activity was done in triplicates on each repeated sample (a total of 9 replicates). The analysis of volatile compounds was done once on each the repeated sample (a total of 3 replicates). The significant difference of means between each processing step was determined using one-way ANOVA with Turkey grouping at 95% confidence level (Minitab Express, version 1.2.0, Sydney, Australia). Heatmap was produced using R (R, version 3.5.3, Vienna, Austria). Principle component analysis (PCA) was performed using XLSTAT (XLSTAT, version 2019.1.2, Boston, USA).

3 Results and Discussion

3.1 Phenolic contents

The phenolic contents of the raw grains were significantly ($P \leq 0.5$) different between the sorghum varieties (Table 1). Black raw sorghum Shawaya Short Black 1 (Raw-S) had significantly higher TPC (5.99 ± 0.21 mg GAE/g db), TFC (6.12 ± 0.08 mg CAE/g db) and CTC (57.49 ± 1.99 mg CAE/g db), than red raw sorghum MR Buster (Raw-M): TPC (0.96 ± 0.04 mg GAE/g db), TFC (1.54 ± 0.06 mg CAE/g db) and CTC (9.26 ± 0.43 mg CAE/g db). While, white raw sorghum Liberty (Raw-L) from

our previous work⁸ had the lowest TPC (0.34 ± 0.02 mg GAE/g db), TFC (0.71 ± 0.02 mg CAE/g db) and CTC (3.26 ± 0.18 mg CAE/g db). The same trend was shown in all soaked, steamed and roasted sorghum; the phenolic contents in the black sorghum were much higher than red (Table 1) and white sorghum varieties⁸. This variation in phenolic contents between varieties and the trend are in agreement with Dykes *et al.*⁹. The level and types of phenolic compounds in sorghum grains depend on the plant environmental and growth conditions and genotype, with genetic factors strongly influencing plant colour, grain pericarp colour, pericarp thickness, and presence of pigmented testa^{9,11}. Sorghum with dark and thick pericarp (i.e. Shawaya Short Black 1) had the highest phenolic contents while sorghum with white and thin pericarp (e.g. Liberty) had the lowest⁹ and sorghum with pigmented testa have high a level of condensed tannin¹¹.

Processing had a significant influence on the phenolic content in sorghum, but the effect varied by variety (Table 1). Soaking reduced ($P \leq 0.5$) the TPC, TFC and CTC in both black and red sorghum. This is likely due to soaking damaging the physical structure of the grain leading to leaching of some phenolic compounds⁶. In contrast, soaking had no ($P > 0.5$) effect on previously reported white sorghum⁸; white sorghum had very low phenolic content compared to other varieties and there was no observable significant change by soaking. The steaming process greatly reduced ($P \leq 0.5$) the TPC, TFC and CTC in black sorghum, and this is in agreement with that of Wu *et al.*⁶ and Qin *et al.*¹⁴. However, no significant ($P > 0.5$) change of phenolic contents was observed for red sorghum, and this is consistent with white sorghum⁸. Steaming could have damaged the grain cellular structure and cause leaching of phenolic compounds⁶, and heat could result in degradation of some phenolic compounds such as vanillic and *p*-coumaric acids⁶. A significant increase in TPC, TFC and CTC were observed upon the roasting process in both sorghum varieties. This result is consistent with that

obtained by Wu *et al.*⁶. Roasting might have damaged the cellular structure, released non-extractable polymeric phenolics, and increased the extractability of some phenolic acids¹⁵.

3.2 Antioxidant activity

The antioxidant activities were significantly different between the two sorghum varieties (Table 1). The ABTS and DPPH radical scavenging activity of Raw-S (31.15 ± 1.8 and 31.15 ± 1.81 mg TE/g db respectively) were significantly ($P \leq 0.5$) higher than Raw-M (4.90 ± 0.21 and 3.62 ± 0.19 mg TE/g db respectively), while Raw-L had the lowest antioxidant activity (2.01 ± 0.15 and 1.42 ± 0.07 mg TE/g db respectively)⁸; the same trend was found in soaked, steamed and roasted sorghum. Wu *et al.*¹² also reported that the antioxidant activity of black sorghum varieties is higher than that of red and then white ones.

The effect of processing techniques on the antioxidant activity was similar to that on the phenolic contents. The radical scavenging activity in black sorghum was greatly influenced by the processing steps, whereas no significant impact was found on red sorghum. This might due to the phenolic compounds in red sorghum was relatively low, and the effect of processing on the antioxidant activity might be too small to be observed.

Phenolic compounds are the most abundant antioxidants in plant materials, which play an important role in the plant shelf-protective mechanism against oxidative stress¹⁶. High levels of phenolic content are associated with high levels of antioxidant activity in sorghum⁹. The black sorghum had the highest level of antioxidant activity. This was associated with the highest level of phenolic contents especially

the condensed tannin (Table 1), and condensed tannin is associated with stronger antioxidant capacity than simple phenolic compounds⁹. The results suggest that black sorghum may be more suitable for making sorghum tea due to its high phenolic content and antioxidant activity, but the aroma, a key indicator of tea quality, also need to be considered for making sorghum grain tea.

3.3 Sorghum volatile profile

Sorghum volatile compounds were identified by mass spectra (MS) and retention index (RI) from in-house NIST library 11.0 and NIST webbook¹³; all compounds were then semi-quantified by internal standard using 4-octanol. The presentative GC-MS chromatograms of raw (Raw-M and Raw-S) and roasted (Roasted-M, Roasted-S) sorghum were provided in the supplementary material (Fig. S-1, S-2, S-3 & S-4, respectively). The identified volatile compounds include alcohols, alkanes, aldehydes, carboxylic acids, esters, ketones, pyrazines and phenylenediamine. The unidentified compounds were short chain alkanes or their derivatives but were not distinguishable by the NIST (11.0) Mass Spectra database. The volatile compound data of white sorghum Liberty is adapted from Xiong *et al.*⁸, and reanalysed and compared with red and black sorghum varieties (Table S-1 & S-2); volatile compound groups were shown in Fig. 1 & 3 and individual volatile compounds were shown in Fig. 2.

3.3.1 The volatile profile of raw sorghum

The volatile profile of raw sorghum grains was significantly ($P \leq 0.5$) different among sorghum varieties (Fig. 1, 2, S-1 & S-2). Raw-M had 52 identified volatile compounds, with the total volatile concentration of $40.61 \pm 1.44 \mu\text{g/g db}$, and Raw-S had 51 identified volatile compounds, with the total volatile concentration of $47.94 \pm 7.27 \mu\text{g/g db}$. In comparison, the volatile compounds from

previous reported Raw-L were more diverse and abundant, with a total of 57 identified volatile compounds and a total volatile concentration (TVC) of $81.29 \pm 3.25 \mu\text{g/g db}^8$.

Esters were the dominant volatile compounds in all raw sorghum (Fig. 1). The total ester content in Raw-M and Raw-S were 20.90 ± 1.36 and $37.81 \pm 6.63 \mu\text{g/g db}$ respectively. Nonanoic acid, methyl ester (**32**), hexanoic acid, methyl ester (**3**), benzoic acid, methyl ester (**42**), 9-octadecenoic acid, methyl ester, (E)- (**64**), 9,12-octadecadienoic acid (Z,Z)-, methyl ester (**63**) and pentadecanoic acid, 14-methyl-, methyl ester (**62**) were the most abundant esters in raw sorghum. The concentration of pentadecanoic acid, 14-methyl-, methyl ester (**62**) was remarkably high in both raw sorghum, particularly in Raw-S where the level reached $19.63 \pm 4.96 \mu\text{g/g db}$ and significantly ($P \leq 0.5$) higher than Raw-M (Fig. 2). These esters are generally responsible for the fruity, green, sweet, and floral aroma^{17,18}. Esters usually have high odour threshold values, and they do not make a significant contribution to the overall aroma in some foods but they are important volatile compounds found in fermented foods such as soy source¹⁹. However, none of these ester compounds was reported in previous studies on sorghum volatile compounds²⁰⁻²³.

Alkanes were the second most abundant volatile compounds in the red sorghum but third in black sorghum, with the total alkane content in Raw-M and Raw-S of 8.39 ± 0.15 and $2.36 \pm 0.19 \mu\text{g/g db}$ respectively (Fig. 1 & 2). The alkane content was also high in previous Raw-L ($15.28 \pm 0.61 \mu\text{g/g db}$), especially the tridecane (**46**) compound⁸. Alkanes are responsible for unpleasant waxy and gasoline-like odour^{17,18,24}, but often have low odour intensity and high odour threshold values; and have less sensory significance than alcohols^{22,24}. Dodecane (**28**) and tridecane (**46**) were previously

reported in Chengari, F-114, JS-263, JS-2002, MR-Sorghum, PC-1 and Sandal Bar sorghum varieties²³; while tridecane (**46**) was only found in fragrant (E228) sorghum, and tetradecane (**54**) and pentadecane (**59**) were reported in both fragrant (E228) and non-fragrant (M35-1) sorghum²².

Alcohols were the third most abundant volatiles in red sorghum but second in black sorghum (Fig. 1). The total alcohol contents in Raw-M and Raw-S were 3.87 ± 0.14 and 3.21 ± 0.21 $\mu\text{g/g db}$ respectively. Similarly, Raw-L (13.31 ± 0.61 $\mu\text{g/g db}$) also had a high level of alcohol, but much higher in comparison to Raw-M and Raw-S⁸. Those alcohols are responsible for sweet, floral and fruity odour^{17,18}. Alcohols including 1-octanol (**1**), 1-nonanol (**25**), phenylethyl alcohol (**19**), 1,6-octadien-3-ol, 3,7-dimethyl- (**17**) and 1-hexanol (**1**), were found in all sorghum varieties. In previous studies 1-octanol (**13**), 1-nonanol (**25**) and 1-hexanol (**1**) were reported in seven commercial sorghum²³; and 1-octanol (**13**) was also found in both fragrant (E228) and non-fragrant (M35-1) sorghum²².

Aldehydes, carboxylic acids and ketones were detected in all sorghum and contributed to a small proportion of the total volatile content (Fig. 1). The aldehyde nonanal (**18**) was found in all sorghum. Nonanal (**18**) have been widely found in many sorghums including Serena cultivar²⁰; both fragrant (E228) and non-fragrant (M35-1) sorghum²²; and Chengari, F-114, JS-263, JS-2002, MR-Sorghum, PC-1 and Sandal Bar sorghum varieties²³. These compounds are products of lipid oxidation, and responsible for the off-flavour and deterioration^{25,26}. The carboxylic acids including 3-thiophenecarboxylic acid (**37**) and hexanoic acid (**5**) that reported in previous Raw-L were not found here⁸. Hexanoic acid (**5**) is responsible for fatty, sweat and cheese odour, and have been commonly found in many nuts such as almonds, walnuts and beechnuts^{17,27}. Ketones including 4-octanone (**4**),

2(3H)-furanone, 5-ethyldihydro- (**10**), 2(3H)-furanone, dihydro-5-pentyl- (**25**) and 2-decanone (**27**) were all found in Raw-L⁸, while only (**4**) and (**50**) were found in Raw-M and (**4**), (**50**) and (**27**) were found in Raw-S. Among them, furanones (**10** and **50**) have a pleasant sweet and creamy-like aroma¹⁷. Both furanones have been used as flavouring agents²⁸.

In previous studies, Lwande and Bentley²⁰ reported eight volatile compounds, including 1 alcohol, 1 carboxylic acid, 3 alkenes and 3 aldehydes in Serena cultivar sorghum. Jambunathan *et al.*²¹ identified a total of eight volatile compounds including 1 alcohol, 5 aldehydes and 2 ketones, in mould-susceptible and mould-resistant sorghum varieties. Chughtai *et al.*²³ found a total of 35 volatile compounds from seven different sorghum varieties. Recently, Zanan *et al.*²² detected a total of 29 volatile compounds in a fragrant (E228) and non-fragrant (M35-1) sorghum. However, among all these reported volatile compounds, only 8 volatiles were detected in our study (compound No. **13**, **25**, **1**, **54**, **59**, **28**, **46**, **18**), and the remaining 37 identified volatile compounds (Fig. 2) were detected for the first time in our study.

3.3.2 Effect of processing

Processing had a great impact on the volatile compounds in all sorghum, and the effect on the volatile profile (i.e. both individual and group of volatile compounds) was similar for the three sorghum varieties, although the composition and the concentration of volatile compounds were slightly different (Fig. 1 & 2).

Soaking dramatically increased the TVC in all sorghum varieties, and such an increase was mainly contributed by the ester compounds. Specifically, 9-octadecenoic acid, methyl ester, (E)- (**64**), 9,12-

octadecadienoic acid (Z,Z)-, methyl ester (**63**) and pentadecanoic acid, 14-methyl-, methyl ester (**62**), were the most abundant esters in all soaked sorghum and were greatly influenced. Other volatile compounds only accounted for a small portion of the TVC in sorghum after soaking. The alkane content was significantly increased in Soaked-M, while slightly decreased in Soaked-S. Aldehyde was increased and was mainly due to the increase of nonanal (**18**). However, the alcohol, carboxylic acid and ketone content were greatly reduced after soaking and some volatile compounds, such as 1-nonanol (**25**) in soaked-M and soaked-S, were not detected.

In contrast, the steaming process dramatically decreased the TVC, and such a significant reduction in steamed sorghum was mainly due to the loss of ester content. After the steaming process, many esters were no longer detected, and the most abundant ester compounds (i.e. **64**, **63** and **62**) that previously found in raw and soaked sorghum were not detected in both Steamed-M and Steamed-S. The alkane content was significantly increased where it becomes the dominant volatile compound in steamed sorghum, while other volatile compounds were not significantly influenced.

The roasting process resulted in the formation of pyrazine and phenylenediamine but did not significantly alter other volatile compounds. Pyrazine, 2,5-dimethyl- (**2**) and pyrazine, 2-ethyl-3,5-dimethyl- (**14**) were the dominant pyrazines in all roasted sorghum. Pyrazine, tetramethyl- (**15**) was only found in Roasted-M and in a small quantity. It has been well known that pyrazines are responsible for the unique roasted aroma in many heated or roasted foods²⁹. 1,2-Benzenediamine, 4-methyl- (**7**) was also exclusively found in roasted sorghum, and this compound is likely to be produced via a similar mechanism as pyrazine and possibly contributes to the roasted aroma.

3.3.3 The volatile profile of roasted sorghum

Roasted sorghum is the final processed product ready for infusion, and volatile compounds including 13 alkanes, 9 esters, 3 alcohols, 2 aldehydes, 2 ketones, 1 carboxylic acid, 3 pyrazines and 1 phenylenediamine were found in roasted sorghums (Fig. 2, S-3 & S-4). The volatile profile varied among sorghum varieties (Fig. 1). Roasted-M was high in pyrazine, phenylenediamine and ester, which was associated with roasted, nutty, fruity, sweet and green aroma; while Roasted-S was low in all volatile compounds, indicating that black sorghum tea might have a low total aroma intensity than other two sorghums.

The aroma profile characterised in our sorghum tea is different from that of Tartary buckwheat tea. Qin *et al.*¹⁴ identified 24 pyrazines, 10 ketones, 6 aldehydes and 5 fatty acids and Guo *et al.*⁷ detected 22 pyrazines, 11 alkanes, 6 esters and 4 aldehydes in Tartary buckwheat tea. Pyrazines were the main volatile compounds in Tartary buckwheat tea and contributed to its unique roasted, nutty, sweet and malty aroma. Pyrazines are found in many heat-processed foods. Because of its intense smell and low odour threshold, it contributes significantly and positively to the aroma and flavour in food³⁰. However, only three pyrazines were found in our samples (Fig. 2). Pyrazines are formed via the reaction of amino acids with sugars during heating, mainly at 120-150 °C, and have low vapour pressure and evaporate easily³⁰. Since our samples were roasted at 150 °C for 60 min, reducing the roasting time may increase the pyrazine compounds in sorghum, which is an interesting aspect for processing optimisation and needs further investigation.

In addition, we previously reported Roasted-L had carboxylic acid and rich in alkane, alcohol and aldehyde, suggesting that white sorghum tea might have an overall more waxy, fatty, floral and sweet

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aroma⁸. Roasted-L had the most abundant volatile compounds than Roasted-M and Roasted-S had the least volatile compounds (Fig. 1 (a)). Since the results in the previous section showed that black sorghum had remarkably high phenolic contents and antioxidant activities, it was expected that Roasted-S might have the most abundant volatile compounds. However, the TVC of Roasted-S was very low. A possible explanation for this is that black sorghum has high levels of condensed tannins, especially those with high molecular weight, and these compounds can interact with starches and proteins to form very stable bulk complexes and matrixes and which may trap the volatile compounds and made it difficult to extract³¹. Nevertheless, the quantity of volatile compounds is not necessarily representative to the aroma quality or consumers' acceptance. For example and as mentioned above, some volatile compounds such as esters and alkanes have high odour threshold while pyrazines have low odour threshold^{19,24,30}, which means same amount of different type of volatile compounds may cause different sensation to consumers. In addition, some volatile compounds such as aldehydes may be formed as a result of lipid oxidation, which contributes to unpleasant off-flavour^{25,26}. Further study is needed to find the key volatile compounds contributing to the sorghum aroma quality.

3.4 Principle component analysis and correlation matrix

Principle component analysis (PCA) for detected chemical indexes were performed to better illustrate the difference among sorghum varieties and during sorghum grain tea processing (Fig. 3). The PCA analysis revealed that the phenolic contents, antioxidant activity and volatile profile were different among sorghum varieties, and processing significantly altered the volatile composition.

PC 1 (52.72%) and PC 2 (21.95%) accounted for 74.67% of the total variance. TPC, TFC, CTC, DPPH and ABTS were the main compounds contributing to the positive side of PC 1, indicating

sorghum on the right side of the plot had higher levels of phenolic contents and antioxidant activity. Whereas the volatile compounds were mainly located on the negative side of PC 1; and alcohol, ketone and carboxylic acids are on the positive PC 2 while pyrazine, phenylenediamine and aldehyde are on the negative PC2. Sorghum on the left of the plot had more volatile compounds; rich in alcohol, ketone and carboxylic acid on the top left while high in pyrazine and phenylenediamine on the bottom left.

Sorghum samples were classified into three groups (ellipses) according to their variety. The ellipses showed that white sorghum (Group 1) had more abundant volatile compounds than red (Group 2) and followed by black (Group 3) sorghum, but in reverse order in terms of antioxidative phenolics. Within each group, a distinct trend was observed for all sorghum varieties. There was an increasing correlation with pyrazine, phenylenediamine, aldehyde and alkane volatiles but a decreasing correlation with ester volatiles, during the tea processing from soaking to steaming and to roasting step.

4 Conclusion

The effect of processing on the phenolic contents and antioxidant activity varied between sorghum varieties. Generally, soaking and steaming decreased the phenolic contents and antioxidant activity in sorghum; roasting increased the phenolic contents while not significantly affecting the antioxidant activity. The phenolic contents (TPC, TFC, CTC) and antioxidant activity (DPPH, ABTS) of Shawaya Short Black 1 are all significantly higher than that of MR-Buster. Black sorghum may be more suitable for making sorghum tea in terms of potential health benefits.

The effect of sorghum tea processing on the volatile compounds was similar for all sorghum varieties but the extent varied by the volatile composition. In general, the soaking process dramatically increased the ester content, while steaming significantly reduced the ester content, and the roasting process resulted in the formation of pyrazine and phenylenediamine. The volatile profile varied significantly among sorghum varieties. The volatile compounds in Raw-M (52 identified volatiles) and Raw-S (51 identified volatiles) were less diverse and abundant than previously reported Raw-L (57 identified volatiles). The volatile compounds in white and red sorghum tea was more abundant and diverse than that of black sorghum. Research is underway to investigate the sensory analysis of sorghum grain tea to identify what type of sorghum flavour could be more acceptable by consumers.

Conflict of interest

The authors declare no conflict of interest.

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Tables and figures

Table 1: Effect of processing on TPC, TFC, CTC, and ABTS and DPPH radical scavenging activity of sorghum grains.

Analysis	Variety	Processing			
		Raw	Soaked	Steamed	Roasted
TPC	Mr-Buster	0.96 ± 0.04^{aB}	0.73 ± 0.03^{bB}	0.75 ± 0.04^{bB}	0.77 ± 0.02^{bB}
	Shawaya Short Black 1	5.99 ± 0.21^{aA}	5.67 ± 0.11^{bA}	2.68 ± 0.18^{dA}	3.11 ± 0.10^{cA}
TFC	Mr-Buster	1.54 ± 0.06^{aB}	1.37 ± 0.06^{bB}	1.37 ± 0.03^{bB}	1.48 ± 0.04^{abB}
	Shawaya Short Black 1	6.12 ± 0.08^{aA}	5.67 ± 0.14^{bA}	4.24 ± 0.09^{cA}	4.33 ± 0.12^{cA}
CTC	Mr-Buster	9.26 ± 0.43^{aB}	7.02 ± 0.34^{bB}	7.20 ± 0.40^{bB}	7.45 ± 0.20^{bB}
	Shawaya Short Black 1	57.49 ± 1.99^{aA}	54.38 ± 1.04^{bA}	25.39 ± 1.72^{dA}	29.63 ± 0.96^{cA}
ABTS	Mr-Buster	4.90 ± 0.21^{aB}	3.82 ± 0.21^{bB}	3.74 ± 0.20^{bB}	3.91 ± 0.10^{bB}
	Shawaya Short Black 1	31.15 ± 1.81^{aA}	31.86 ± 1.69^{aA}	15.94 ± 1.06^{bA}	17.31 ± 1.01^{bA}
DPPH	Mr-Buster	3.62 ± 0.19^{aB}	2.77 ± 0.22^{bB}	2.69 ± 0.30^{bB}	2.79 ± 0.14^{bB}
	Shawaya Short Black 1	30.63 ± 1.88^{aA}	28.66 ± 1.86^{aA}	13.00 ± 1.11^{bA}	14.80 ± 0.60^{bA}

TPC = total phenolic content (mg GAE/g db).

TFC = total flavonoid content (mg CAE/g db).

CTC = condensed tannin content (mg CAE/g db).

ABTS and DPPH = ABTS and DPPH radical scavenging activity (mg TE/g db).

a, b, c = values with different superscripts in the same row are significantly different ($P \leq 0.5$).

A, B, C = values with different superscripts in the same column within each analysis are significantly different ($P \leq 0.5$).

Figure captions

Fig. 1. The concentration (a) and proportion (b) of the volatile compound group in Liberty, MR-Buster and Shawaya Short Black 1 sorghum varieties at different processing point. Data of white sorghum Liberty are adapted from Xiong *et al.*⁸.

Fig. 2. Heat map of individual volatile compounds in Liberty, MR-Buster and Shawaya Short Black 1 sorghum varieties at different processing stages. Heat map legends show the relative concentration range of (a) all identified volatiles detected and (b) selected volatiles. TCIPIS: Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester. Data of white sorghum Liberty are adapted from Xiong *et al.*⁸.

Fig. 3. Variable projections of phenolic contents, antioxidant activities and volatile compounds after principal component analysis (PCA) on three sorghum genotypes during tea processing: soaking, steaming and roasting. Data of white sorghum Liberty are adapted from Xiong *et al.*⁸.

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