Asian Journal of Chemistry; Vol. 26, No. 23 (2014), 7954-7958

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16817

Physico-Chemical Attributes and Nutritional Ingredients of Fresh Chinese Rice Wines from Different Regions

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Received: 4 December 2013; *Accepted*: 16 April 2014; *Published online*: 15 November 2014; AJC-16271

Fresh rice wine is fermented for a couple of days and very popular in the world, especially in Asian countries. Fresh rice wines differ in how they made and where they come from. For better understanding the differences among these fresh rice wines, the physical and chemical attributes and nutritional ingredients of nine kinds of rice wines from different region of China were detected in this paper. Bradford protein assay and colourimetric method, *etc*. were performed in this paper. The results showed that solid content ranged from 2.0435 g/100 mL to 19.2735 g/100 mL, acidity was 0.29-1.05 g/100 mL, alcoholic content was 1.9-5.1 %, protein content was 43.63-85.06 mg/100 mL, total sugar content was 0.31-15.15 g/100 mL. Amino acid was almost same in these rice wines but quite different in quantity. Three kinds of monosaccharide and two kinds of disaccharides were detected. Eight kinds of rice wines contained glucose, three kinds of rice wines contained maltose, two kinds of rice wines contained sucrose, one kind of rice wine contained fructose and xylose was undetected.

Keywords: Rice wine, Physical and chemical attributes, Amino acid, Monosaccharide, Disaccharide.

INTRODUCTION

Rice wine is a fermented beverage with high nutritious value and popular among most of Asian countries, including China, Korea, Japan, India and Thailand. Rice wine is mainly made from glutinous rice, grain or cereal, but with different name and material in different country, such as sweet fermented rice or yellow wine in China, sake in Japan and Ruou in Viet Nam. Even so, rice wine has a similar fermented process inherited from ancestors. Most rice wines are fermented under the natural circumstances with interaction of fungi, yeast and bacteria especially lactic acid bacteria, which is a complicated procedure[1-6]. After the fermentation from grain or cereal, the production was obtained with non-filtration, filtration or distillation. In China, some kinds of rice wine with distillation, such as Kaoliang with a higher alcoholic content, which can reach to 40 % by volume. Otherwise, we call the wine without distillation as "yellow wine" and the ethanol content ranges up to 20 % by volume. Sometimes, there is fermented residue left in rice wine and only fermented for a couple of days. These rice wines are not deep fermented, which means they are fresh. We also called them sweet fermented rice or "Laozao" in China. The sweet ferm- ented rice has a lower alcoholic content about 5 % by volume.

Rice wine with abundant nutritious substances in it attracts more and more investigators. Ferulic Acid, which is the basic component of the medicine to cure cardiovascular and cerebrovascular diseases and leucopenia, in rice wine was detected lately. The result showed that the concentration of ferulic acid increased during the process of fermentation[7]. The comparisons of amino acids between two different rice wine derived from traditional natural fermentation and pure molded mass fermentation, respectively were studied and found out that the rice wine fermented by natural environment have more abundant of amino acids[8]. The organic acid in this two kinds of rice wine was also detected, the result showed that the amount of organic acid in these two kinds of rice wine was equivalent and increased with time consuming[9]. And later investigations showed that there were a variety of amino acids[10], a large amount kinds of sugars[11], biogenic amine contents[12], antioxidant activities[13] and aroma compounds[14] in rice wine, which determined the flavor substances, nutritious components and biologic activities of rice wine.

Nowadays, researches about Chinese rice wine are increased, but most of which were from markets. In this paper, we compared physico-chemical attributes and nutritional ingredients of nine kinds of rice wines, which were diverse in how they made and where they came from. A variety of analytical methods were applied in this paper to analyze the constituents in nine kinds of rice wines from different regions of China. The aim of this study was to support the evaluation of diverse functional and nutritional ingredients in traditional fermented rice wine.

EXPERIMENTAL

Nine kinds of rice wines were obtained from three provinces of China. The samples named as NA1, NA2 and NA3 were from native families in Ning'an, Heilongjiang province. The samples named as MDJ1 and MDZ2 were from native families in Mudanjiang, Heilongjiang province. The FS1 sample was from the market in Fushun, Liaoning province. The six samples which mentioned above were all liquid. The samples named as X1, X3 and X2 were from markets and families respectively in Xiaogan, Hubei province, which were semi-solid. All samples were stored at 4 °C prior to screening for their functional properties.

Physico-chemical analysis: Semi-solid samples were analyzed after centrifuged at 300×g for 1-2 min in a centrifuge (Sigma 3-30k, SIGMA Laborzentrifugen GmbH, Germany) and the supernatant was obtained to be the objects of study in the following experiments. Total solid content was determined using the GB/T method (GB/T 13662-2008). Electric constant temperature drying oven (202-3A, Taisite, China) was applied at 103 ± 2 °C for 4 h to obtain the constant weight of samples. The acidity of rice wine was measured by acid-base titration method. 2 to 5 mL each of samples was placed in a triangular flask with 250 volume, using phenolphthalein as an acid-base indicator. Quantitative analysis was used by definite volumes of base standard solutions (0.9995 mol L^{-1}). Acidity quantity was calculated by the formula of GB/T 15038-2006 method. Alcoholic content was determined by an alcohol meter. Accurately 100 mL sample was placed in a 500 volume of distilling flask and then washed with 50 mL distilled water. The total samples with water were distilled until the volume of liquid condensed from vapor in distillation reached 100 mL. The distilled liquid was held at a temperature of 20 ± 0.1 °C for 0.5 h. Then the distilled liquid was performed in 100 mL graduated cylinder and the amount of ethanol was measured by alcohol meter, the temperature was also measured at the same time. The alcohol result was translated into the temperature of 20 °C according to the sheet of transform (GB/T 15038-2006).

Total protein content determination: Protein content was determined by the Bradford protein assay15.1 mL of each sample with 10 × dilution was placed in a 20 × 200 nm glass tube and added 5 mL of protein reagent, which contained 0.01 % (w/v) Coomassie Brilliant Blue G-250 (Sigma, Germany), 4.7 % (w/v) ethanol and 8.5 % (w/v) phosphoric acid. Then protein concentrations were determined by the absorbance at 595 nm using a spectrophotometer (analytikjena SPECORD/200, analytikjena, Germany). A standard curve was prepared using bovine serum albumin as standard solution.

Amino acids determination: Free amino acids were analyzed by an amino acid analyzer (L-8800, Hitachi Ltd., Japan). 2 mL of each sample was placed in a flask with the volume of 10 mL and then added 2 mL of aqueous solution of sulfosalicylic acid (50 g/L). The flask was swirled for 30 s and made up to 10 mL with HCl solution (0.02 mol/L) after staying for 0.5 h. Then the mixture was filtered through a 0.45 μm membrane. An aliquot of 20 μL was injected into automatic amino acid analyzer and the samples were determined by comparing with standard peak areas.

Hydrolysis amino acids in samples were also determined. The diluent sample with approximately 12 mg crude protein was placed in a 20 mL text tube and then added with 9 mL HCl (6 mol/L). The test tube was sealed up after blowing into the nitrogen and dried at 110 °C for 22 h. The cooled solution was placed in a flask with the volume of 100 mL and neutralized by 9 mL NaOH (6 mol/L). 0.02 mol/L of HCl was added to the flask until to the calibration tail. The solution filtered through a 0.45 μm membrane and 20 μL filtrate was injected into automatic amino acid analyzer to detect the hydrolysis amino acids[16]

Total sugar determination: Amount of sugars in rice wine was analyzed by colourimetric method, which called phenol-sulphuric acid method[17]. Samples were treated with dilutions of 10^3. 20 mL of dilution sample was placed in a test tube and then added with 1 mL phenol and 5 mL sulphuric acid in proper order. The absorbance readings of each test tube were measured at 490 nm using a spectrophotometer.

Determination of monosaccharide and disaccharides in rice wines: Monosaccharide and disaccharides were measured by performing high-performance liquid chromatography (Agilent 1100 series) with a differential refractive index detector, using a chromatographic column (ZORBAX Carbonhydrate 4.6 × 250 mm 5μm). A thermostat was set at 30 °C. The mobile phase was methyl cyanide and water (in the ratio of 75:25 (v/v) methyl cyanide: water) at a flow rate of 1 mL/min. Samples were filtered though 0.22 μm membrane filter before injection into the HPLC system and then 10 μL each of samples was injected into volume for analyzing[18]. Sugar standards of D-sucrose, D-glucose, D-fructose, D-maltose and D-xylose were purchased from Aladdin Industrial Inc.

Statistical analysis: Data analysis was carried out with SPSS software (ver.16.0; SPSS, Chicago, IL). One-way ANOVA was used to determine significant differences between means at a significance level of $\alpha = 0.05$.Tukey's test was used to perform multiple comparisons between means. All analyses were performed in triplicate (n = 3).

RESULTS AND DISCUSSION

Physico-chemical attribution in rice wine: As we discussed above, acidity and alcoholic content are the important index for evaluating the rice wine's quality. Solid content, the kinds of amino acids and monosaccharide represent the nutritious value of rice wine. In this study, we detected the total solid, acidity, pH value and alcoholic content in nine kinds of rice wines from different regions of China and from Table-1, we could see that the solid content in these rice wine ranged from 2.0435 g/100 mL to 19.2735 g/100 mL. Three rice wine samples from Ningan province and the semi-solid sample X2 were the top four acidity samples and the highest acidity in these nine kinds of rice wine reached up to 1.05 g/100 mL. Acidity in the following samples were in turn with X1 > MDJ2

TABLE-1
SOLID CONTENT, ACIDITY AND ALCOHOLIC CONTENT IN THE NINE KINDS OF RICE WINES(x ± SD, n = 3)

Sample	Solid content (g/100 mL)	Acidity (g/100 mL)	Ethanol content (% vol)
NA1	3.7930 ± 0.0410[c]	0.74 ± 0.01[g]	4.3 ± 0.1[d]
NA2	4.0088 ± 0.0473[d]	0.65 ± 0.02[f]	3.3 ± 0.4[c]
NA3	2.0435 ± 0.1775[a]	1.05 ± 0.01[i]	2.6 ± 0.3[b]
MDJ1	4.0883 ± 0.0117[d]	0.29 ± 0.01[a]	1.9 ± 0.2[a]
MDJ2	4.1047 ± 0.0180[d]	0.52 ± 0.01[d]	2.0 ± 0.1[a]
FS1	2.2180 ± 0.0005[b]	0.31 ± 0.01[b]	5.1 ± 0.3[e]
X1	13.3930 ± 0.0070[e]	0.55 ± 0.01[e]	3.0 ± 0.1[c]
X2	19.2735 ± 0.0585[g]	0.81 ± 0.02[h]	4.3 ± 0.1[d]
X3	18.2510 ± 0.0250[f]	0.44 ± 0.01[c]	5.0 ± 0.0[e]

[a,b,c]Means with different letters are significantly different, $p < 0.05$

> X3 > FS1 > MDJ1 and have a significant difference between each other ($p < 0.05$). The alcoholic content in the nine kinds of rice wines ranged from 1.9 % (vol) to 5.1 % (vol).

The result showed that there was higher solid content in three semi-solid rice wines from Xiaogan province than others and there were significant differences among the three samples. The two kinds of rice wines MDJ2 and MDJ1 from Mudanjiang province had similar solid contents and lower than the top of three. The sample NA3 from Ningan province had the lowest solid content in the nine kinds of rice wine. Solid content in rice wine contained ash, simple proteins, fat and sugars, *etc*., which endowed high nutritious value to rice wine. The sample FS1 had the highest alcoholic content and the two samples from Mudanjiang province had the lowest alcoholic contents with no significant difference ($p < 0.05$).

Protein content: The protein content of rice wine was detected as Fig.1 showed. The rice wine MDJ2 that contained 85.06 mg/100 mL protein and X3 that contained 82.22 mg/100 mL protein were the top of two samples of protein containing. There were no significant differences ($p < 0.05$) of protein content in FS1, X1 and X2, which determined as 61.70 mg/100 mL, 55.51 mg/100 mL and 63.99 mg/100 mL, respectively. The lowest protein contained samples were identified as NA1, NA2, NA3 and MDJ1, which determined as 43.63 mg/100 mL, 44.53 mg/100 mL, 46.10 mg/100 mL and 47.98 mg/100 mL and there were no significant differences ($p < 0.05$) in these four samples.

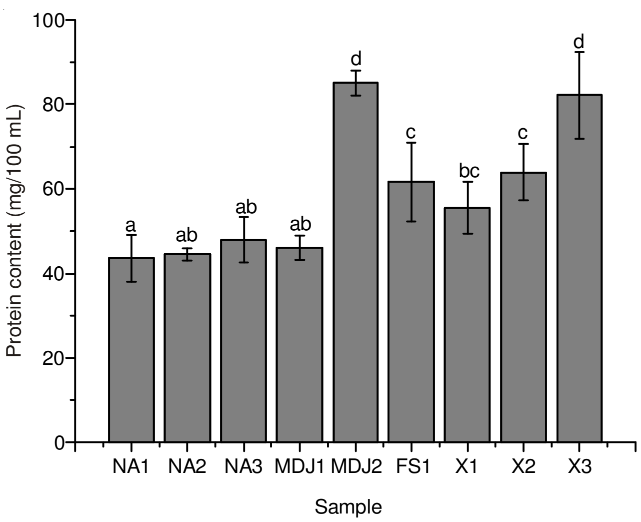

Fig. 1. Protein content in the nine kinds of rice wines a, b, c, d: Different letters indicate significant differences at $p < 0.05$

Acidity, alcoholic content, which were detected above and protein content had little dependency with the type of rice wine but had significant differences among rice wines with different classifications and places of origin, which may be caused by the quality decided factors that usually called raw materials, yeast strains, brewing conditions and maturation conditions[19].

Analysis amino acids: Free amino acids and hydrolysis amino acids were determined by an amino acid analyzer. Free amino acids in nine kinds of rice wine was showed in Table-2. There were some limited amino acids in rice wines, but different in different rice wines. Isoleucine was absent in NA2 and MDJ2 rice wines. Valine, leucine and isoleucine were absent in MDJ1 sample. Tyrosine and arginine in FS1 sample were undetected, so did cysteine in X2 rice wine and histidine in X3 rice wine. Tryptophan, which was hydrolyzed in acid environment, was undetected in all samples, because acid hydrolyzation method was applied in this article. From Table-2 we could see the most abundant amino acids represented as proline, leucine, tyrosine, phenylalanine and alanine in different rice wines. The ratio of essential amino acids in these rice wine samples ranged from 7.24 to 54.57 % and there were more than one sample with high ratio of 40 %. The X2 turned out to be the most essential amino acids containing sample.

The seventeen kinds of hydrolysis amino acids were also determined as showed in Table-2 and ranged from 17.90 mg/100 mL to 166.25 mg/100 mL. Glutamate was the most abundant kind of amino acid in all nine kinds of samples and the content of this kind of amino acid was 3.52-31.49 mg/100 mL. The essential amino acids calculated as hydrolysis amino acids ranged from 23.50 to 34.59 %. The total amino acid content of X2 rice wine, which is semi-solid sample, was served as the highest total amino acid content sample.

Thus, it is seen that the main free amino acid in nine kinds of rice wines are different. On the contrary, main hydrolysis amino acid was represented as glutamate identically, which corresponding with previous report[11], possibly because Glutamate is the maximum kind of amino acid in rice which served as the raw material of rice wine.

Total sugar content: Total sugar content in nine kinds of rice wines was detected as showed in Fig. 2 and X2 sample represented as the highest total sugar contend sample with the value of 15.15 g/100 mL. Total sugar contents in rest of samples was 0.31-11.06 g/100 mL, from which we could see the sugar content in semi-solid samples was higher than liquid samples with significant differences ($p < 0.05$).

TABLE-1
FREE AMINO ACIDS AND HYDROLYSIS AMINO ACIDS IN THE NINE KINDS OF DIFFERENT RICE WINES (mg/100 mL)

Amino acid	Sample																	
	NA1		NA2		NA3		MDJ1		MDJ2		FS1		X1		X2		X3	
	F[1]	H[2]	F[1]	H[2]	F[1]	H[2]	F[1]	H[2]	F[1]	H[2]	F[1]	H[2]	F[1]	H[2]	F[1]	H[2]	F[1]	H[2]
Asp	0.5138	1.6217	0.1342	1.4201	3.9173	1.8892	0.0736	1.6996	0.4982	3.1438	1.2690	5.6351	1.0997	5.6884	9.6245	18.3512	0.7580	7.7863
Thr	0.8956	0.8800	0.3766	0.7716	2.9840	1.0566	0.2643	0.9140	0.5158	1.5861	1.1765	3.0252	0.6805	1.9940	16.7331	6.0625	0.3042	2.7259
Ser	0.4388	1.0757	0.2112	0.8960	0.5696	0.9619	0.1261	1.2975	0.2376	2.3718	0.9595	5.1126	0.3969	3.2888	8.3913	9.9387	0.3829	4.5646
Glu	6.4086	5.3170	1.9162	3.5210	17.0944	4.6858	0.6247	4.3461	0.9426	7.5363	1.4626	26.2006	5.3701	10.7775	21.6447	31.4882	6.3058	14.9764
Gly	1.9980	1.0111	0.8091	0.7454	4.3183	1.2024	0.1317	0.8496	0.2322	1.5834	0.7890	3.7324	1.1386	2.9841	7.3271	8.6309	1.5037	3.9544
Ala	4.0986	1.5842	1.3324	1.2775	9.6830	2.2068	0.3991	1.7260	0.9456	3.2963	3.3331	4.1482	9.6711	3.8568	23.3677	10.3297	11.9544	5.0849
Cys	1.2206	1.0997	1.0994	1.0703	1.6258	1.1437	1.9976	1.1083	0.9817	1.2993	1.0106	2.4293	1.3257	1.6023	<0.1000	2.2205	1.2413	2.4511
Val	1.2856	0.8411	0.4707	0.8832	8.8142	1.7721	<0.1000	1.0906	0.6021	1.5967	1.7281	2.9207	4.2572	2.4221	25.8966	7.6054	5.3390	3.7712
Met	0.3600	0.3776	0.2089	0.3298	2.6720	0.5129	0.1101	0.2174	0.1056	0.3749	0.5846	0.6934	0.6975	0.0814	7.6157	0.5454	0.9660	0.2159
Ile	0.1835	0.3893	<0.1000	0.3174	5.3732	0.8442	<0.1000	0.3712	<0.1000	0.6985	0.8782	1.8430	1.4383	1.0475	12.3600	4.7292	1.9603	1.5358
Leu	1.2486	1.2015	0.4786	1.0900	17.3449	2.7666	<0.1000	2.4093	0.3858	4.3855	3.5552	6.5689	6.8043	3.6125	52.2401	13.3215	9.1760	5.2366
Tyr	1.2606	1.1044	0.8109	0.9794	0.3491	0.1728	1.2587	1.1899	0.9372	1.8061	<0.1000	3.6055	9.6822	2.6049	41.6354	5.0363	11.3778	3.6700
Phe	0.6745	0.6231	0.0823	0.5113	10.3246	1.2056	0.1297	0.7872	0.2144	1.6704	1.8142	4.4618	8.7723	2.5420	59.1985	10.2011	11.6811	3.6129
Lys	0.3532	0.8944	0.2921	0.8085	3.3312	0.9861	0.0961	0.8208	0.1006	1.4433	0.8148	2.5191	0.1461	2.1415	2.6597	6.5691	0.1110	2.8721
His	0.4335	0.5503	0.1311	0.5168	2.3235	0.9117	0.0711	0.7099	0.1815	1.0624	0.3862	1.7916	0.0935	1.6840	0.7467	4.4741	<0.1000	2.4447
Arg	0.1752	0.7329	0.1115	0.6233	2.3823	0.6859	0.0954	0.9178	0.1043	1.6555	<0.1000	4.2635	3.8566	5.1673	21.5249	14.8784	4.9276	7.1289
Pro	9.3795	2.1956	8.2285	1.6969	14.9772	2.4459	2.6575	1.8914	5.6612	3.6902	0.7959	9.0737	3.9293	3.1827	9.9344	8.5738	3.5497	4.1560
Total	32.6692	22.1543	16.9409	17.8975	114.7172	26.4380	8.2901	22.7899	12.8532	39.9177	21.9218	90.5276	60.9377	55.8806	323.8273	166.2480	73.9742	77.7680

[1]Means free amino acids in the nine kinds of different rice wines; [2]Means hydrolysis amino acids in the nine kinds of different rice wines; "< 0.1000" means this kind of amino acid did not reach the detected line

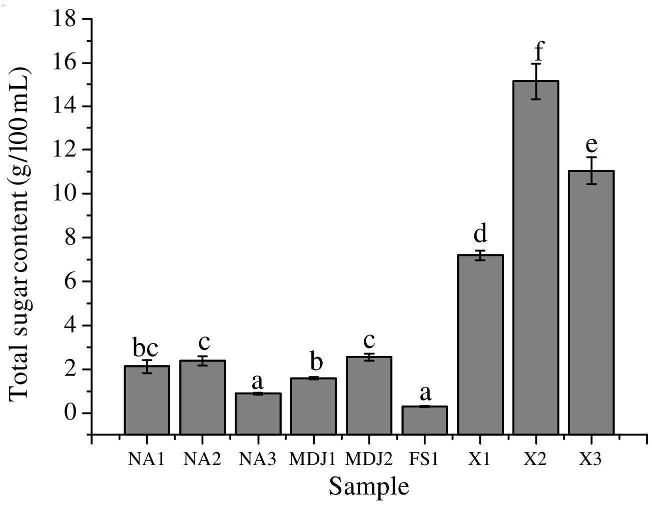

Fig. 2. Total sugar contents in nine kinds of rice wines, a, b, c, d, e, f: Different letters indicate significant differences at $p < 0.05$

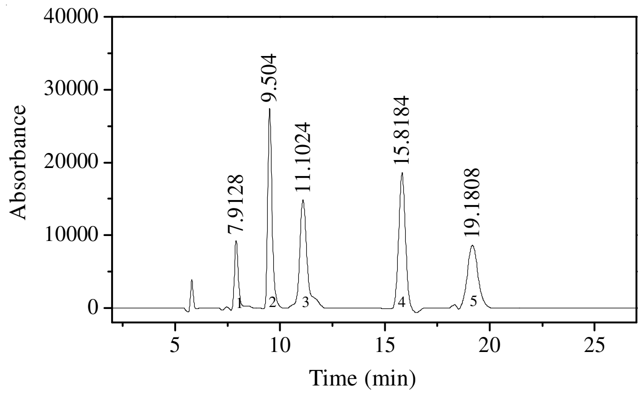

Fig. 3. Spectrogram of standard monosaccharide and disaccharides, 1-xylose, 2-fructose, 3-glucose, 4-sucrose, and 5-maltose

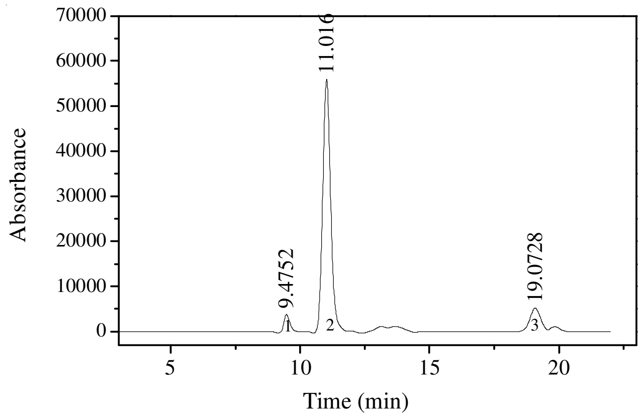

Fig. 4. Spectrogram of monosaccharide and disaccharides in X1 rice wine sample, 1-fructose, 2-glucocose, and 3-maltose

Solid content detected above and total sugar content in nine kinds of rice wines tended to be relative with type of rice wine. Solid content and total sugar was higher in semi-solid samples than liquid samples and there was significant difference between each other.

Monosaccharide and disaccharide: Five kinds of monosaccharide and disaccharide were determined by HPLC method. Sugar standards were run through the column and the spectrogram showed in Fig. 3. There were different kinds of monosaccharide and disaccharide in different kinds of rice wines. HPLC spectrogram of X1 rice wine sample was showed in Fig. 4 (spectrogram of the rest of samples were not showed). Glucose, which ranged from 0.05 g/100 mL to 16.59 g/100 mL, was discovered in eight kinds of rice wines except FS1 sample as showed in Table-3. Sucrose was found in NA2 and MDJ2 samples and fructose was found in X1 sample. Besides, maltose was found in three semi-solid samples. Fructose was absent in all nine kinds of rice wines. Five kinds of sugar in FS1 sample were not touched the detecting underline.

Starch in rice as the raw material of rice wine broken down to the monosaccharide and disaccharides, such as glucose, fructose, maltose, *etc*., which were also found in Yong's research[20]. Five kinds of monosaccharide and disaccharides were determined in this paper and found that the kinds of sugar were different in different samples. The kinds of sugar in rice wine may have relationships with the microflora in rice wine.

TABLE-3
AMOUNT OF FIVE KINDS OF MONOSACCHARIDE AND DISACCHARIDES IN RICE WINES (g/100 mL)

Sample	D - xylose	D -fructose	D -glucose	D -sucrose	D -maltose
NA1	-	-	0.17	-	-
NA2	-	-	0.05	0.07	-
NA3	-	-	0.22	-	-
MDJ1	-	-	0.09	-	-
MDJ2	-	-	0.10	0.19	-
FS1	-	-	-	-	-
X1	-	0.11	1.53	-	0.27
X2	-	-	16.59	-	0.27
X3	-	-	4.45	-	0.85

"-"Means this kind of monosaccharide undetected

Conclusion

The results indicated that rice wine is full of nutritious gradients, which is different in different rice wines. This paper provided a basic research for better understanding the relationships between the physico-chemical attributes, nutritional ingredients and environmental conditions. Furthermore, these findings could be a guide to produce more nutritional and delicious rice wines. More researches need to be conducted on rice wine because of its nutritional potential and multipurpose use.

ACKNOWLEDGEMENTS

This work was supported by Technology of the People's Republic of China (2007AA10Z354).

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