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Research Article

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Volatiles components and total flavonoids in buckwheat and highland barley brewing wine

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ABSTRACT

Three kinds of brewing wine were made by using tartary buckwheat, highland barley and mixed them 1:1 as raw material. The volatile flavor compounds of wines were evaluated by HS-SPME-GC-MS. Total flavonoids content were detected by $Al(NO_3)_3$-$NaNO_2$ colorimetric method. The results showed it was 13.404% 1,3-prop anediol in buckwheat wine, 23.328% 1-pentanol in highland barley, has different characteristic components in three kind wines. The flavonoids content of tartary buckwheat wine was higher than both highland barley and mixture wine and it was 2.4 mg/g.

Key words: buckwheat, highland barley, wine, volatile flavor, flavonoids

INTRODUCTION

Highland barley*(Hordeum vulgare L.)* named in Chinese as Qing Ke, is an indispensable and important ingredient in China Tibet cuisine, which is listed in a coarse grains and used as feed grain [1]. In terms of annual production, barley is the fourth most important cereal around the world, after wheat, rice and corn. It contains about various phytochemicals such as phenolic acids[2], β-glucan[3], which have the potential to lower cholesterol and blood glucose levels. It's adaptable to high altitude and also tolerate low temperature.Unlike phenolic acids and -β-glucan, which were studied and applied widely, the aroma compound of fermented barley production was neglected.

Tartary buckwheat (*Fagopyrum tataricum*) is a functional food, one of the ancient crops cultivated all over the world[4]. As a nutritional food , there are proteins, fats, amino acids, vitamins, mineral elements in human diets[5]. A large variety of buckwheat foods have been produced, including tea, cookies, noodle, vinegar, etc[6-7]. In the recent decades, flavors research from tartary buckwheat was increased. Volatiles were extracted by distillation and evaluated with HS-SPME-GC-MS. It is buckwheat that can be distinguished by analysis of aroma compound[8]. Aroma compounds contributed to varieties of plant materials, which are usually present in trace,even ultratrace amounts, comprising a diverse range of chemical compounds.

As one of the traditional Chinese alcohol drink especially in South China, rice wine is a favorite alcohol beverages for its flavor, nutritional and aromatic compounds. Rice wine is benefit for human health mainly due to their phytochemical and functional effects[9]. Unlike Chinese liquors and beer, which were used as alcohol drinking widely, rice wine,commercially available fermented alcoholic beverages, made from cereal was ignored. Novel fermentation rice wine was based on buckwheat or barley as material,after stewing, then mixing koji and finally fermenting, tahat made fermented rice wine. The increasing number of people pay more attention to the natural and functional brewing wine.

This study mainly used tartary buckwheat and highland barley instead of glutinous rice to make ferment rice wine, choosing solid-state fermentation method for processing wine. Thus, the objective of this study was (1) to determine

the flavonoids contents in the polyphenol extract from three kinds of wine by colorimetric method, and (2) investigate the characteristic flavor compounds in finished fermented rice wine processed replace glutinous rice.

EXPERIMENTAL SECTION

Seeds

Heifeng No.1(tartary buckwheat, Fagopyrum tararicum Gaertn) and highland barley No.1 were provided from Shanxi Academy of Agricalture Science in Taiyuan, P.R. China in 2015.

Reagents and Materials

Koji was purchased by supermarket. Rutin (≥95%) were purchased from Sigma-Aldrich Co. Ltd.(Shanghai,China). All chemicals were analytical grade. The SPME manual holder and the fibers of polydimethylsiloxane (Carboxen/PDMS,75 μm), were purchased from Supelco, USA. The GC-MS 6820/5973 was Aglient Technologies.

Preparation of Samples

Tartary buckwheat seeds(TB) were placed in a 4 ℃ room before initiation of experiments. A 200.000 g amounts of TB put into 250 mL flasks and soaked in distilled water for 12 h. Water saturated seeds were placed in a chamber and cooked with steam for 2 h. After steaming, it was placed in room and cooled at 30 ℃. Take the TB and 0.4% koji mixed , sealed in flasks and stored for 36 - 48 h at 30 ℃ to. After fermentation (36-48 h) in incubator, the solution was centrifuged at 5 ℃ for 15 min at 4000 rpm. The supernatant collected and used for the total flavonoids assays and GC-MS. Extractions were done in triplicate.

The highland barley seeds(HB) was replaced the TB, using the method of processing of TB fermented wine , which were weight 200.000 g, to make highland barley rice wine.

The mixture fermented rice wine was made from TB and HB, including 100.000 g for each one.

Determination of total flavonoids content

The total flavonoids content in samples were analyzed by colorimetric method reported by Liu et al. with some modifications[10]. Extract (1 mL), as prepared after fermentation,was processed with a commercial grinder and dispersed in 5 mL of 70% aqueous ethanol. 0.75 mL 5% sodium nitrite solution was added and the mixture allowed to stand for 5 min. Next, 10 mL of 1 mol/L sodium hydroxide solution was added and the volume topped to 20 mL with 70% ethanol. Absorbance was read at 510 nm against a blank. A standard curve of concentration (0, 6, 12, 18, 24 and 30 μg/mL) versus absorbance was constructed using rutin standard. All tests were done in triplicate and results expressed as mg equivalent of rutin/100 g.

HS-SPME volatiles isolation

Vials were immediately capped and placed on a temperature controlled tray for 60 min at 40 ℃ with the SPME fiber inserted into the headspace above the wine sample. Adsorption was timed for 50 min. A system blank containing no plant material was run as a control.

GC-MS analysis

The analyses were performed using a gas chromatographer(Agilent 7860A) fitted with a splitless injection with a liner suitable for SPME analysis and an Agilent 5973C mass spectrometer (MS) detector. SPME fibers were desorbed at 245 ℃ for 5 min in the injection port, and volatiles were separated on a DB-5 capillary column (60 m length, 0.25mminner diameter, and 0.25 μm film).

The sample (8 mL) was placed inside 20mL clear glass vials contained 3.000 g of sodium chloride and magnetic stirrers. The vials were sealed with polyethylene and silicone septum cap. The samples were conditioned in thermostatic bath at 40±1.0 ℃ for 5 min. Vials were then immediately capped and placed on a temperature controlled tray for 1 h at 40±1.0 ℃ with the SPME fiber inserted into the headspace above the wine sample. SPME Fibre 75 μm Carboxen/PDMS and manual holder were purchased from Supelco Park (USA). The extracts were desorbed in the injection port of GC-MS. Helium was used as the carrier gas at a flow rate of 1 mL•min$^{-1.}$

Samples were analyzed on a DB-INNWAX capillary column 30 m×0.25 mm×0.25 μm. The injector temperature was 245 ℃. The oven temperature was programmed as follows: 5 min at 40 ℃→5 ℃•min^{-1}→230 ℃. The MS transfer line was kept at 250 ℃.

STATISTICAL ANALYSIS

Results were presented as mean ± standard deviation (S.D). Linear regression was applied for the working range, and mean values, standard deviations, and variances were computed using SAS 9.0. The P values less than 0.05 were regarded as significant differences, and the P values less than 0.01 were very significant differences.

RESULTS AND DISCUSSION

Total flavonoids in material and wine

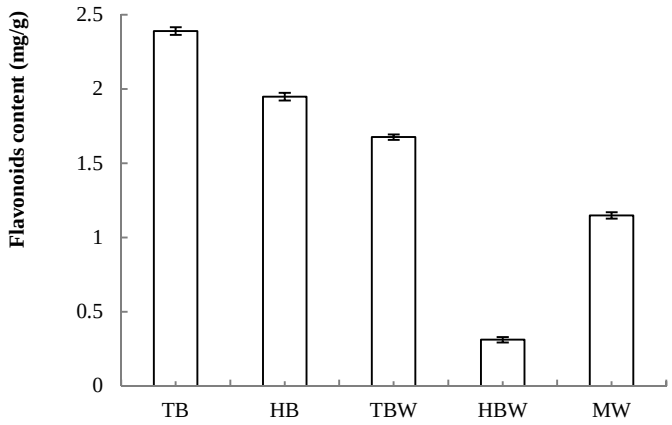

Fig1. flavonoids content in fermented wine and raw materials

The standard deviations (n =3); TB=tartary buckwheat seed; HB=highland barley seed; TBW=tartary buckwheat wine; HBW=highland barley wine; MW=mixture wine with tartary buckwheat and barley

The amount of total flavonoids in the fermented wine was investigated using the $Al(N0_3)_3$-$NaNO_2$ colorimetric method. During the test, the total flavonoids content in among three wine decreased. And the total flavonoids level of TB were 15% more than HB(Fig.1). A standard linear was A=0.6919 C - 0.0144 (r^2 = 0.9995) suggested that the curve was reliable and indispensable. After brewing with koji, content was decreased for wine samples ranged from 0.3 to 2.4 mg/g, and the contents order was confirmed, TB＞HB＞TBW＞MW＞MW. Then macromolecules were decomposed into small molecules in the process of koji settlement. The contents was decreased with fermentation from raw materials to productions. The content was reduced from 2.390 mg/g(raw materials) to 1.676 mg/g(fermented wine). The same results as barley, the content was decreased from 1.947 mg/g to 0.312 mg/g.

Identification of flavor compounds using GC-MS

Vials were immediately capped and placed on a temperature controlled tray for 1 h at 40±1.0 ℃. The optimization of the combined treatment was possessed by detecting sensitivity and metabolites, which were used to determine the best condition of extraction on flavor.

In terms of number of constituents and chromatographic separation, the best response was obtained with SPME fibre, whose application was used for low to high polarity volatile and non-volatile compounds[11]. The compositions of three kinds of fermented wine were detected with HS-SPME. A total of 19 volatile compounds were detected.

Eight chemicals were detected and classified into four categories in TBW. Twelve compounds were detected and classified into seven categories in HBW and nine compounds were identified and classified into five categories in MW (as shown in table 1).

Alcohols were main volatile components in the wine, and amine compounds just were detected in TBW, but not detected in others. The most chemical constituents in HBW were shown in Table 1. These volatile aroma differ considerably in composition, according contribute different to overall wine composition. Because of certain substances can be manipulated during fermentation to produce particular product.

Table 1 Chemical components comparison in three kinds of fermented wine

Wine species	Alcohols	Esters	Alkanes	Aldehydes	Ketone	Phenolic	Acids	Amine
TBW	4	n.d.	n.d.	n.d.	n.d.	1	1	2
HBW	5	1	1	1	1	1	2	n.d.
MW	4	n.d.	1	n.d.	1	2	1	n.d.

n.d.= no detected

Determination of volatile flavor in TBW, HBW and MW

Volatile flavor compositions were analyzed using GC-MS after extraction by SPME. Eight volatile compositions were identified in TBW (Table 2), i.e. acetic acid, 2-propanol 1-propoxy, 2,3-butanediol, phenol, propanamide 2-hydroxy, phenylethyl alcohol, 1,3-prop anediol, sec-butylamine. According to aromatic compounds, it may be present smell as fruits or flowers even bitter taste. Some compounds were no detected such as esters, alkanes, aldehydes and ketone.

Table 2 GC-MS results of volatile aromatic compounds from TBW

Number	Retention time（min）	Compounds	Molecular formula	Molecular mass
1	2.359	acetic acid	$C_2H_4O_2$	60.02
2	2.542	2-propanol,1-propoxy	$C_6H_{14}O_2$	118.10
3	3.406	2,3-butanediol	$C_4H_{10}O_2$	90.07
4	8.365	phenol	C_6H_6O	94.04
5	10.363	propanamide,2-hydroxy	$C_3H_7NO_2$	89.05
6	12.019	phenylethyl alcohol	$C_8H_{10}O$	122.07
7	16.430	1,3-prop anediol	$C_3H_8O_2$	76.05
8	17.934	sec-butylamine	$C_4H_{11}N$	73.09

Twelve volatile flavor compounds in HBW were analyzed(Table 3), i.e. 1-pentanol, formic acid, acetic acid ethoxy-, 2-propanone 1-hydroxy, hydrazinecarboxylic acid ethyl ester, propane 1-methoxy-2-methyl, 2,3-butanediol, 2-furanmethanol, isopropyl alcohol, acetaldehyde methoxy, phenol, phenylethyl alcohol.

A certain co-operativity may be existed among different aromatic characteristics of volatile substances. Aldehyde material chemistry was lively, to some extent, which was restored into the corresponding alcohols. Aldehyde material was detected in this wine, and then it provides a synergistic to this flavor from the characteristics of the material. Alkanes compounds can present a sweet fragrance, and ketone substance can show fruit and flower fragrance.

The increase amount of the carbon chain reflects the odor, which was detected hydroxy acetone from HBW, such as green fragrance existing in beer, tobacco and honey. In addition, heterocyclic substances, such as furan, could present a pleasant fruit odor.

Table 3 GC-MS results of volatile aromatic compounds from HBW

Number	Retention time（min）	Compounds	Molecular formula	Molecular mass
1	1.475	1-pentanol	C_5H1_2O	88.09
2	1.876	formic acid	CH_2O_2	46.01
3	1.947	acetic acid,ethoxy-	$C_4H_8O_3$	104.05
4	2.191	2-propanone,1-hydroxy	$C_3H_6O_2$	74.04
5	2.267	hydrazinecarboxylic acid,ethyl ester	$C_3H_8N_2O_2$	104.06
6	2.400	propane,1-methoxy-2-methyl	$C_5H_{12}O$	88.09
7	3.370	2,3-butanediol	$C_4H_{10}O_2$	90.07
8	4.346	2-furanmethanol	$C_5H_6O_2$	98.04
9	6.938	isopropyl alcohol	C_3H_8O	60.06
10	7.873	acetaldehyde,methoxy	$C_3H_6O_2$	74.04
11	8.726	phenol	C_6H_6O	94.04
12	12.131	phenylethyl alcohol	$C_8H_{10}O$	122.07

Nine volatile compositions in MW were identified (table 4), i.e. 1-butanol 3-methyl, propane 1-methoxy-2-methyl, acetic acid, 2-butanone 3-hydroxy, 2,3-butanediol, isopropyl alcohol, phenol, phenol 2-methoxy, phenylethyl alcohol. Overall, the three fermented wine can be used as an alternative resource for further experiment.

Kur-Kononowicz[12] studied the names of the beverages which made of tartary buckwheat and highland barley. Nine kinds of flavor compositions was studied by Manach C[13]and classified into two compounds. Phenolics could

prevent cancer, atherosclerosis and other diseases. The two kinds of cereals are rich in phenolic substances, but some phenolic chemicals was deteced only in the mixed liquor fermented wine. The mechanism of which need to be further studied, the same as antioxidant properties. And then specific effects will be valued in vitro.

Table 4 GC-MS results of volatile aromatic compounds from MW

Number	Retention time （min）	Compounds	Molecular formula	Molecular mass
1	1.470	1-butanol,3-methyl	$C_5H_{12}O$	88.09
2	2.344	propane,1-methoxy-2-methyl	$C_5H_{12}O$	88.09
3	2.267	acetic acid	$C_4H_8O_3$	104.05
4	2.501	2-butanone,3-hydroxy	$C_4H_8O_2$	88.05
5	3.131	2,3-butanediol	$C_4H_{10}O_2$	90.07
6	6.694	isopropyl alcohol	C_3H_8O	60.06
7	8.452	phenol	C_6H_6O	94.04
8	11.211	phenol,2-methoxy	$C_7H_8O_2$	124.05
9	12.045	phenylethyl alcohol	$C_8H_{10}O$	122.07

CONCLUSION

In aluminum nitrate colorimetric method, we used rutin as a standard compound because of its widely spread flavonoids in propolis, and has strong absorbance at concentrations lower than 30 ppm at 510 nm. The results demonstrated that TPC in brewing wine were gradually decreased.

The volatile flavors were extracted from 3 fermented wines with headspace solid phase microextraction technology in the extraction, using GC-MS for analysis. A total of 19 compounds were detected. The species were detected from three wines,7 kinds of alcohols, 1 kind of ester, 1 kind of alkane, 1 kind of aldehyde, 2 kinds of ketones, 2 kinds of phenol, 2 kinds of acids and 1 kind of amine. These flavors were contributed to the formation of raw materials, amino acid metabolism, microbial activities and the interaction. Aromatic substances were absorbed by SPME and analyzed by GC - MS for qualitative analysis. Most of the volatile components among them are not exactly same.

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