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Changes in phenolic content, antioxidant activity and volatile compounds during processing of fermented sorghum grain tea

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Abstract:

Background and objective: Sorghum is an important cereal crop with abundant content of polyphenols and may be used to develop nutraceutical sorghum grain tea. This study investigated the changes of phenolic content, antioxidant capacity and volatile compounds in sorghum grain during the production of fermented sorghum grain tea using *Lactobacillus plantarum* subsp. *Argentoratensis* (LAB) and *Saccharomyces cerevisiae*. **Findings**: Significant ($P < 0.05$) changes in total phenolic content (TPC), total flavonoids content (TFC) and condensed tannins content (CTC) were observed during the soaking, fermentation, steaming and roasting process. TPC, TFC and CTC were significantly decreased after soaking, steaming and fermentation stages, whereas roasting led to a significant increase. A total of 53 volatile compounds were recorded from raw and processed sorghum. Pyrazines, phenols and esters were the most abundant volatile compounds. Alcohols, carboxylic acids, ketones and alkanes decreased during processing. Esters increased during soaking, steaming and fermentation but decreased during roasting.

Conclusions: Processing affected the phenolic content significantly ($P < 0.05$) and changed the volatile profiles of sorghum grain. Fermented sorghum grain tea by *Saccharomyces cerevisiae* showed a higher possibility of being developed as sorghum grain tea.

Significance and novelty: The study developed a practical method to process novel sorghum grain tea products.

Keywords: Antioxidant capacity; Fermented sorghum tea; Phenolic compounds; Volatile compounds

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench), the fifth primary cereal crop in the world, has been attracting much attention because of its high content of phytochemicals (Ragaee et al. 2006). Sorghum grains contains high portion of bioactive phytochemicals such as phenolic acids, procyanidins, 3-deoxyanthocyanidins and condensed tannins (Vanamala., 2017; Xiong et al.,

2019a). Epidemiological studies have consistently shown that the consumption of sorghum products is associated with reduced risk of chronic diseases, including type II diabetes, cardiovascular diseases and cancer owing to the phenolic profile (Awika and Rooney, 2004). Recently, a large variety of cereal grain tea emerged in the market due to their unique flavor and function, such as Tartary buckwheat tea, barley tea and wheat bran tea (Guo et al., 2011; Wang et al., 2019). Therefore, sorghum grain may be used to produce a novel nutraceutical tea beverage.

Processing is a prerequisite to produce grain-based food. Different processing methods (soaking, fermentation and thermal processing) may significantly affect physical characteristics and chemical compositions of grains (Wu et al., 2013). For example, microorganisms have the ability to assimilate condensed tannins by producing tannase which catalyzes the biodegradation of large tannin molecules during fermentation (Chávez-González et al., 2011). Additionally, aroma and flavor are important quality indicators of the fermented sorghum tea, which are primarily affected by the composition of volatile substances. Xiong et al. (2019b) identified a total of 63 aromatic compounds, mainly alcohols, aldehydes and esters in processed sorghum grain tea, which were highly influenced by the processing methods.

However, limited information is available on the effects of processing methods on the phytochemicals and volatile compounds in fermented sorghum. The present study used *Lactobacillus plantarum* subsp. *argentoratensis* and *Saccharomyces cerevisiae* to ferment sorghum grain and examined the effect of fermentation together with other processing methods (soaking, steaming and roasting) on the phenolic content, antioxidant and volatile compounds of the fermented sorghum tea. This study may have provided a practical method to produce fermented sorghum tea as a novel value-added grain beverage product.

2. Materials and methods

2.1 Materials and chemicals

Sorghum sample (MR-Buster) was obtained from Pacific Seeds, Toowoomba, QLD, Australia. It is a red color, medium maturity hybrid sorghum cultivar well established in Australia. Methanol, Folin & Ciocalteu's phenol reagent, sodium carbonate, sodium nitrite, aluminum chloride, sodium hydroxide, vanillin, hydrochloric acid, 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), gallic acid, (+)-catechin hydrate, n-alkanes (C8–C22), 4-octanol, de Man, Rogosa and Sharpe (MRS) broth and yeast extract peptone- dextrose (YEPD) broth were all purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). All chemicals were of analytical or HPLC grade.

2.2 Microorganisms and Media

Lactobacillus plantarum subsp. *argentoratensis* (DSM-16365) was obtained from German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) and cultivated in MRS broth at 30 ºC under anaerobic conditions without shaking. *Saccharomyces cerevisiae* was collected from the Microbiology Laboratory of Faculty of Agricultural and Veterinary Sciences, University of Melbourne, Australia and cultured aerobically at 25 ºC in YEPD broth.

2.3 Preparation of starter cultures

Activation of LAB or yeast was conducted by mixing 1.0 g of freeze-dried strain pellet with 0.5 mL of MRS broth. The pellet was allowed to rehydrate for up to 30 min and aseptically transferred to a 10 mL centrifuge tube containing 9 mL MRS broth. Then the liquid culture

was incubated at 30 ºC anaerobically to obtain inoculums with a cell concentration of approximate 10^7 CFU/mL, which was determined by standard curves between optical densities (OD) and CFU/mL. The total counts for both lactic acid bacteria and yeast were performed using a spread plate technic following the methods of Bhatia et al. (1989). The correlation between CFU/ml and optical densities (OD) was also established by measuring the OD of various serially diluted solution using M501 Single Beam Scanning UV-Vis spectrophotometer (Camspec, Leeds, UK) at 600 nm (Emamifar 2011).

2.4 Processing of fermented sorghum tea

The fermented sorghum tea was prepared through four main processing procedures including: soaking, fermenting, steaming and roasting **(Fig. 1)**. About 50 g of materials were collected from each processing step, packed separately in plastic bags and stored at -20ºC for further analyses. The processing was performed in triplicates.

2.4.1 Soaking

Raw red sorghum (350 g) was soaked in reverse osmosis (RO) water (1:4 w/v) for 24 h at room temperature (25 ºC). Then the soaked grains were drained through paper towels and dried to constant weight at room temperature (25 ºC). About 50 g dried grains were stored at -20 ºC for analyses and the rest were subjected to further processing steps (Xiong et al., 2019b).

2.4.2 Fermenting

The remaining soaked sorghum (300 g) was divided into two equal amounts. Each portion (150 g) was weighted into 500 mL Erlenmeyer flask, mixed with 150 mL sterilized water (1:1 w/v), and then autoclaved at 121 ºC for 20 min. After cooling to room temperature (25 ºC),

the two samples were inoculated with 30 mL *Lactobacillus plantarum* subsp. *argentoratensis* and *Saccharomyces cerevisiae* active cultures, respectively. These inoculated samples were incubated anaerobically for 5 days at optimal temperature (*Lactobacillus plantarum* at 30 ºC; *Saccharomyces cerevisiae* at 25 ºC). The fermented grains were drained, dried and stored as described (section 2.4.1). The remaining fermented samples were subjected to steaming.

2.4.3 Steaming

Each type of fermented sample (100g) was steamed at 100 ºC for 55 min (steam mode, fan level 1) using the Convotherm 4 easyDial 10.10 multifunctional oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany). The sample was cooled to room temperature (25 ºC) and drained on paper towel. A proportion of the steamed grain (50 g) was stored at -20 ºC for analyses, and the remaining was subjected to roasting.

2.4.4 Roasting

Roasting was performed at 150 ºC for 60 min in a Convotherm 4 easyDial 10.10 multifunctional oven (Wolfratshausen, Germany) to produce the final fermented sorghum grain tea. After cooled to room temperature (25 ºC), the samples were vacuum packed in high density polyethylene bags and stored in a fridge at -20 °C for further analysis. This product is ready for consumption by infusing into hot water, just like traditional leaf tea.

2.5 Total polyphenol and antioxidant capacity assay

2.5.1 Extraction of phenolic compounds

Phenolic compounds in various treated sorghum grains were extracted following the method of Wu et al. (2013). Briefly, 5 g of sorghum sample was ground into power in liquid nitrogen using a FCML2012 coffee grinder (Home & Co., Mulgrave, Australia), and all ground

144 samples were sieved through a 500 μm sieve. About 1 g of sieved sorghum powder was
145 extracted with 45 mL 80% (v/v) aqueous methanol for 2 h in 50 ºC water bath and then
146 centrifuged at 5,400 rpm at 4 ºC for 10 mins. The supernatant was moved into a new container
147 and the residue was extracted and centrifuged again following the same procedures. The
148 supernatant of two extract were combined and evaporated to dryness at 60 ºC using a Hei-vap
149 rotary evaporator (Heidolph, Schwabach, Germany). Solids were reconstituted in 10 mL
150 methanol and stored at -20 ºC in the dark until analysis. All extraction and following
151 measurements were repeated in triplicates.

152

153 *2.5.2 Determination of total phenolic content (TPC)*

154 The TPC of each extract was determined using a modified Folin–Ciocalteu method (de la
155 Rosa et al., 2011). The absorbance was determined at 760 nm using the M501 Single Beam
156 Scanning UV-Vis spectrophotometer. Gallic acid was used as a standard and the results were
157 expressed as mg gallic acid equivalents (GAE)/g dried sample basis (db).

158

159 *2.5.3 Determination of total flavonoid content (TFC)*

160 The TFC was determined following an established method (de la Rosa et al. 2011). Briefly,
161 the extracts (0.2 mL) were mixed with 0.8 mL of water and 0.06 mL of 5% $NaNO_2$ solution
162 (w/v). After 5 mins of reaction, 0.06 mL 10 $AlCl_3$ solution (w/v) was added to the mixture
163 and incubated for another 3 min. 0.5 mL of 0.5 M NaOH was then added and the mixture was
164 incubated in the dark for 30 min at room temperature. The absorbance was read immediately
165 at 510 nm. Catechin was used as standard and the results were expressed as mg catechin
166 equivalents (CE)/g db.

167

168 *2.5.4 Determination of condensed tannins content (CTC)*

169 The CTC was determined by the vanillin assay (Price, Van Scoyoc and Butler, 1978). The
170 absorbance was measured at 500 nm. Catechin was used as standard and the results were
171 expressed as mg catechin equivalents (CE)/g db.

172

173 *2.5.5 Determination of ABTS radical scavenging activity*

174 ABTS assay was performed following the protocol of Thaipong et al. (2006). The stock
175 solution was prepared by mixing the 7mM $ABTS^{\cdot+}$ and 2.6 mM potassium persulfate in equal
176 amounts and allowed to react in the dark for 12 h at room temperature. The stock solution of 1
177 mL was diluted with 50 mL of methanol to obtain an absorbance of 1.1 ± 0.02 units at 734 nm.
178 Each sample extract (1 mL) was diluted with 10 ml of 80% methanol, and 0.2 mL of the
179 diluted extracts were mixed with 1 mL of fresh $ABTS^{\cdot+}$ working solution. After incubation at
180 room temperature for 2 h in dark, the absorbance was measured at 734 nm. Trolox was used
181 as standard and the results were expressed as mg Trolox equivalents (TE)/g db.

182

183 *2.5.6 Determination of DPPH radical scavenging activity*

184 DPPH assay was performed following the method of Thaipong et al. (2006). Briefly, 24 mg of
185 DPPH was dissolved in 100 ml of 80% methanol to prepare the stocking solution, then stored
186 at -20 ºC in the dark until analysis. The working solution was prepared by diluting 10 mL of
187 the stock solution with 45 mL of 80% methanol to obtain an absorbance of 1.1 ± 0.02 units at
188 515 nm. Then 0.2 mL of sample extract was mixed with 3.8 mL of DPPH working solution
189 and allowed to react at room temperature for 8 h at dark. The final absorbance was determined
190 at 515 nm. Trolox was used as standard and the results were expressed as mg Trolox
191 equivalents (TE)/g db.

192

193 *2.6 Headspace solid phase microextraction and gas chromatography mass spectrometry*
194 *(HS-SPME-GC-MS) for volatile compounds analyses*

195 The extraction of volatile compounds was modified from our previous experimental
196 conditions (Xiong et al. 2019b). Each sorghum sample (5 g) was ground into powder in liquid
197 nitrogen using a FCML2012 coffee grinder (Home & Co., Mulgrave, Australia). Sorghum
198 powder (2 g) were transferred into a 20 mL vial with 20 µL of internal standard (4-octanol,
199 0.01 g/100 mL) and sealed with a magnetic PTFE silicon cap. Vial was equilibrated at room
200 temperature for 20 min. A 65 µm PDMS/DVB SPME fibre (Agilent, Palo Alto, USA) was
201 then exposed to the headspace for 35 min at 80 ºC for adsorption. The fibre was then desorbed
202 in the GC injector at 250 ºC for 30 min in splitless mode.

203

204 The analysis of volatile compounds was performed using a 6850 series II gas chromatograph
205 connected to a 5973 mass selective detector and coupled with a PAL 3 multi-purpose
206 automated sampler (Agilent Technologies, Palo Alto, USA). Volatiles were separated on a
207 J&W DB-5MS capillary column (30 m x 250µm x 0.25µm, Agilent Technologies, Palo Alto,
208 USA). Helium (99.999%) was used as the carrier gas under a constant flow rate at 1 mL/min.
209 The oven temperature was programmed as follows: an initial temperature of 50 ºC for 5 mins,
210 3 ºC/min to 125 ºC and held for 3 mins, 2 ºC/min to 180 ºC with 3 mins holding, 15 ºC/min to
211 230 ºC without holding, 20 ºC/min to a final temperature of 280 ºC with 5 mins holding. The
212 MS interface temperature, ion source and quadrupole temperature were set at 280 ºC, 230 ºC
213 and 150 ºC, respectively. MS was operated in scan mode from *m/z* 35 to 400. Blank and
214 internal standard were analyzed using the same sample program and performed at the
215 beginning and end of each sample analysis.

216

217 Volatile compounds were identified by comparing their mass spectra and linear retention
218 indices (RIs), with those reported in the NIST reference database (NIST 11.0) and NIST
219 Chemistry Webbook (NIST 2018). The RIs were calculated from the retention time of a series

of n-alkanes (C7-C30) (Sigma-Aldrich, Germany). For quantification, 15 external standards diluted with methanol were analyzed as samples to establish standard curves. Volatiles without standards were semi-quantified by comparing the peak areas of target compounds to the peak area of internal standard.

2.7 Statistical analysis

One-way ANOVA was used to investigate the differences in TPC, TFC, CTC, ABTS, DPPH among samples from each process steps using Minitab Express software (Version 1.2.0, Minitab Pty Ltd, Sydney, Australia). Principal component analysis (PCA) was conducted for TPC, TFC, CTC, ABTS, DPPH and volatile compounds using Matlab® (Version 9.3, Mathwork Inc, Natick, MA, USA).

3. Results and discussion

3.1 Effects of processing on the phenolic compounds in sorghum grain tea

The influences of processing treatments on the phenolic compounds and antioxidant capacity of the sorghum grain tea are summarized in **Table 1**. Soaking process significantly decreased TPC, TFC and CTC compared to untreated sorghum, which was likely due to the rupture of sorghum cell wall and accelerated the leaching of conjugated water-soluble polyphenols into soaking water during the process (Xiong et al., 2019b).

Fermentation significantly ($P < 0.05$) decreased TFC and CTC but not TPC when compared with the soaked sorghum (**Table 1**). No significant differences ($P > 0.05$) were observed between the two types of fermentations in sorghum polyphenol content and antioxidant capacity. The significant losses of flavonoids and condensed tannins during fermentation may due to the degradation of phenolic compounds (Adebo et al., 2018). *Lactobacillus plantarum*

245 can decompose condensed tannins into oligomeric tannins and other derivatives through the
246 action of tannase and gallate decarboxylase (Jiménez et al., 2014). In addition, oxidation of
247 diffused phenolic compounds may also lead to reduced TPC. Towo et al., (2006) reported that
248 oxidation induced by polyphenol oxidase (PPO) in red sorghum varieties might also reduce
249 TPC. On the contrary, fermentation may also lead to the release of bound polyphenols from
250 cell walls, and therefore leading to an increase in the detectable phenolic content (Đorđević et
251 al., 2010). Enzymes such as amylases and proteases produced by starter culture could
252 hydrolyze phenolic conjugates with one or more bounded sugar into free polyphenols
253 (Shrestha et al., 2013). Kadiri (2017) reported that *Saccharomyces cerevisiae* could synthesize
254 some enzymes, including β-glucosidase, cellulase and xylanase, which have the capability to
255 hydrolyze the β-glucosidic bonds of certain polyphenols. Therefore, the significant decrease
256 in TFC and CTC but limited change of TPC were the result of the combined effects of
257 enzymatic, physical and biochemical activities during the fermenting process.

258

259 TPC, TFC and CTC of the extracts were significantly ($P < 0.05$) decreased after steaming
260 process. Wu et al., (2013) suggested that steaming could degrade phenolic compounds such as
261 vanillic and *p*-coumaric acids. Similar phenomenon was also observed by Xiong et al.,
262 (2019b), who indicated that the losses of phenolic compounds may due to the breakdown and
263 oxidation of heat-labile phenolic compounds.

264

265 However, different to the steaming effect, roasting process significantly ($P < 0.05$) increased
266 TPC, TFC and CTC, which may be the result of depolymerization of conjugated phenolic
267 compounds (Wu et al., 2013). High temperature could lead to breakdown of high molecular
268 weight polyphenols, such as condensed tannins, and convert into lower molecular weight and
269 more extractable compounds (Xiong et al., 2019b). Additionally, Randhir et al., (2008)
270 demonstrated that roasting treatment led to the release of bound phenolic compounds
271 originally combined to macromolecules such as structural proteins, cellulose and pectin in the

cell wall matrix, which could have contributed to the increase of phenolic compounds as well.

3.2 Effects of processing on the antioxidant capacity in sorghum grain tea

Compared with the processed samples, the raw sorghum grain showed the highest antioxidant capacity (**Table 1**). The soaking process significantly ($P < 0.05$) decreased the DPPH and ABTS levels, which could be attributed to the lower TPC as discussed in section 3.1. However, the antioxidant capacity of sorghum was significantly enhanced after the roasting process. Wu et al., (2013) suggested that the increase may be attributed to the formation of Maillard reaction products such as melanoidins. Rufián-Henares & Morales (2007) reported the antioxidant ability of melanoidins through scavenging oxygen radicals or chelating metals. TPC and CTC were correlated directly to antioxidant capacity, and significant positive correlation were observed between polyphenols and antioxidant capacity (**Table 2)**. TPC in both yeast-fermented and LAB-fermented sorghum tea was positively related to the DPPH and ABTS assays, which reflected the contribution of TPC to the antioxidant capacity in sorghum tea. On the other hand, CTC was also found to weakly but significantly correlated with DPPH and ABTS in both yeast-fermented and LAB-fermented sorghum tea. However, large amounts of condensed tannins may be degraded and modified as previous studies have reported that tannin content in sorghum grain was reduced by 36%-80% after high temperature treatment (Dlamini et al., 2007; Babiker et al., 2008). This may be the result of the formation of insoluble complexes by reacting between phenolic hydroxyl groups of tannin and proteins, minerals (Wu et al., 2016) or heat induced degradation of condensed tannin molecules (Xiong et al., 2019b). Therefore, the total phenolic acids affected the major antioxidant capacity, which explained that TPC has stronger correlation with antioxidant capacity than condensed tannins.

297 *3.3 Volatile compounds in sorghum grain tea*

298 A total of 53 volatile compounds were detected in all sorghum samples. Among these
299 compounds, 51 were identified, including 4 alcohols, 5 alkanes, 4 aldehydes, 3 phenols, 3
300 ketones, 2 carboxylic acids, 4 pyrazines, 2 pyrroles and 25 esters (**Table 3**). However, another
301 2 isolated compounds were unable to be identified. The total volatile content (TVC) did not
302 change after soaking. Only LAB fermentation significantly ($P < 0.05$) increased TVC, but not
303 yeast fermentation. A significant ($P < 0.05$) decrease in the TVC after the steaming process
304 was observed in all samples followed by an increment of TVC in roasted sample of yeast
305 fermented sorghum, but not in that of LAB fermented sorghum. Another important finding is
306 that different fermentation influenced the volatile compounds significantly. *S. cerevisiae*
307 fermented sorghum tea showed a higher content of pyrazine, pyrrole and aldehyde than LAB
308 fermented sorghum tea. Both fermentation lead to a high content of esters (**Table 3**).

309

310 *3.3.1 Effects of processing on volatile compounds in sorghum tea*

311 In raw sorghum, esters and alcohols are the major volatiles followed by alkanes, which was
312 consistent to a previous study (Xiong et al., 2019b). The major alcohol and ester observed
313 were 1-hexanol, 1-nonaol and methyl hexanoate, while hexanal and nonanal were detected in
314 low concentrations, as in consistent to past studies. The two detected ketones of 4-octanone
315 and 2(3H)-furanone, 5-pentyl-, were previously reported in literature (Xiong et al., 2019b).
316 Two carboxylic acids, nonanoic acid and 6-Octadecenoic acid, were firstly detected in
317 sorghum in the current study, which may give a waxy, green and fatty flavor (The Good
318 Scents Company, 2019).

319

320 The soaking process decreased the total alcohol, ketone and carboxylic acid content in
321 sorghum (**Table 3**), likely due to the leaching effect (Lucas, Le Ray and Mariette, 2007).
322 However, the content of aldehyde and ester were increased significantly. Two main factors
323 may have contributed to this increment i) soaking disrupted the physical structure, and

released more volatile compounds; ii) soaking increased the activity of lipoxygenase, which accelerate lipid oxidation and formation of aldehyde (Lucas, Le Ray and Mariette, 2007;).

Both yeast and LAB fermentation resulted in the increment of phenols and esters, and reduction of aldehyde and alkanes (**Table 3**). Alcohols were disappeared in yeast fermented samples but remained consist in LAB samples. The change of phenols during both fermentations has been reported by previous studies, which might be due to decarboxylation of ferulic acid (Zhu and Cui, 2013). The abundant fatty acids content in sorghum and the esterase activity of microorganisms were responsible for the elevated esters in fermented sorghum (Mehmood et al., 2008). Among all esters, butanoic acid, butyl ester and hexadecenoic acid, and methyl ester had the highest concentration, which could be formed by lipase-catalyzed esterification (Mamede, 2005).

During the steaming process, the alkanes content was increased while the esters content decreased (**Table 3**). Decrease in aldehydes were only observed in yeast-steamed sorghum, while the alcohols content was only reduced in LAB-steamed sorghum. The increment of aldehyde was due to the appearance of benzeneacetaldehyde, which was formed with amino acids during the Strecker degradation (Ji et al., 2015). However, no aldehydes were detected in lactic fermented samples, which may due to the synthesis of amino acid in yeast fermentation but biodegradation of amino acid in LAB fermentation (Fernández and Zúñiga, 2006). The increment of alkanes may due to the thermal damage of sorghum structure during steaming, which released more alkanes (Wu et al., 2013). Reduction in alcohols and esters content post steaming was also reported in previous study, mainly due to the leaching effect and thermal evaporation (Xiong et al., 2019b).

During the roasting process, pyrrole and pyrazine were formed and became the major volatile compounds in yeast-fermented sorghum, but not in LAB fermented sorghum (**Table 3**). These compounds formed during the Strecker degradation with amino acids and therefore contributed to the nutty and roasted flavor in final product (Arkadaş, 2018). It can be deduced that the absence of pyrazine and pyrrole in LAB-roasted samples was the result of low amino acids concentrations present in LAB fermented sorghum. It has been suggested that amino acids were generally in low concentrations after fermentation due to LAB growth (Pozo-Bayón et al., 2005). However, some studies mentioned the fact that the release of amino acids post-fermentation of *Saccharomyces cerevisiae* (Valero et al., 2003). Therefore, the difference of amino acids concentrations affected Maillard reaction. Roasting process also increased total aldehydes content in yeast-fermented sorghum due to the formation of 2,4-decadienal, (E,E)-. 2,4-decadienal, (E,E)-, which could be formed by autoxidation and lipoxygenase action on linoleic acid, and giving a fatty, cucumber and melon flavor to final sorghum tea product (Gassenmeier and Schieberle, 1994). The roasting process further reduced the alcohol contents in LAB-roasted samples and the esters contents in both type of fermented tea, which were likely due to the evaporation caused by high roasting temperature. Alkanes are relatively heat-resistant, which explained their stable contents during processing.

3.4 Principal components analysis of sorghum grains during processing

Principal component analysis was performed to illustrate the differences in sorghum grain samples subjected to different fermentation and processing treatments in phenolic content, antioxidant capacity and volatiles profile (**Figure 2**). The first (48.14%) and second (25.82%) component explained 73.96% of total variance. Total alkanes and ketones are the major contributors to the positive aspect of PC1, while the total pyrazine, pyrrole and phenol are the major contributors to the positive aspect of PC2. The total ester is the major contributor to the negative aspect of PC1 and total aldehyde contributes to the positive aspect of PC1 and PC2; and total alcohol contributed to the positive aspect of PC1 and the negative aspect of PC2.

Phenolics, ketones, alkanes, carboxylic acids and alcohols are the key compounds in raw sorghum, which gradually decreased during processing steps. Ester becomes the key volatile after fermentation process, while phenol, pyrrole and pyrazine are the major volatiles in yeast-roasted samples. Most processing steps, soaking, fermenting and roasting resulted in dramatic changes on sorghum chemical profile, while the influences of steaming were less than that of other steps.

4. Conclusion

In summary, the present study produced novel fermented sorghum tea with *Saccharomyces cerevisiae* or *Lactobacillus plantarum* and demonstrated that soaking, fermenting, steaming and roasting significantly changed its phenolic content, antioxidant capacity and volatile compounds. No significant differences in TPC were observed between the two types of fermentation whereas significant changes in aroma profiles were detected. Processing treatments, soaking, fermenting and steaming led to significant decreases in the concentration of phenolic compounds, while roasting significantly enhanced the TPC and antioxidant capacity. Significant ($P < 0.05$) positive correlations were observed between total phenolic compounds, condensed tannins content and antioxidant capacity. A total of 53 volatile compounds were detected. The abundance and composition of these volatiles were significantly influenced by each processing step, especially the two types of fermentation. Yeast fermentation showed a better performance regarding volatile profiles with esters, phenols and pyrazines being predominant. However, further studies are required to evaluate the sensory characteristics of the fermented sorghum grain tea and optimize the processing methods.

Conflict of interest

The authors declare no conflict of interest.

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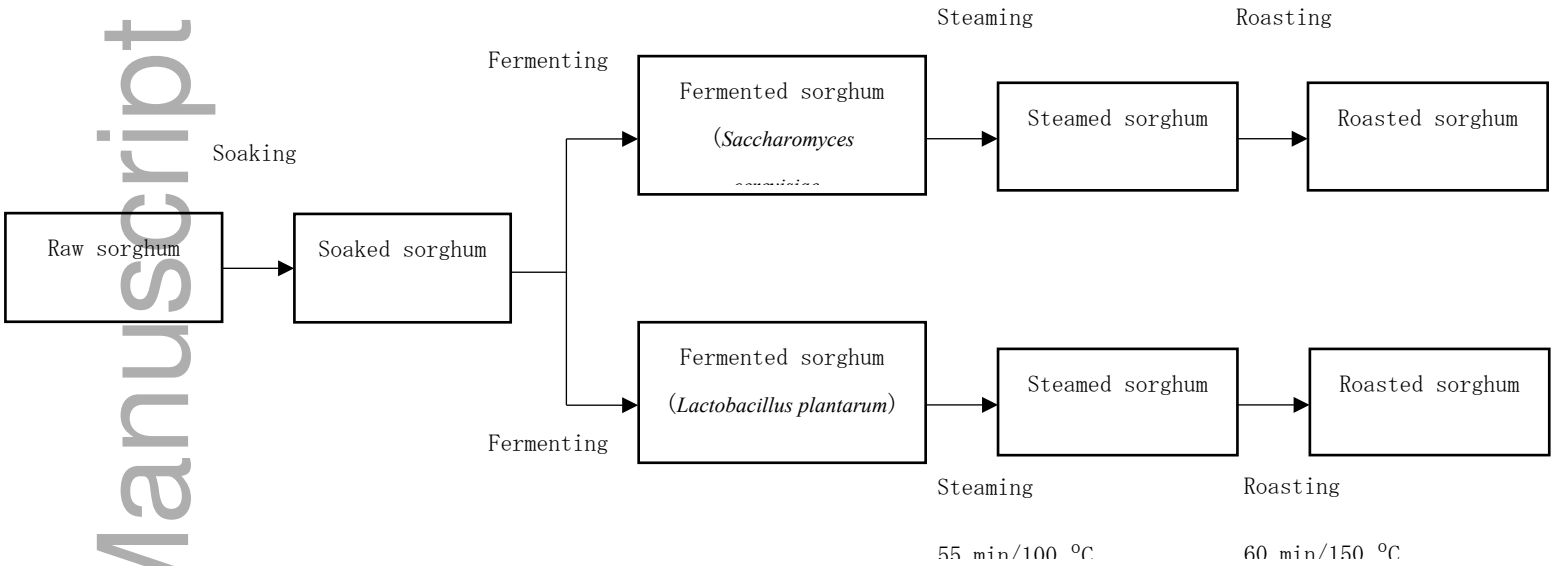

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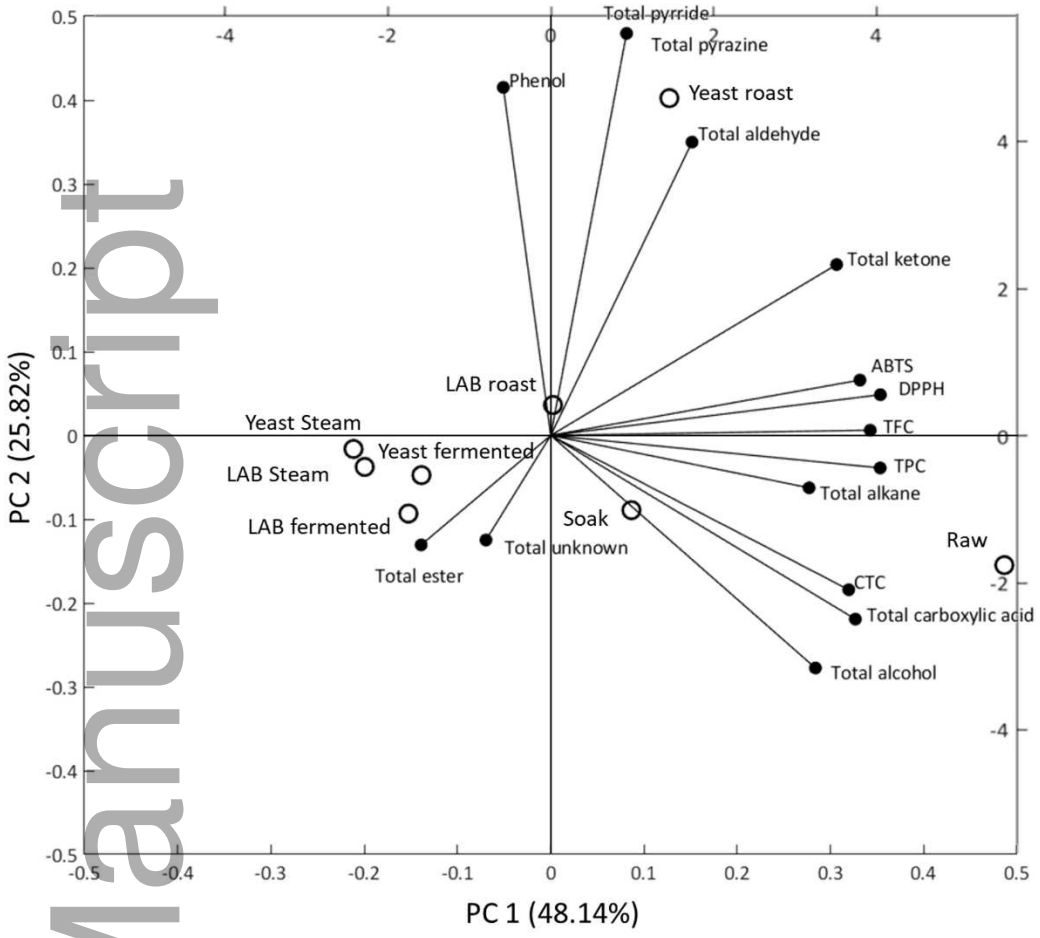
506 **Figure 1.** Flow chart of processing procedures for fermented sorghum tea with two different species of microorganisms



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508 **Figure 2.** Principal component analysis biplots of wheat bran tea processing. PC1
509 (48.14%) and PC2 (25.82%) explained 73.96% of total variance. Left and bottom axes
510 represent loading plot, right and top axes represent score plot.

	Yeast					LAB				
	Raw	Soak	Fermentation	Steam	Roast	Raw	Soak	Fermentation	Steam	Roast
TPC (mg GAE/g)	6.93 ± 0.62^{aA}	3.92 ± 0.26^{cA}	3.80 ± 0.12^{cA}	3.23 ± 0.10^{dA}	4.61 ± 0.14^{bA}	6.93 ± 0.62^{aA}	3.92 ± 0.26^{aA}	3.87 ± 0.39^{cA}	3.02 ± 0.25^{dA}	4.49 ± 0.38^{bA}
TFC (mg CAE/g)	1.07 ± 0.02^{aA}	0.83 ± 0.11^{bA}	0.63 ± 0.05^{cA}	0.39 ± 0.12^{dA}	0.80 ± 0.08^{bA}	1.07 ± 0.02^{aA}	0.83 ± 0.11^{bA}	0.61 ± 0.08^{cA}	0.44 ± 0.09^{dA}	0.85 ± 0.06^{bA}
CTC (mg CAE/g)	38.02 ± 1.63^{aA}	29.85 ± 2.13^{bA}	19.55 ± 2.22^{cA}	9.95 ± 2.46^{dA}	18.12 ± 2.82^{cA}	38.02 ± 1.63^{aA}	29.85 ± 2.13^{bA}	21.68 ± 1.63^{cA}	11.02 ± 2.68^{dA}	20.26 ± 1.85^{cA}
DPPH (mg TEAC/g)	1.37 ± 0.05^{aA}	1.10 ± 0.09^{bA}	0.97 ± 0.20^{cA}	0.93 ± 0.17^{dA}	1.17 ± 0.04^{bA}	1.37 ± 0.05^{aA}	1.10 ± 0.09^{bcA}	0.96 ± 0.05^{cA}	0.93 ± 0.08^{cA}	1.20 ± 0.05^{bA}
ABTS (mg TEAC/g)	12.62 ± 0.68^{aA}	7.07 ± 0.53^{cA}	6.06 ± 0.30^{cdA}	5.80 ± 0.09^{dA}	9.54 ± 0.11^{bA}	12.62 ± 0.68^{aA}	7.07 ± 0.53^{cA}	6.63 ± 1.11 ^{cdA}	6.36 ± 0.58^{dA}	10.31 ± 0.52^{bA}

511 **Table 1. Changes of phenolic compounds and antioxidant activity during fermented sorghum grain tea processing (mg/g)**

Data were displayed as mean ± standard deviation (n=3) on a dry basis.

a-d indicate significant difference within each pretreatment group, while A, B indicate the significant difference between two types of fermentation ($p<0.05$)

Abbreviation: LAB, fermented by *Lactobacillus plantarum subsp. argentoratensis*; Yeast, fermented by *Saccharomyces cerevisia*; TPC, Total phenolic content; TFC, Total flavonoid content; CTC, Condensed tannins content.

Table 2. Pearson's correlation coefficients of TPC, CTC and antioxidant activities after fermentation with different starter cultures.

Correlation coefficient (r)	Treatment	DPPH	ABTS
TPC	Yeast-treated	0.96	0.97
	LAB-treated	0.93	0.92
CTC	Yeast-treated	0.83	0.72
	LAB-treated	0.80	0.66

Correlation is significant at $P < 0.05$ (two-tailed)

Abbreviation: TPC, total phenolic content; CTC, condensed tannins content; N/A, Not applicable; LAB, Lactic acid bacteria

Table 3. Identification and quantification of volatile compounds (ng/g dried sample basis) in raw and processed sorghum grains.

	RI	RI Nist	Compound	Quantification method	Yeast					LAB					Odor description
					Raw	Soaked	Fermentation	Steaming	Roasting	Raw	Soaked	Fermentation	Steaming	Roasting	
1	874	871	1-Hexanol	ES	1062.0 ^{aA}± 590.4	280.2 ^{bA}±35.4	ND	ND	ND	1062.0 ^{aA}± 590.4	280.2 ^{bA}±35.4	ND	ND	ND	Ethereal, fruity, alcoholic
2	1070	1070	1-Octanol	ES	631.3 ^{aA}±78.9	ND	ND	ND	ND	631.3 ^{aA}±78.9	ND	ND	ND	ND	Waxy, green, citrus, aldehydic
3	1107	1103	Phenylethyl Alcohol	IS	ND	ND	ND	ND	ND	ND	ND	1029.9 ^{a} ± 290.1	428.8 ^{b} ± 144.6	164.9 ^{c} ± 41.7	Sweet, flora, fresh, honey
4	1169	1172	1-Nonanol	ES	1080.1 ^{aA} ± 72.6	741.6 ^{bA} ±8.1	ND	ND	ND	1080.1 ^{aA} ± 72.6	741.6 ^{bA} ±8.1	ND	ND	ND	Fresh, fatty, citrus, floral
			Alcohol		2773.4 ^{aA} ± 741.9	1021.8 ^{bA}± 35.7	ND	ND	ND	2773.4 ^{aA} ± 741.9	1021.8 ^{bA}± 35.7	1029.9 ^{bA} ± 290.1	428.8 ^{cA} ± 144.6	164.9 ^{dA} ± 41.7	
5	1099	N/A	Undecane	IS	74.5 ^{aA} ±14.3	40.3 ^{bA} ± 4.3	ND	ND	ND	74.5 ^{aA} ±14.3	40.3 ^{bA} ± 4.3	ND	ND	ND	N/A
6	1197	N/A	Dodecane	IS	58.2 ^{bA} ± 14.4	106.1 ^{aA} ± 10.6	ND	ND	28.2 ^{cA} ± 1.1	58.2 ^{bA} ± 14.4	106.1 ^{aA} ± 10.6	ND	ND	ND	Alkane
7	1297	N/A	Tridecane	IS	367.7 ^{aA}± 82.7	396.1 ^{aA} ± 30.0	186.7 ^{cA}± 12.5	252.9 ^{bA} ± 39.6	270.2 ^{bA} ± 69.6	367.7 ^{aA}± 82.7	396.1 ^{aA} ± 30.0	57.0 ^{e} ± 19.4	194.6 ^{bA} ± 59.2	150.9 ^{bB} ± 59.7	N/A
8	1396	N/A	Tetradecane	IS	76.0 ^{aA} ± 20.0	35.2 ^{c}± 7.5	85.7 ^{aA} ± 4.9	55.9 ^{bA}± 0.9	48.8 ^{bA} ± 5.9	76.0 ^{aA} ± 20.0	35.2 ^{c}± 7.5	ND	58.9 ^{bA} ± 7.3	51.7 ^{bA} ± 18.8	Mild waxy
9	1497	N/A	Heptadecane	IS	13.2 ^{aA} ±2.64	ND	ND	ND	ND	13.2 ^{aA} ±2.64	ND	ND	ND	ND	N/A
			Alkane		589.6 ^{aA} ± 133.7	577.9 ^{aA} ± 41.7	242.6 ^{cA} ± 27.4	307.6 ^{bA} ± 34.2	347.1 ^{bA} ± 64.1	589.6 ^{aA} ± 133.7	577.9 ^{aA} ± 41.7	57.0 ^{cB} ± 19.4	253.6 ^{bA} ± 54.2	202.6 ^{bB} ± 78.4	

810	812	Hexanal	ES	144.4 ^{bA}±9.8	255.4 ^{aA}± 64.4	ND	ND	ND	144.4 ^{bA}±9.8	255.4 ^{aA}± 64.4	ND	ND	ND	Fresh, green, fatty
1041	1044	Benzeneacetaldehyde	ES	ND	ND	ND	186.7 ^{a}±4.9	164.3 ^{b} ± 4.4	ND	ND	ND	ND	ND	Green, honey, flora
1103	1102	Nonanal	IS	31.9 ^{bA} ± 10.4	66.9 ^{aA} ± 21.0	22.2 ^{bA} ± 1.7	27.4 ^{bA}± 11.9	37.4 ^{bA} ± 8.3	31.9 ^{bA} ± 10.4	66.9 ^{aA} ± 21.0	ND	ND	ND	Waxy, aldehydic, citrus, fresh
1313	1314	2,4-Decadienal, (E,E)-	ES	ND	ND	ND	ND	375.5 ^{a}± 19.9	ND	ND	ND	ND	ND	Fatty, cucumber, melon,
		Aldehyde		176.3 ^{dA}± 4.7	322.3 ^{bA} ± 63.2	22.2 ^{eA}± 1.7	214.4 ^{cA} ± 16.0	577.2 ^{aA} ± 31.3	176.3 ^{dA}± 4.7	322.3 ^{bA} ± 63.2	ND	ND	ND	
1160	1163	Phenol, 4-ethyl-	IS	ND	ND	56.3 ^{bB} ± 21.6	112.8 ^{aB} ± 40.4	58.1 ^{bB} ± 7.7	ND	ND	254.9 ^{aA} ± 73.4	399.2 ^{aA} ± 80.0	128.2 ^{cA} ± 63.8	Phenolic, castoreum, smoky
1266	1268	Phenol, 4-ethyl-2-methoxy-	IS	ND	ND	693.1 ^{aA} ± 237.6	671.4 ^{aA} ± 188.0	570.5 ^{aA} ± 65.8	ND	ND	524.7 ^{aA} ± 168.4	246.7 ^{bB} ± 77.5	130.4 ^{cB} ± 20.9	Spicy, clove, woody, sweet
1302	1301	2-Methoxy-4-vinylphenol	ES	ND	ND	1468.9 ^{bA}± 37.7	1621.2 ^{bA}±20.8	7277.0 ^{aA}±1186.6	ND	ND	1456.5 ^{bA} ±20.4	1663.0 ^{bA}±30.2	7405.0 ^{aA}± 285.7	Woody, fresh, amber

		Phenol		ND	ND	2405.4± 246.6^{aA}	2716.1^{aA} ± 166.4	7905.5^{bA} ± 1249.7	ND	ND	2236.0^{aA} ± 225.9	2308.9^{aB}± 129.1	7663.6^{aB} ±34.5	
973	977	4-Octanone	IS	47.0^{aA} ± 8.2	ND	ND	ND	ND	47.0^{aA} ± 8.2	ND	ND	ND	ND	N/A
1116	1118	Isophorone	IS	ND	ND	ND	ND	79.9 ^{aA}± 29.1	ND	ND	ND	ND	ND	Woody, sweet, green, fruity
1350	1350	2(3H)-Furanone, dihydro-5-pentyl-	IS	39.9^{aA} ± 13.5	ND	ND	ND	ND	39.9^{aA} ± 13.5	ND	ND	ND	ND	Sweet, coconut, caramel
		Ketone		86.9 ^{aA}± 21.3	ND	ND	ND	79.9 ^{aA} ± 29.1	86.9 ^{aA}± 21.3	ND	ND	ND	ND	
1264	1260	Nonanoic acid	ES	33.3^{aA} ± 20.4	5.0^{bA} ± 1.0	ND	ND	ND	33.3^{aA} ± 20.4	5.0^{bA} ± 1.0	ND	ND	ND	Waxy, green, fatty,
2358	N/A	6-Octadecenoic acid	IS	10.7^{aA} ± 3.7	10.3^{aA}± 1.6	ND	ND	ND	10.7^{aA} ± 3.7	10.3^{aA}± 1.6	ND	ND	ND	N/A

		Carboxylic acid		43.9 [aA] ± 23.6	15.3 [bA] ± 2.5	ND	ND	ND	43.9 [aA] ± 23.6	15.3 [bA] ± 2.5	ND	ND	ND	
2								3859.6 [aA] ±						
	917	915	Pyrazine, 2,6-dimethyl-	ES	ND	ND	ND	ND		ND	ND	ND	ND	ND	Nutty, burnt, almond, roasted
2								1230.6						
2								5517.1 [aA]±						
	1072	1069	Pyrazine, 3-ethyl-2,5-dimethyl-	ES	ND	ND	ND	ND		ND	ND	ND	ND	ND	Nutty, potato, cocoa, roasted
3								2046.9						
2														
	1147	1148	Pyrazine, 2,3-diethyl-5-methyl-	IS	ND	ND	ND	ND	81.5 [aA] ± 7.9	ND	ND	ND	ND	ND	Nutty, meaty, vegetable, roasted
4														
2														
	1149	1150	Pyrazine, 3,5-diethyl-2-methyl-	IS	ND	ND	ND	ND	165.4 [aA] ± 116.0	ND	ND	ND	ND	ND	Nutty, meaty, vegetable
5														
								9623.7 [aA] ±						
		Pyrazine		ND	ND	ND	ND		ND	ND	ND	ND	ND	
								1815.8						
2		1H-Pyrrole-2-carboxaldehyde,												
	1045	1046		IS	ND	ND	ND	ND	68.1 [aA] ± 17.5	ND	ND	ND	ND	ND	Vegetable, green, fruity, cereal
6		1-ethyl												

1173	1172	1H-Pyrrole, 1-(2-furanylmethyl)-	IS	ND	ND	ND	ND	54.2 ^{aA} ± 2.0	ND	ND	ND	ND	ND	Burnt, roasted, smoky
		Pyrrole		ND	ND	ND	ND	122.3 ^{aA} ± 16.4	ND	ND	ND	ND	ND	
937	938	Hexanoic acid methyl ester	ES	1204.3 ^{aA} ± 128.5	360.0 ^{bA} ± 107.9	ND	ND	ND	1204.3 ^{aA} ± 128.5	360.0 ^{bA} ± 107.9	ND	ND	ND	Fruity, pineapple, ethereal
998	995	Butanoic acid, butyl ester	ES	ND	ND	3074.3 ^{bB} ± 308.2	4795.9 ^{aB} ± 751.8	2719.8 ^{bB} ± 1279.8	ND	ND	83799.8 ^{aA} ± 12787.1	20076.4 ^{bA}± 6770.1	19725.1 ^{bA} ± 10219.9	Fruity, sweet, banana, green
1035	N/A	Pantolactone	IS	ND	ND	ND	ND	146.8 ^{a} ± 28.7	ND	ND	ND	ND	ND	Cotton, candy
1056	1056	Butanoic acid, 3-methylbutyl ester	IS	ND	ND	57.2 ^{bB} ± 4.4	70.1 ^{aB} ± 6.1	99.8 ^{aB} ± 34.3	ND	ND	378.3 ^{aA} ± 106.7	491.3^{aA}± 197.4	358.9 ^{a} ± 145.9	Fruity, sweet, estery, green,
1090	1094	Benzoic acid, methyl ester	IS	264.0 ^{bA} ± 74.2	413.9 ^{aA} ± 28.7	142.5 ^{cA} ± 17.2	ND	ND	264.0 ^{bA} ± 74.2	413.9 ^{aA} ± 28.7	ND	ND	ND	Phenolic, wintergreen, almond
1122	1120	Octanoic acid methyl ester	ES	228.0 ^{aA} ± 17.3	171.4 ^{bA} ± 19.1	ND	ND	ND	228.0 ^{aA} ± 17.3	171.4 ^{bA} ± 19.1	ND	ND	ND	Waxy, green, sweet, orange, aldehydic

1142	1145	Methyl nicotinate	IS	11.6 ^{bA} ± 3.8	42.9 ^{aA} ± 11.7	13.2 ^{bA} ± 4.2	ND	ND	11.6 ^{bA} ± 3.8	42.9 ^{aA} ± 11.7	ND	ND	ND	Warm, herbal, tobacco
1166	1168	Benzoic acid, ethyl ester	ES	ND	ND	ND	ND	ND	ND	ND	405.8 ^{aA}± 31.6	151.5 ^{bA} ± 14.3	75 ^{cA} ± 5.2	Fruity, sweet, wintergreen
1189	1186	Hexanoic acid, butyl ester	IS	ND	ND	ND	ND	ND	ND	ND	143.8 ^{aA}± 22.2	117.9 ^{aA} ± 59.4	88.8 ^{aA} ± 9.1	Fruity, berry, pineapple
1223	N/A	Nonanoic acid methyl ester	IS	145.3 ^{aA} ± 48.1	27.8 ^{bA} ± 7.1	ND	ND	ND	145.3 ^{aA} ± 48.1	27.8 ^{bA} ± 7.1	ND	ND	ND	Sweet, fruity, pear, waxy
1365	N/A	Butyl benzoate	IS	92.6 ^{bA} ± 15.6	34.9 ^{cA} ± 7.8	125.3 ^{abA}± 21.7	46.9 ^{cb} ± 7.5	47.9 ^{cb} ± 6.0	92.6 ^{bA} ± 15.6	34.9 ^{cbA} ± 7.8	356.5 ^{aA} ± 97.0	463.0 ^{aA} ± 123.2	199.5 ^{bA} ± 35.8	Amber, balsamic, fruity
1375	1373	Butyl caprylate	IS	ND	ND	ND	ND	ND	ND	ND	83.0 ^{aA}± 13.3	67.8 ^{aA} ± 24.7	81.0 ^{aA} ± 20.6	Buttery, ethereal, herbal
1431	N/A	Benzoic acid, 2-methylbutyl ester	IS	72.7 ^{bA} ± 12.3	136.7 ^{aA} ± 11.0	93.5 ^{abA} ± 33.7	70.6 ^{bA} ± 17.6	46.8 ^{cA} ± 4.3	72.7 ^{bA} ± 12.3	136.7 ^{aA} ± 11.0	ND	ND	ND	N/A
1432	1439	.beta.-Phenylethyl butyrate	IS	ND	ND	ND	ND	ND	ND	ND	292.3 ^{aA} ± 99.5	415.8 ^{aA} ± 152.1	183.5 ^{aA} ± 31.2	Fruity, sweet, berry, juice

1520	1521	Dodecanoic acid, methyl ester	IS	20.4 ^{aA} ± 9.0	22.4 ^{aA}± 4.2	ND	ND	ND	20.4 ^{aA} ± 9.0	22.4 ^{aA}± 4.2	ND	ND	ND	Waxy, creamy, coconut, mushroom
1921	1921	Hexadecanoic acid, methyl ester	IS	656.5 ^{bA} ± 195.2	2892.1^{aA}± 497.5	3871.9 ^{a} ± 1027.2	38.7 ^{cA} ± 4.0	ND	656.5 ^{bA} ± 195.2	2892.1^{aA}± 497.5	11176.1 ^{aA} ± 1998.2	ND	ND	Oily, waxy, fatty, orris
1993	1994	Hexadecanoic acid, ethyl ester	ES	25.1 ^{cA} ± 4.0	363.7 ^{aA} ± 149.7	151.5 ^{bB} ± 88.7	17.2 ^{cB} ± 2.4	13.1 ^{db} ± 0.9	25.1 ^{cA} ± 4.0	363.7 ^{aA} ± 149.7	1051.9 ^{aA} ± 238.8	119.1 ^{cA} ± 12.5	91.6 ^{cA} ± 39.7	Waxy, fruity, creamy, milky, balsamic
2089	2087	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	IS	337.4 ^{aA} ± 98.2	402.5 ^{aA} ± 61.9	801.3 ^{aA} ±530.9	26.8 ^{bA} ± 6.6	ND	337.4 ^{aA} ± 98.2	402.5 ^{aA} ± 61.9	690.0 ^{aA} ± 82.0	ND	ND	Bland
2095	N/A	9-Octadecenoic acid, methyl ester, (E)-	IS	223.4 ^{bA} ± 54.9	583.4 ^{aA} ± 209.1	554.2 ^{aA} ± 327.8	23.6 ^{cA}± 6.6	ND	223.4 ^{bA} ± 54.9	583.4 ^{aA} ± 209.1	386.1 ^{bB} ± 49.2	ND	ND	N/A
2128	2128	Methyl stearate	IS	18.3 ^{cA} ± 3.1	26.0 ^{bA} ± 5.9	71.3 ^{aA}± 40.4	ND	ND	18.3 ^{cA} ± 3.1	26.0 ^{bA} ± 5.9	59.1 ^{aA} ± 11.6	ND	ND	Oily, waxy
2159	2155	Linoleic acid ethyl ester	IS	39.3 ^{bA} ± 10.8	51.4 ^{bA} ± 29.3	172.5 ^{aA} ±52.0	14.2 ^{cB} ± 0.6	ND	39.3 ^{bA} ± 10.8	51.4 ^{bA} ± 29.3	256.4 ^{aA} ± 46.4	100.6 ^{b} ± 17.9	47.1 ^{cA} ± 14.6	Fatty, fruity
2180	2180	Ethyl Oleate	IS	27.5 ^{bA} ± 7.8	36.5 ^{bA} ± 14.0	105.6 ^{aA} ±52.0	11.4 ^{cB} ± 0.7	ND	27.5 ^{bA} ± 7.8	36.5 ^{bA} ± 14.0	147.8^{aA}± 36.5	86.4 ^{bA}± 26.9	27.3 ^{cA} ± 4.2	N/A

2185	N/A	Hexadecanoic acid, butyl ester	IS	44.9 ^{aA} ± 7.2	19.1 ^{bA} ± 3.2	ND	ND	ND	44.9 ^{aA} ± 7.2	19.1 ^{bA} ± 3.2	504.3 ^{aA} ± 299.1	99.4 ^{bA} ± 36.2	68.3 ^{bA} ± 10.6	N/A
2353	N/A	Butyl 9, 12-octadecadienoate	IS	44.9 ^{aA} ± 5.9	ND	ND	ND	ND	44.9 ^{aA} ± 5.9	ND	93.8 ^{aA} ± 56.3	37.3 ^{bA} ± 25.2	11.6 ^{cA}± 3.7	N/A
		Ester		2935.4 ^{cA} ±607.3	6427.0 ^{bA} ± 450.8	9410.7 ^{a} ± 2379.5	5103.4 ^{b} ± 780.9	3127.6 ± 1178.9 ^{c}	2935.4 ^{cA} ±607.3	6427.0 ^{bA} ± 450.8	93354.1 ^{aA} ± 12422.0	23159.4 ^{bA} ± 6070	20958.5 ^{cA}± 10311.8	
1303	N/A	Unknown1	N/A	160.0 ^{cA} ± 19.6	201.0 ^{bA} ± 134.0	309.6 ^{bA} ± 220.3	666.6 ^{aA} ± 37.1	ND	160.0 ^{cA} ± 19.6	201.0 ^{bA} ± 134.0	ND	ND	ND	N/A
1362	N/A	Unknown2	N/A	46.4 ^{aA} ± 13.3	ND	ND	ND	ND	46.4 ^{aA} ± 13.3	ND	ND	ND	ND	N/A
		Unknown		206.3 ^{cA} ± 28.3	201.0 ^{cA} ± 134.0	309.6 ^{bA} ± 220.3	666.6 ^{aA} ± 37.1	ND	206.3 ^{cA} ± 28.3	201.0 ^{cA} ± 134.0	ND	ND	ND	
		Total		6881.8 ^{A} ± 1457.0 ^{c}	8563.6 ^{cA} ± 540.4	12094. ^{bB} 7 ± 2567.9	8655.0 ^{cB}± 863.9	21783.3 ^{aA} ± 1760.9	6881.8 ^{A} ± 1457.0 ^{c}	8563.6 ^{cA} ± 540.4	96898.8 ^{aA} ± 12998.6	25897.1 ^{bA} ± 5654.6	28989.6 ^{bA} ±10763.8	

a-d indicate significant difference within each pretreatment group, while A, B indicate significant difference between two types of fermentation ($p<0.05$)

Odor description were obtained from The Good Scents Company (2018).

Volatile compounds were identified by mass spectra (NIST library 11.0) and retention index from NIST Webbook (NIST 2018).

Semi-quantified was used by comparing the peak areas of target compounds to the peak area of internal standard, external quantification method was used by calculation of standard curve

Abbreviation: RI = Retention index; RI NIST = Retention indices obtained from NIST Webbook; N/A = Not Applicable; ND = Not Detected; IS=internal standard; ES=external standard