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Effects of Processing on The Phenolic Contents, Antioxidant Activity and Volatile Profile of Wheat Bran Tea

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Abstract

Wheat bran is a major milling by-product with significant nutritional importance. This study investigated the influence of processing on the antioxidant activity and volatile profile of wheat bran tea. The processing protocol involved two different pretreatments: soaking and cellulase treatment, followed by steaming and roasting. The results indicated that total phenolic content and antioxidant activity were reduced post both pretreatments, but increased after steaming, and dramatically increased after roasting. The cellulase-roasted samples had significantly higher antioxidant activity compared to soak-roasted samples. A total of 40 key volatile compounds were identified in the wheat bran tea. The total volatile compounds were significantly

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increased by both soaking and cellulase treatments and steaming, but reduced by roasting. Compared to soaking pretreatment, cellulase pretreated samples had significantly higher total volatile compounds at each processing step. This study offers a practical method in processing of wheat bran into novel grain tea products.

Key words: *Wheat bran tea; food processing; polyphenols; GC-MS; aroma profile*

1. Introduction

Bread wheat (*Triticum aestivum*) is one of the most important staple foods worldwide, with annual global production of 754.8 million tons in 2017 (FAO, 2018). Wheat bran is the major by-product during wheat processing. Assuming all wheat was used in the flour industry, approximately 173.6 to 203.8 tons of wheat bran was produced annually under the current extraction rate. However, 90% of wheat bran is still used as low value animal feed, while only 10% was used for human consumption, mainly as bakery, fried food and breakfast cereal (Prückler et al., 2014).

Wheat bran is a rich source of nutritional compounds. Phenolic acids are the most abundant phytochemicals and ferulic acid accounts up to 60 % of the total phenolic content of wheat bran (Shahidi and Ho, 2005). Other important phenolic acids include syringic, *p*-coumaric, *p*-hydroxybenzoic and vanillic acid. In addition, other nutritional compounds such as magnesium, zinc, copper, arabinoxylan, methionine and cystine are also abundant in wheat bran (Fardet, 2010). Due to its high nutritional value, wheat bran can be used to develop novel functional food products. The application of wheat bran in the food industry has been increased significantly in the past two decades. In 2001, only 80 wheat bran-incorporated foods were developed, while this was increased to approximately 800 by 2011 (Prückler et al., 2014).

Grain-based beverages have attracted increasing interests due to its unique flavor, potential health benefits, and caffeine/alcohol-free characteristics (Hui, 2007). For example, tartary buckwheat (Guo et al., 2017) and sorghum (Wu et al., 2013; Xiong et al., 2019) have been developed as functional tea products. Soaking, steaming, roasting and fermentation are the most common methods for these grain-based beverage processing. However, different grain responses differently to the same processing method. In terms of wheat bran tea, there is only one study investigated the effect of processing on the product quality of a traditionally fermented drink, by using mixed corn flour and wheat bran as raw materials (Pasqualone et al., 2018). However, to the best of our knowledge, study on the non-fermentation beverage based on wheat bran only has not been reported. This study investigated the influence of different processing methods on the antioxidant activity and volatile profiles of wheat bran tea, which could be developed as a value-added novel functional grain beverage.

2. Material and Method

2.1 Materials and chemicals

Methanol (for HPLC, ≥99.9%), gallic acid, Folin & Ciocalteu's phenol reagent, sodium carbonate (>99.5%), (+)-catechin hydrate (≥98% HPLC), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), aluminum chloride, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate SPME fibre assembly polydimethylsiloxane/divinylbenzene (df 65 μm, fused silica/SS, fiber length 1 cm) (PDMS/DVB), 4-octanol, n-alkanes (C8–C22), analytical standards for all volatile compounds and magnetic PTFE/sil hdsp cap were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). Sodium nitrite (reagent grade), citric acid monohydrate (reagent grade) and sodium hydroxide pellets (reagent grade) were purchased from Chem-Supply (Gillman, SA, Australia). Headspace screw top clear vials (20 ml) was purchased from Agilent Technologies (Mulgrave, VIC, Australia). Food grade cellulase were purchase from Alchemist's Pantry Company (Copenhagen, Denmark).

2.2 Processing of wheat bran tea

Commercial wheat bran (100% wheat bran) was purchased from Woolworth supermarket in Melbourne, Australia. The experimental design and sampling points are shown in **Figure 1**. A total of 350 g raw wheat bran was weighed, and 50 g was sampled for extraction of phenolic compounds and chemical assay. The rest 300 g were divided into two groups of 150 g each and two pretreatments were applied respectively. The first pretreatment was soaking the sample with 1.5 L tape water for

24 hours under room temperature. The second pretreatment was immersing the wheat bran (150 g) in 1.5 L tape water with 0.015 g food grade cellulase (Alchemist's Pantry Company, Copenhagen, Denmark). The pH was adjusted to 4.8 by citric acid and then incubated in a water bath at 50 °C for one hour to achieve maximum cellulase activity.

After pretreatment, the sample was filtered through a sieve (mesh number 60, Greer & Ashburner Pty Ltd, VIC, Australia) and dried at room temperature. A subsample of 50 g was taken from each pretreatment. The remaining 100 g pretreated wheat bran was then steamed by a food steamer (model XJ-92214, Kmart, VIC, Australia) for 40 minutes, and 50 g steamed sample was collected for chemical analysis (**Figure 1**). The rest 50 g steamed wheat bran was roasted by an oven (WFE946SB electric free-standing cooker, Westinghouse Electric Corporation, VIC, Australia) at 180 °C for 15 minutes. The experiments were conducted in triplicate and all samples were freeze-dried for 72 hours and stored at -20 °C until use. The freeze-dried samples were then subjected to phenolic compound extraction and analysis.

2.3 Extraction of phenolic compounds

The phenolic extraction method was adapted from Wu et al. (2017) with minor modifications. Briefly, each replicate (1 g) was dissolved in 45 ml 80% methanol, incubated at 50 °C in water bath for one hour, and then centrifugated under 2500 g, 4 °C for 10 minutes using a Beckman Allegra X-12 centrifuge (Beckman coulter Inc, Indianapolis, IN, USA). The supernatant was then collected in a 250 ml flask and the extraction process was repeated three times. The collected supernatants were pooled and evaporated by a Hei-VAP Value rotary evaporator (Scitek Australia Pty Ltd, NSW, Australia) to dryness under 50 °C. The extract was dissolved in 10 ml methanol and kept at -20 °C until analysis.

2.4 Antioxidant activity analysis

2.4.1 Total phenolic content (TPC) determination

The TPC assay was adapted from (Wu et al., 2017) with modifications. Phenolic extraction solution (0.2 ml) was reacted with 1 ml 10% Folin-Ciocalteau reagent for 2 minutes under room temperature. Then 0.8 ml 7.5% Na_2CO_3 was added and the mixture was incubated in a 60 °C water bath for 30 minutes. The measurement was performed at 765 nm using a Multiskan Go plate reader in a 96 well plate (Thermo Fisher Scientific Cooperation, VIC, Australia). Methanol was used as blank and gallic acid was used as reference. The results were expressed as milligram of gallic acid equivalent per gram (GAE μg /g) of sample.

2.4.2 Total flavonoid content (TFC) determination

The TFC assay method was adapted from (Wu et al., 2017) with minor modifications. First, 0.15 ml phenolic extraction, 0.66 ml distilled water and 0.045 ml 5% $NaNO_2$ were mixed and kept in darkness under room temperature for 5 minutes. Then 0.045 ml of 10% $AlCl_3$ was added and incubated for 3 minutes. Finally, 0.6 ml of 0.5 M NaOH was added and reacted for 30 minutes. Measurement was performed at 415 nm in a 96 well plate using the Multiskan Go plate reader with methanol as blank and catechin as reference. The final result was express as milligram catechin equivalents per gram (GAE μg /g) of sample.

2.4.3 Determination of ABTS radical scavenging activity

The ABTS assay was adapted from Zheng, Zhang and Liu (2015). A mixture of 10 ml 7.4 mM $ABTS^+$ solution and 10 ml 2.6 mM $K_2S_2O_8$ solution was prepared and reacted overnight. The solution was then diluted by methanol to achieve an absorbance of 1.1+ 0.1 as working solution. Then, 0.2 ml of the phenolic compounds were reacted with 1.3 ml of $ABTS^+$ working solution in darkness under room temperature. Methanol was used as blank and Trolox as standard. Then the final absorbance was measured at 734 nm using a 96 well plates on the Multiskan Go plate reader and results expressed as milligram Trolox equivalent antioxidant capacity per gram (TEAC mg/g) of sample.

2.4.4 Determination of DPPH radical scavenging activity

The DPPH assay was modified from Zheng, Zhang and Liu (2015). DPPH solution was prepared by diluting 12 mg DPPH with 50 ml methanol. The solution was further diluted by methanol to achieve a working solution (absorbance of 1.1 + 0.1). Then 0.2 ml phenolic extraction sample was mixed with 1.3 ml DPPH working solution and reacted in darkness for 8 hours under room temperature. Methanol was used as blank with Trolox as standard. Final absorbance was measured at 517 nm using a 96 well plates on the Multiskan Go plate reader and results were expressed as milligram Trolox equivalent antioxidant capacity per gram (TEAC mg/g) of sample.

2.5 Headspace Solid Phase Microextraction and Gas Chromatography Mass Spectrometry (HS-SPME-GC-MS) for volatile compounds analysis

Each sample of 5 g was immersed in liquid nitrogen and ground to powder by a grinder (Sunbeam EM0405 Multigrinder II, VIC, Australia), and 2 g of the powder was transferred to a 20 ml vial, and 20 µL 4-octanol (0.01 g/100 ml) was added as internal standard. The processing step samples were analysis in triplicates. Between each triplicate, a blank vial with 20 µL 4-octanol (0.01 g/100 ml) was run as as a normal sample to ensure that there was no significant change on the machine condition during sample analysis. The vials were tightly sealed with a magnetic PTFE/sil cap, kept for 20 minutes at 50 ℃ with agitation to achieve equilibrium, and a blue SPME fiber (65 µm PDMS/DVB fiber, Sigma Aldrich, Castle Hill, NSW, Australia) was exposed to the headspace with a penetration depth of 35 mm at 50 ℃ for 35 minutes for adsorption.

An Agilent 6850 series II gas chromatograph connected to a mass spectrometer (Agilent Technologies 5973 mass selective detector, USA) coupled with an SPME PAL 3 multi-purpose automated sampler (Agilent Technologies, Foster City, CA, USA) were used. Separation was achieved using an Agilent J & W DB-5ms capillary column (30 m x 250µm x 0.25µm, Agilent Technologies, Apple Valley, MN, USA). The carrier gas was helium with a constant flow velocity of 0.8 ml/min. Sample desorption at inlet was 7 minutes in splitless mode at 250 °C. The solvent delay was set as 2 minutes. The initial oven temperature was held at 50 ℃ for 5 minutes; increased to 125 ℃ at a rate of 2 ℃/min and held for 3 minutes; then increased to 180 ℃ at a rate of 3 ℃/min and held for 3 minutes; then increased to 230 ℃ at a rate of 15 ℃/min; and final increased to 280 ℃ at a rate of 20 ℃/min and held for 10 minutes. The mass spectrometer was operated in scan mode at 70 eV, with a mass scan range of 35-400 AMU. The transfer line, ion source and quadrupole temperatures were 280 °C, 230 °C and 150 °C, respectively. Alkanes (C7-C22) were used to establish retention index (RI). Blank samples and internal standard were analyzed regularly for quality control. The data were analyzed using Agilent G1701EA MSD ChemStation software. Target compounds were identified based on RI, mass spectra and external standards. NIST (NIST 11.0) reference database and NIST webbook were used for reference RI. The compounds were only confirmed if the difference between actual RI and reference RI was less than 10. The volatile compounds were semi-quantified by comparing the peak area of the target compounds to the peak area of the internal standard (4-octanol).

2.6 Statistics

One-way ANOVA was performed to compare the differences in TPC, TFC, ABTS, DPPH and volatiles among treatment groups and samples from each processing stage using Minitab Express (Version 1.2.0, Minitab Pty Ltd, Sydney, Australia). Principal component analysis (PCA) was performed for TPC, TFC, ABTS, DPPH and total volatile component of each chemical group using Matlab® (Version 9.3, Mathwork Inc, Natick, MA, USA).

3. Result and discussion

3.1 Effect of processing on phenolic compounds and antioxidant activity

The phenolic compounds and antioxidant activity of raw wheat bran were present in **Table 1**. The TPC in the raw material was about 1,032 µg GAE/g, which is in the range of 1,000 µg /g to 4,000 µg GAE /g in wheat bran (Vaher et al., 2010). The TFC in the raw bran material was about 87 µg CAE/g (**Table 1**). Flavonoids are not major phenolic compounds in wheat bran, and the reported TFC value was ranging from 200 µg /g to 400 µg /g (Feng and Mcdonald 1989). The TPC and TFC values in the present study were at the lower end within the reported ranges, which may due to different cultivars, agronomical practices, or variations between winter and spring wheat (Vaher et al., 2010).

Changes in phenolic compounds and antioxidant activity at each processing stage and between different pretreated samples were compared (**Table 1**). Significantly decreased TPC was observed in both pretreated samples compared to the raw wheat bran; however, no change was observed in the steamed samples compared to the pretreated ones, whereas significantly increased TPC was observed in the roasted samples. A similar pattern was observed for TFC change, where both pretreatments significantly reduced TFC, steaming significantly increased TFC in the soaked samples but not the cellulase treated samples, and roasting significantly increased TFC value (**Table 1**). Between the two pretreatments, cellulase treated samples had significant higher TPC and TFC values compared to the soaked samples at each processing stage. The antioxidant activity of wheat bran was significantly decreased after both pretreatments, then raised after steaming and roasting processes. Cellulase pretreated samples had a significantly higher antioxidant activity compared to the soaking pretreated samples.

Two main factors might have contributed to the reduction of TPC and TFC during the bran soaking process: i) soluble phenolic compounds could be leached to water, ii) break down of phenolic compounds by activation of polyphenol oxidase (Wu et al., 2013). The cellulase treated bran had a significantly higher TPC and TFC value compared to soaked sample, but still dramatically lower than the raw bran. Similar results were observed in previous studies, where cellulase treated rice bran had significantly higher TPC value than milled rice, but lower than raw rice (Das, Banerjee and Bal, 2008). This may be due to the breakdown of the cell wall by cellulase, which released the bounded phenolic compounds (Das, Banerjee and Bal, 2008).

The steaming process significantly increased the TFC value of soaked samples, and no changes for the TPC value for both soak and cellulase treated samples (**Table 1**). This may be due to the variations of degradation temperature for flavonoids and phenolic acids. Wheat bran contains a number of phenolic acids, including ferulic acid, vanillic acid, syringic acid, caffeic acid and sinapic acid (Verma, Hucl and Chibbar, 2009), which are sensitive to heat treatment (Réblová, 2012). However, flavonoids have higher heat stability compared to phenolic acids, and moderate heat treatment (80 °C -100 °C) was previously found to enhance the TFC value (Sharma et al., 2015).

Roasting process significantly increased TPC and TFC in both soaking and cellulase pretreated samples. Wu et al. (2013) suggested that roasting significantly increased the TPC and TFC values in processing of sorghum tea, likely due to the following reasons: i) the high temperature during roasting may destroy the cell wall, which leads to releasement of more phenolic compounds; ii) conjugated polyphenolics may break down to form simple phenolics under high temperature; and iii), some polyphenol compounds may form during Maillard reaction (Wu et al., 2013). It was also reported that the extractability of phenolic compounds may increase at high temperature (Patras et al., 2009). In addition, the TPC value was measured by the Folin-Ciocalteu method, which may be interfered by non-phenolic reducing compounds, such as amino acid, organic acid, reducing sugar and Maillard reaction products (Wu et al., 2013).

Both DPPH and ABTS were used to estimate the antioxidant activity of raw wheat bran and processed samples. The ABTS value was higher than the DPPH value in each analysis sample, which is consistent to a previous study (Floegel et al., 2011). This is probably because DPPH can detect the antioxidant activity only in lipophilic system, but ABTS can detect in both hydrophilic and lipophilic systems (Floegel et al., 2011).

The TPC values in the roasted wheat bran were similar to the raw wheat bran. However, antioxidant activities in roasted bran were much lower than that of the raw wheat bran. This could be the non-phenolic compounds in the raw wheat bran such as vitamins (carotene, vitamin B and E) have also contributed to the antioxidant activity. These vitamins are unstable under heat treatment, which could be lost during processing and therefore lower antioxidant activity in the roasted wheat bran samples (Wu et al., 2013).

3.2 Headspace Solid Phase Microextraction and Gas Chromatography Mass Spectrometry (HS-SPME-GC-MS) for volatile compounds analysis

A total of 40 volatile compounds was identified in the wheat bran samples, including 3 alcohols, 3 pyrazines, 11 esters, 1 furan, 3 ketones, 9 aldehydes, 3 carboxylic acids, 6 alkanes and 1 phenol (**Table 2**). Both soaking and cellulase pretreatments significantly increased the total volatile compounds, then, a substantial increase in total volatile compounds was observed after the steaming process, but decreased by the roasting process.

In the raw wheat bran, high concentration of 1-hexanol was detected (**Table 2**), which is in consistent to the literature that 1-hexanol is the main alcohol in wheat (Ji, Kang and Baik, 2017). This alcohol compound has a green and herbal aroma (**Table 2**). A total of 8 esters were observed in wheat bran, which provide fruity and waxy aroma. Amongst all esters, methyl hexanoate was the most abundant. 2-Pentyl-furan was detected in raw wheat bran, which was also reported in whole wheat with a fruity and green aroma (Ji, Kang and Baik, 2017). It was reported that whole wheat flour had a significantly higher level of 2-pentyl furan compared to the refined wheat flour (Moskowitz et al., 2012), indicating it mainly existed in the bran. Only one aldehyde ((E)-2-nonenal) and one ketone (2(3H)-furanone, 5-ethyldihydro-) were detected in the raw wheat bran. Both compounds were previously reported in wheat and (E)-2-nonenal has a fatty and green scent, whereas 2(3H)-furanone, 5-ethyldihydro- has a tonka aroma (Lippolis et al., 2014). Nonanoic acid was the only carboxylic fatty acid detected in the raw wheat bran. It is commonly found in the lipid layer of grain with a waxy and fatty scent (Ji, Kang and Baik, 2017). Alkane is frequently reported in raw wheat, but do not contribute to the aroma profile due to its high odor detection threshold (Hui, 2007). Two alkanes, nonadecane and heneicosane were detected in this studied material, which were also previously reported in wheat by Zhang (2012) and Ji, Kang and Baik (2017).

Compared to the raw wheat bran, the alcohol, ester and carboxylic acid concentrations were significantly reduced after both soaking and cellulase pretreatments (**Table 2**). This may due to the leaching effects of the process, which has been reported in a past study (Lucas, Le Ray and Mariette, 2007). Both pretreatments dramatically increased the concentration of 2-pentyl furan compared to raw wheat bran, which could be produced by the oxidation of linoleic acid in the sample (Moskowitz et al., 2012). Cellulase treatment led to a higher increment of 2-pentyl furan compared to the soaked sample, this could be caused by the relatively higher temperature (50 ℃) during the enzyme incubation (Xu et al., 2017). Aldehydes are products of lipid peroxidation (Pizzimenti et al., 2013) and the aldehyde contents in the wheat bran were significantly increased after both soaking and cellulase pretreatments (**Table 2**). The water immersion during the pretreatments could have increased the activity of lipoxygenase, thus cause more lipid oxidation and the formation of aldehydes (Zhang et al., 2018). In this study, cellulase samples incubated in 50 ℃ water bath had the highest aldehydes content, which could be explained by the higher lipoxygenase activity at a relatively higher temperature. 2(3H)-Furanone, 5-ethyldihydro- was disappeared after both pretreatments, likely due to the leakage into the water (Xu and Chang, 2008). However, a new compound of 2-butanone was detected after pretreatment, probably due to the increased extraction rate after this process (Wu et al., 2013). The alkane contents in the soaked sample were also increased. These are plant lipids and insoluble in water, thus soaking may break the cell wall to increase the alkane content without leaching into solvent (Wentzel et al., 2007).

After the steaming treatment, the concentrations of aldehydes and furans were increased rapidly and became the most abundant aromatic compounds in the bran sample (**Table 2**). (E, E)-2,4-decadienal was the major aldehyde. Previous study reported that (E)-2-nonenal and (E, E)-2,4-decadienal were of high concentrations in whole wheat flour (Moskowitz et al., 2012). Most aldehydes in the wheat sample might be produced through autoxidation and lipoxygenasic activity of linoleic acid (Moskowitz et al., 2012). (E, E)-2,4-decadienal provides fatty and chicken aroma at high concentrations, while at low concentration it has a flavor of citrus, orange and grapefruit flavor (Moskowitz et al., 2012). 2-Pentyl furan was further increased in the steamed sample, which is in consistent with the study of Dong et al. (2018) that steaming process significantly increased the concentration of 2-pentyl furan in whole wheat flour. One possible explanation is that steaming accelerated the oxidation of linoleic acid at high temperature (Zhang, Li and Zhang, 2013). Octanoic acid was also detected in cellulase pretreated samples, likely due to the elevated extraction rate of carboxylic acid at higher temperature (Canari and Eyal, 2003). For the aroma compounds of ketones, 2-butanone disappeared from all samples after the steaming process due to its low boiling point of 79.64 ℃ (Pubchem.ncbi.nlm.nih.gov, 2019). A new ketone 3-hydroxy-2-butanone appeared in soak-steamed samples, which was reported in wheat in a previous study (Dong et al., 2018).

Roasting was the last processing step of wheat bran tea processing, pyrazines and phenol were only detected in the roasted samples (**Table 2**). Pyrazines could be formed by the reaction of reducing sugar and amino acid through Strecker degradation of Millard reaction (Misnawi et al., 2004). Three pyrazines were identified and two of them (2,6-dimethyl- pyrazine and 2-ethyl-3-methyl-pyrazine) were previously reported in roasted whole wheat (Moskowitz et al., 2012). Pyrazines provide nutty and chocolate aroma and are responsible for the aroma of many roasted food products (Misnawi et al., 2004). Soak-roasted samples have a significant higher pyrazine content than the cellulase pretreated sample after the roasting process. This may due to the higher phenolic content in the cellulase treated sample. It was reported that polyphenols were able to react with reducing sugars, thus decreases its availability to produce pyrazines (Misnawi et al., 2004). The aldehyde contents of all roasted samples were decreased compared to the steamed samples. Wu, Xu and Akoh (2014) reported that the concentrations of medium-chain aliphatic aldehydes (nonanal, 2,4-nonadienal, 2,4-decadienal, (E, E)-2,4-nonadienal, and (E, E)-2,4-decadienal) were reduced after roasting. Likely the loss in aldehydes was due to their low boiling temperature and then evaporated during the roasting process. The concentration of 2-pentyl furan was further increased in the roasted sample and became the most abundant aroma compound, which was in consistent to the previous reports (Moskowitz et al., 2012; Dong et al., 2018). 2-Methoxy-4-vinylphenol was detected in the roasted samples of both cellulose-roasted and soak-roasted samples. It has a woody and smoky flavor and commonly observed in wheat and buckwheat (Yajima et al., 1983). Previous study reported that 2-methoxy-4-vinylphenol could be formed by thermal decarboxylation of ferulic acid (Fava et al., 2009), which could be further converted into vanillin. The convention of ferulic acid to 2-methoxy-4-vinylphenol and vanillin could relieve the bitterness of ferulic acid, but decrease the antioxidant activity (Yan et al., 2016). The ketones of 3-hydroxy-2-butanone was disappeared in the soaking pretreated samples after the roasting process, possibly because of its low boiling point of 147.8 °C (Pubchem.ncbi.nlm.nih.gov, 2019). The alkane concentration was increased in both samples after the roasting due to the breakdown of cell wall and increment of alkane extraction rate (Wu et al., 2013). Carboxylic acids content in soak-roasted samples were increased and nonanoic acid appeared in cellulase-roasted samples. As aforementioned, this may due to the elevated extraction rate at higher temperature (Canari and Eyal, 2003).

3.3 *Principal component analysis (PCA) of wheat bran tea processing*

PCA of wheat bran tea processing was performed based on the data from aroma and antioxidant profile **(Figure 2)**. Total pyrazines and phenol are the major contributor to the positive aspect of the X-axis (PC1) and the total ester is the major contributor to the positive aspect of Y-axis (PC2). The total aldehyde is the major contributor to the negative aspect of Y-axis and total ketone is the major contributor to the negative aspect of X-axis. Furan has an equal contribution to both the positive aspect of X-axis and the negative aspect of Y-axis.

Processing significantly influenced the antioxidant and aroma profile of wheat bran tea. In raw wheat bran, alcohols and esters are the key compounds and decreased during processing. Soaking, steaming and cellulase treated samples have high total ketone content, and steaming and cellulase pretreated samples have a higher concentration of aldehyde and furan. Soak-roasted samples have a higher concentration of pyrazine and cellulase-roasted samples have a higher concentration of phenol. Samples were varied in polyphenols and antioxidant activity, with cellulase, soaking and steaming processed samples of much lower TPC and antioxidant activity compared to raw and roasted samples.

4. Conclusion

This study investigated the impacts of processing technologies on the antioxidant activity and volatile profile of wheat bran tea. Raw bran was either soaking or cellulase treated, then subjected to steaming and roasting to produce a novel cereal beverage. Both soaking and cellulase pretreatments significantly decreased the phenolic contents and antioxidant activities of wheat bran, while they were increased after steaming and roasting. Cellulase treated wheat bran tea has significantly higher antioxidant activity compared to soaking treated ones. A total of 40 volatile compounds were identified in wheat bran tea. Similar change trend of volatile compounds during processing was observed in both soaking and cellulase pretreated samples. Alcohol and esters were the major volatile compounds in raw wheat bran. After soaking and cellulase treatments, alcohol and esters content were decreased, while aldehyde and furan concentrations were increased. After steaming, aldehydes and furan concentration were increased and became the major volatile compounds. Roasting process further increased furan content and 2-pentyl furan became the most abundant volatile compound. Phenols and pyrazines were only detected after the roasting process. Comparing the two pretreatments, cellulase pretreated wheat bran tea could be better than the soaking pretreated wheat bran tea because of higher antioxidant activity and higher contents of volatile compounds. However, a sensory test may be required in the future study to examine consumers' acceptance of these products.

Conflict of interest

All authors declare no conflict of interest.

5. Reference

Canari, R. and Eyal, A. (2003). Extraction of carboxylic acids by amine-based extractants: apparent extractant basicity according to the pH of half-neutralization. *Industrial & Engineering Chemistry Research*, 42(7), pp.1285-1292.

Das, M., Banerjee, R. and Bal, S. (2008). Evaluation of physicochemical properties of enzyme treated brown rice (Part B). *LWT - Food Science and Technology*, 41(10), pp.2092-2096.

Di Gioia, D., Sciubba, L., Ruzzi, M., Setti, L. and Fava, F. (2009). Production of vanillin from wheat bran hydrolyzates via microbial bioconversion. *Journal of Chemical Technology & Biotechnology*, 84(10), pp.1441-1448.

Fao.org. (2019). *Wheat - the largest primary commodity*. [online] Available at: http://www.fao.org/resources/infographics/infographics-details/en/c/240943/ [Accessed 4 Jan. 2019].

Fardet, A. (2010). New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre?. *Nutrition Research Reviews*, 23(1), pp.65-134.

Feng, Y. and Mcdonald, C. (1989). Comparison of flavonoids in bran of four classes of wheat. *Cereal chemistry*, 66(2), pp.516-518.

Floegel, A., Kim, D., Chung, S., Koo, S. and Chun, O. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis*, 24(7), pp.1043-1048.

Hui, Y. (2007). *Handbook of food products manufacturing*. 1st ed. Hoboken (New Jersey): Wiley-Interscience, pp.241-290.

Ji, T., Kang, M. and Baik, B. (2017). Volatile organic compounds of whole-grain soft winter wheat. *Cereal Chemistry Journal*, 94(3), pp.594-601.

Kiritsakis, A. (1998). Flavor components of olive oil-A review. *Journal of the American Oil Chemists' Society*, 75(6), pp.673-681.

Li, Y., Zhang, L. and Wang, W. (2012). Formation of aldehyde and ketone compounds during production and storage of milk powder. *Molecules*, 17(8), pp.9900-9911.

Lippolis, V., Pascale, M., Cervellieri, S., Damascelli, A. and Visconti, A. (2014). Screening of deoxynivalenol contamination in durum wheat by MOS-based electronic nose and identification of the relevant pattern of volatile compounds. *Food Control*, 37, pp.263-271.

Lucas, T., Le Ray, D. and Mariette, F. (2007). Kinetics of water absorption and solute leaching during soaking of breakfast cereals. *Journal of Food Engineering*, 80(2), pp.377-384.

Marty, C. and Berset, C. (1990). Factors affecting the thermal degradation of all-trans-beta-carotene. *Journal of Agricultural and Food Chemistry*, 38(4), pp.1063-1067.

Misnawi, Jinap, S., Jamilah, B. and Nazamid, S. (2004). Effect of polyphenol concentration on pyrazine formation during cocoa liquor roasting. *Food Chemistry*, 85(1), pp.73-80.

Moskowitz, M., Bin, Q., Elias, R. and Peterson, D. (2012). Influence of endogenous ferulic acid in whole wheat flour on bread crust aroma. *Journal of Agricultural and Food Chemistry*, 60(45), pp.11245-11252.

Pasqualone, A., Summo, C., Laddomada, B., Mudura, E. and Coldea, T. (2018). Effect of processing variables on the physico-chemical characteristics and aroma of borş, a traditional beverage derived from wheat bran. *Food Chemistry*, 265, pp.242-252.

Patras, A., Brunton, N., Da Pieve, S. and Butler, F. (2009). Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innovative Food Science & Emerging Technologies*, 10(3), pp.308-313.

Pizzimenti, S., Ciamporcero, E., Daga, M., Pettazzoni, P., Arcaro, A., Cetrangolo, G., Minelli, R., Dianzani, C., Lepore, A., Gentile, F. and Barrera, G. (2013). Interaction of aldehydes derived from lipid peroxidation and membrane proteins. *Frontiers in*

Physiology, 4, pp.1-17.

Prückler, M., Siebenhandl-Ehn, S., Apprich, S., Höltinger, S., Haas, C., Schmid, E. and Kneifel, W. (2014). Wheat bran-based biorefinery 1: Composition of wheat bran and strategies of functionalization. *LWT - Food Science and Technology*, 56(2), pp.211-221.

Pubchem.ncbi.nlm.nih.gov. (2019). *The PubChem Project*. [online] Available at: https://pubchem.ncbi.nlm.nih.gov/ [Accessed 13 Mar. 2019].

Réblová, Z. (2012). Effect of temperature on the antioxidant activity of phenolic acids. *Czech Journal of Food Sciences*, 30(No. 2), pp.171-175.

Shahidi, F. and Ho, C. (2005). *Phenolic compounds in foods and natural health products*. 1st ed. Washington, DC: American Chemical Society, pp.10-18.

Sharma, K., Ko, E., Assefa, A., Ha, S., Nile, S., Lee, E. and Park, S. (2015). Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. *Journal of Food and Drug Analysis*, 23(2), pp.243-252.

Vaher, M., Matso, K., Levandi, T., Helmja, K. and Kaljurand, M. (2010). Phenolic compounds and the antioxidant activity of the bran, flour and whole grain of different wheat varieties. *Procedia Chemistry*, 2(1), pp.76-82.

Verma, B., Hucl, P. and Chibbar, R. (2009). Phenolic acid composition and antioxidant capacity of acid and alkali hydrolysed wheat bran fractions. *Food Chemistry*, 116(4), pp.947-954.

Wentzel, A., Ellingsen, T., Kotlar, H., Zotchev, S. and Throne-Holst, M. (2007). Bacterial metabolism of long-chain n-alkanes. *Applied Microbiology and Biotechnology*, 76(6), pp.1209-1221.

Wu, G., Johnson, S., Bornman, J., Bennett, S. and Fang, Z. (2017). Changes in whole grain polyphenols and antioxidant activity of six sorghum genotypes under different irrigation treatments. *Food Chemistry*, 214, pp.199-207.

Wu, L., Huang, Z., Qin, P. and Ren, G. (2013). Effects of processing on phytochemical profiles and biological activities for production of sorghum tea. *Food Research International*, 53(2), pp.678-685.

Wu, S., Xu, T. and Akoh, C. (2014). Effect of roasting on the volatile constituents of *Trichosanthes kirilowii* seeds. *Journal of Food and Drug Analysis*, 22(3), pp.310-317.

Xiong, Y., Zhang, P., Luo, J., Johnson, S. and Fang, Z. (2019). Effect of processing on the phenolic contents, antioxidant activity and volatile compounds of sorghum grain tea. *Journal of Cereal Science*, 85, pp. 6-14.

Xu, B. and Chang, S. (2008). Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. *Food Chemistry*, 110(1), pp.1-13.

Xu, L., Yu, X., Li, M., Chen, J. and Wang, X. (2017). Monitoring oxidative stability and changes in key volatile compounds in edible oils during ambient storage through HS-SPME/GC–MS. *International Journal of Food Properties*, 20(sup3), pp. S2926-S2938.

Yajima, I., Yanai, T., Nakamura, M., Sakakibara, H., Uchida, H. and Hayashi, K. (1983). Volatile flavor compounds of boiled buckwheat flour. *Agricultural and Biological Chemistry*, 47(4), pp.729-738.

Yan, L., Chen, P., Zhang, S., Li, S., Yan, X., Wang, N., Liang, N. and Li, H. (2016). Biotransformation of ferulic acid to vanillin in the packed bed-stirred fermentors. *Scientific Reports*, 6, 34644.

Zhang, W., Liu, X., Yang, Z., Song, H., Zhang, Y. and Jin, Y. (2018). Effect of soaking and temperature process on the volatile compounds in soymilk made by soymilk maker. *Journal of Food Science and Technology*, 55, 1591-1598.

Zhang, Z. (2012). Analysis on pyrolytic behavior of wheat straw multifunctional materials by Py-GC-MS. *Advanced Materials Research*, 496, pp.194-197.

Zhang, Z., Li, D. and Zhang, L. (2013). Effect of heating on the fatty acid composition and oxidation products of flaxseed oil. *Asian Journal of Chemistry*, 25(18), pp.10082-10086.

Zheng, X., Zhang, R. and Liu, C. (2015). Extraction and antioxidant activity of phenolic compounds from wheat bran treated by steam explosion. *Tropical Journal of Pharmaceutical Research*, 14(10), pp. 1865-1870.

Figure and table captions

Figure 1. Flow chart of the wheat bran tea processing.

Figure 2. Principal component analysis of wheat bran tea processing. PC1 (44.29%) and PC2 (25.86%) explained in total of 70.15 % of variance.

Table 1. Effect of tea processing on phenolic contents and antioxidant activity of wheat bran

Table 2. Changes in volatile compounds during wheat bran tea processing (ng/g)

Table 1. Effect of tea processing on phenolic contents and antioxidant activity of wheat bran

Analysis	Pretreatment/Sample	[i] Raw	Processed		
			[ii] Pretreatment	Steam	Roast
TPC (µg GAE/g)	Soak	[iii] 1032.0±7.1^{aA}	124.0±5.8^{bB}	213.7±10.3^{bB}	1070.12±16.0^{bA}
	Cellulase	1032.0±7.1^{aB}	323.3±1.9^{aC}	366.8±1.0^{aC}	1258.31±9.1 ^{aA}
TFC (µg CAE/g)	Soak	187.1±1.2^{aA}	32.1±0.2^{bD}	38.4±0.4^{bC}	97.5±2.5^{bB}
	Cellulase	187.1±1.2^{aA}	47.7±0.7^{aC}	49.5±0. 9^{aC}	143.6±1.4^{aB}
DPPH (µg TEAC/g)	Soak	1420.2±2.0^{aA}	765.1±4.7^{bD}	880.0±3.6^{bC}	1141.0±3.7^{bB}
	Cellulase	1420.2±2.0^{aA}	820.2±7.3^{aD}	979.6±5.2^{aC}	1258.3±9.0^{aB}
ABTS (µg TEAC/g)	Soak	1657.1±1.1^{aA}	664.6±0.4^{bD}	785.9±0.7^{bC}	1254.4±0.3^{cB}
	Cellulase	1657.1±1.1^{aA}	807.2±0.9^{aC}	1018.9±12.3^{aB}	1651.8±2.2^{aA}

[i] All samples were processed from the same raw material and measured in triplicates.

[ii] Two different pretreatments, soaking and cellulase, were applied.

[iii] Superscript capital and small letters represent significant difference within a row and column respectively ($p<0.05$)

Table 2. Changes in volatile compounds during wheat bran tea processing (ng/g)

Volatiles	RI actual	RI db	Pre-treatment: Soak				Pre-treatment: Cellulase				Flavor***	Identification Method*****
			Raw	Soak	Steam	Roast	Raw	Cellulase	Cellulase steam	Cellulase roast		
Alcohol			2475.6±193.6^{aA}	1247.9±155.9^{bB}	ND	ND	2475.6±193.6^{bA*}	1658.6±58.9^{aA}	ND	ND		
1-Hexanol	986	990	2475.6±193.6^{a}	1114.7±367.3^{b}	ND	ND	2475.6±193.6^{a}	1267.9±6.4^{b}	ND	ND	Green, herbal	MS/RI
Phenylethyl Alcohol	1107	1109	ND**	133.7±162.2^{a}	ND	ND	ND**	ND	ND	ND	Floral	MS/RI
1-Octen-3-ol	1068	1072	ND	ND	ND	ND	ND	390.6±52.4^{a}	ND	ND	Earthy, mushroom	MS/RI
Pyrazine			ND	ND	ND	2275.2±56.5^{aA}	ND	ND	ND	104.9±21.2^{aB}		
Pyrazine, methyl-	824	825	ND	ND	ND	589.6±138.9^{a}	ND	ND	ND	ND	Nutty	MS/RI
Pyrazine, 2,6-dimethyl-	912	909	ND	ND	ND	943.0±86.0^{a}	ND	ND	ND	104.9±21.2^{aB}	Chocolate, nutty	MS/RI
Pyrazine, 2-ethyl-3-methyl-	999	1001	ND	ND	ND	742.3±49.6^{a}	ND	ND	ND	ND	Nutty	MS/RI
Ester			3298.0±428.8^{aA}	2208.8±48.0^{bA}	66.1±42.4^{cA}	ND	3298.0±428.8^{aA}	34.3±4.0^{bB}	81.2±9.1^{bA}	ND		
Hexanoic acid, methyl ester	924	927	2567.6±372.6^{a}	1094.7±181.0^{b}	10.3±13.6^{c}	ND	2567.6±372.6^{a}	ND	ND	ND	Fruity	MS/RI
Hexanoic acid, ethyl ester	1000	999	126.6±38.6^{a}	50.2±6.8^{b}	4.3±6.6^{c}	ND	126.6±38.6^{a}	ND	ND	ND	Fruity	MS/RI
Heptanoic acid, methyl ester	1022	1021	169.6±17.6^{a}	ND	ND	ND	169.6±17.6^{a}	ND	ND	ND	Fruity	MS/RI
Nonanoic acid, methyl ester	1222	N/A	48.5±6.1^{a}	ND	ND	ND	48.5±0. 61^{a}	ND	ND	ND	Fruity, winey	MS
Hexadecanoic acid, methyl ester	1991	1975	78.6±42.6^{b}	158.7±78.2^{a}	ND	ND	78.6±42.6^{a}	ND	ND	ND	Waxy	MS/RI
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	2088	2082	18.6±6.6^{a}	ND	15.3±12.6^{a}	ND	18.6±6.6^{a}	ND	ND	ND	N/A****	MS/RI
9-Octadecenoic acid (Z)-, methyl ester	2094	2087	6.6±1.6^{b}	ND	12.3±7.6^{a}	ND	6.6±1.6^{a}	ND	ND	ND	N/A	MS/RI
Pentadecanoic acid, 14-methyl-, methyl ester	1925	1925	ND	ND	9.0±116.7^{a}	ND	ND	ND	ND	ND	N/A	MS
Hexadecanoic acid, ethyl ester	1925	1926	ND	905.7±351.3^{a}	ND	ND	ND	34.3±4.0^{a}	ND	ND	Waxy, green	MS/RI
Octanoic acid, methyl ester	1122	1120	282.6±22.6^{a}	ND	ND	ND	282.6±22.6^{a}	ND	ND	ND	Waxy	MS/RI
Octanoic acid, ethyl	1196	1193	ND	ND	ND	ND	ND	ND	81.2±9.1^{a}	ND	Waxy	MS/RI

ester												
Furan			496.6±36.6^{dA}	1099.2±115.3^{cB}	2754.9±246.0^{bB}	4775.1±354.0^{aB}	496.6±36.6^{dA}	4985.2±677.0^{cA}	5641.4±22.5^{bA}	9188.7±2.5^{aA}		
Furan, 2-pentyl-	989	993	496.6±36.6^{d}	1099.2±115.3^{c}	2754.9±246.0^{b}	4775.1±354.0^{a}	496.6±36.6^{d}	4985.2±677.0^{c}	5641.4±22.5^{b}	9188.7±2.5^{a}	Fruity, green	MS/RI
ketone			105.6±7.6^{cA}	169.7±7.6^{bB}	297.2±21.9^{aA}	ND	105.6±7.6^{aA}	1062.3±5.4^{aA}	ND	ND		
2(3H)-Furanone, 5-ethyldihydro-	1047	1047	105.6±7.6^{a}	ND	ND	ND	105.6±7.6^{a}	ND	ND	ND	Tonka, creamy	MS/RI
2-Butanone	814	403	ND	169.7±0.0076^{b}	ND	ND	ND	1062.3±5.4^{a}	ND	ND	Fruity	MS
3-Hydroxy-2-butanone	729	720	ND	ND	297.2±21.9^{a}	ND	ND	ND	ND	ND	Buttery, creamy	MS/RI
Aldehyde			16.6±3.6^{dA}	5935.5±559.7^{bB}	9426.5±474.5^{aB}	590.3±20.0^{cB}	16.6±3.6^{dA}	12010.2±1402.2^{bA}	21885.2±1227.8^{aA}	2587.6±356.1^{cA}		
2-Nonenal, (E)-	1157	1155	16.6±3.6^{a}	132.7±679.1^{b}	ND	33.7±16.0^{c}	16.6±3.6^{c}	437.8±79.1^{a}	79.2±6.1^{b}	ND	Fatty, green	MS/RI
2-Octenal, (E)-	1056	1062	ND	119.7±328.5	ND	ND	ND	1567.3±153.4	ND	ND	Fatty	MS/RI
2,4-Decadienal, (E,E)-	1313	1314	ND	2729.7±544.9^{a}	8459.5±444.0^{b}	ND	ND	1235.2±404.4^{b}	21802.0±1221.1^{a}	1978.7±2.6^{b}	Fatty	MS
2,4-Decadienal	1283	1284	ND	2953.7±377.4^{a}	661.7±44.0^{b}	ND	ND	735.5±219.1^{a}	ND	112.7±2.6^{b}	Fatty	MS
Benzaldehyde	958	961	ND	ND	301.0±7.7^{a}	93.3±8.6^{b}	ND	ND	ND	139.7±2.6^{a}	Fruity	MS/RI
Benzeneacetaldehyde	1040	1045	ND	ND	ND	463.3±33.6^{a}	ND	ND	ND	357.7±2.6^{a}	Green	MS
Hexanal	807	802	ND	ND	ND	ND	ND	7052.8±354.9^{a}	ND	ND	Green, fatty	MS/RI
2-Heptenal, (E)-	956	955	ND	ND	ND	ND	ND	327.4±37.6^{a}	ND	ND	Green	MS/RI
2,4-Nonadienal, (E,E)-	1213	1211	ND	ND	ND	ND	ND	654.1±153.4^{a}	ND	ND	Fatty	MS/RI
Carboxylic cacid			6.6±0.6^{bA}	ND	ND	443.3±133.6^{aA}	6.6±0. 6^{bA}	ND	871.2±62.1^{a}	857.1±89.7^{aB}		
Nonanoic acid	1265	1272	ND	ND	ND	ND	ND	ND	ND	275.7±2.6	Waxy, fatty	MS/RI
Hexanoic acid	986	990	6.6±06^{a}	ND	ND	ND	6.6±0. 6^{a}	ND	ND	ND	Fatty, cheesy	MS/RI
Octanoic acid	1178	1180	ND	ND	ND	443.3±133.6^{a}	ND	ND	871.2±62.1^{a}	581.7±2.6^{b}	Fatty, soapy	MS/RI
Alkane			42.6±1.3^{b}	432.8±100.6^{a}	ND	20.1±6.0^{c}	42.6±1.3^{c}	482.4±62.2^{b}	183.2±24.1^{c}	1301.9±272.6^{a}		
Nonadecane	1098	1900	20.6±1.6^{b}	196.7±517.7^{a}	ND	ND	0.0206±0.0016^{b}	ND	ND	437.7±2.6^{a}	Alkane	MS
Tetradecane, 5-methyl-	1339	1454	ND	ND	ND	20.1±6.0^{a}	ND	ND	ND	ND	Alkane	MS
Tetradecane	1397	1400	ND	ND	ND	ND	ND	111.6±16.0^{a}	ND	ND	Alkane	MS/RI
Dodecane	1200	1200	ND	ND	ND	ND	ND	370.7±46.1^{b}	183.2±24.1^{c}	414.7±2.6^{a}	Alkane	MS/RI
Tridecane	1304	1300	ND	ND	ND	ND	ND	ND	ND	320.7±2.6^{a}	Alkane	MS/RI
Heneicosane	1271	N/A	22.1±1.0^{a}	236.7±95.3^{b}	ND	ND	22.1±1.0^{a}	ND	ND	ND	Alkane	MS
Phenol			ND	ND	ND	37.3±3.6^{aB}	ND	ND	ND	133.7±102.2^{aA}		
2-Methoxy-4-vinylphenol	1302	1309	ND	ND	ND	37.3±3.6^{a}	ND	ND	ND	133.7±102.2^{a}	Woody, smoky	MS/RI
Total volatile compounds			6441.6±672.1^{c}	11093.9±987.1^{b}	12544.7±784.8^{a}	8141.3±573.7^{c}	6441.6±672.1^{d}	20233.0±1980.0^{b}	28662.2±2000.1^{a}	14173.9±844.3^{c}		

* Superscript letters of a, b, c, d indicate significant difference within each pretreatment group, while A, B indicate the significant difference

between two types of pretreatment in total volatiles ($p<0.05$);

**Not detected or value below detection threshold;

***Compared to data from http://www.thegoodscentscompany.com/ (The Good Scents Company, 2018);

****Not available;

****MS (mass spectra) and RI (retention index) were compared with NIST database; external standards were used to identify and quantify volatile compounds.

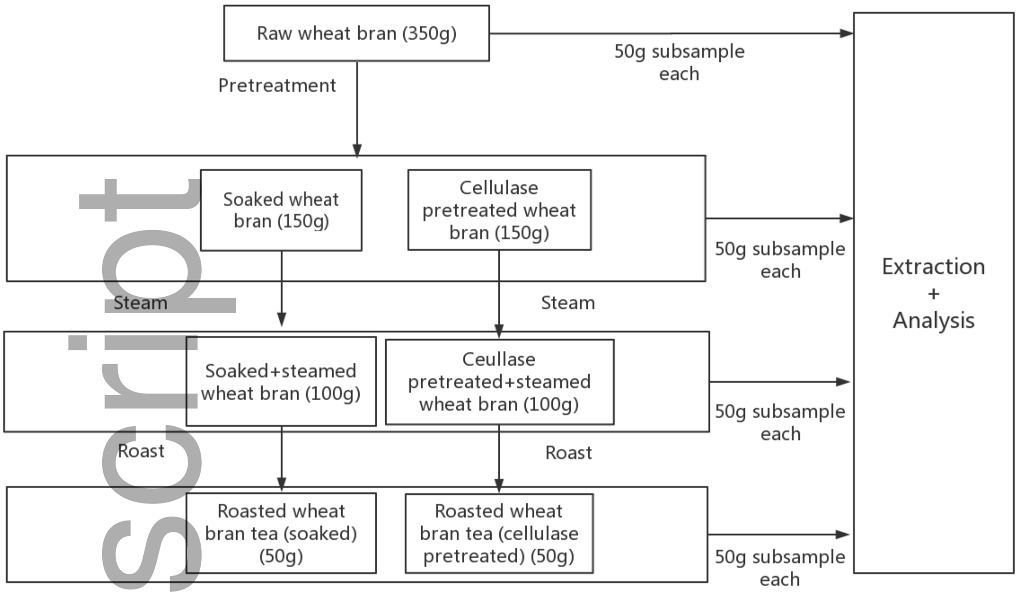

Figure 1. Flow chart of the wheat bran tea processing.

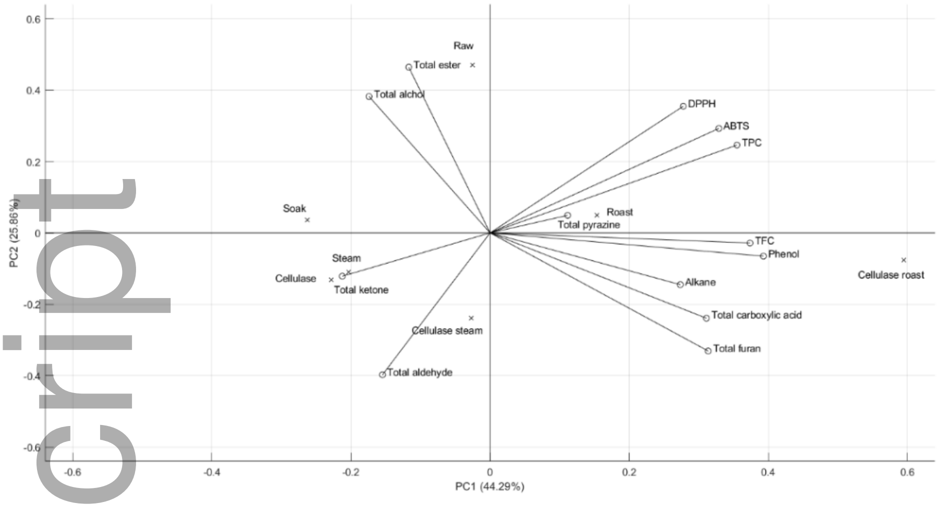

Figure 2. Principal component analysis of wheat bran tea processing. PC1 (44.29%) and PC2 (25.86%) explained in total of 70.15 % of variance.