Change in Soluble Sugar and Organic Acids during Fermentation of Dragon Fruit Wine

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Abstract: Change in soluble sugar and organic acids of dragon fruit wine during fermentation by reversed phase high performance liquid chromatography (RP-HPLC); Organic acids chromatographic conditions: chromatographic column BP-OA, mobile phase A(1% H_3PO_4) and B (UPW), column temperature 35°C , the flow rate of 0.5 ml/min, UV detector (210 nm). Sugar chromatographic conditions: BP-OA chromatographic column, mobile phase(UPW), column temperature 25°C, the flow rate of 0.3 ml/min, differential detector. The results showed that the degree of separation of components in 1.44 above, RSD was between 0.6% and 2.7%, the average recovery is between 93.4% to 108.4%.Citric acid and malic acid decrease by 71.02% and 71.30%, respectively, at the end of the fermentation. Lactic acid content increases 86.71%, succinic acid and acetic acid content increased, but pyruvicrise after falling.Sucrose and glucosefall by 78.54% and 59.06% respectively, fructose content increased by 95.23%.

1. Introduction

Pitaya is Cactacea *Hylocereus undatus* and *Selenicreus*. It is rich in sugar, organic acids and minerals and other nutrients, known as the king of fruit[1-2]. It is cultivated widely in Guangdong, Hainan, Guangxi and other places, dragon fruit cultivation area is about 50000 hectares, annual output of about 1 million tons[3]. Pitaya is good processing properties, it is used in the production of fruit juice, jam, preserved fruit, etc[4-6], but economic value of these rough machining is low, Making wine is not only conforms to the transformation of the food wine to wine, but also improve the economic value of dragon fruit[7].

Sugar and acid is an important nutrients and influence the flavor of wine, it has a direct relationship of the degree of fermentation and is an important precursor of wine aroma, directly affect the taste and quality of fruit wine[8-9]. Method for determination of sugar and acid has titration method, ion chromatography mass spectrometry, and gas chromatographic internal standard method etc[10-12]. Titration method cannot separate the acid, operation of gas chromatography and ion chromatography mass spectrometry are complex[13]. RP-HPLC is commonly used methods for analysis of sugar and acid. It has simple operation and high sensitivity. This experiment is based on the analysis change of sugar and acid content of dragon fruit wine during fermentation, provide theory support for the fermentation of wine.

2. Materials and methods

2.1. Materials and reagents

Pitaya was purchased from Wal-mart Supermarket in Zhanjiang, Guangdong. Dragon fruit wine is brewed wine. Oxalic acid (99.8%), citric acid (99.5%), tartaric acid (99.8%), malic acid (99.5%), pyruvic (N-13167), succinic acid (99.5%), lactic acid (91.2%), acetic acid (99.9%) of the standard products, waspurchased from Dr. Ehrenstorfer GmbH; sucrose (99%), glucose (99%) and fructose (99%) were purchased from sigma-alorich, chromatography phosphoric acid (85-90%) was obtained sigma company.

2.2. Instrument and equipment

HPLC, LC-20A, Shimadzu Corporation; Analytical balance of one over one hundred thousand Sartorius Group, Germany; Pure water processing system Milli-Q, Merck, USA.

2.3. Method

2.3.1. Organic acid chromatographic conditions

Chromatographic column (BP-OA 2000-0, 300 mm x 7.8 mm). Organic acid mobile phase A (UPW): B (1% phosphoric acid solution) = 20:80 (v/v), column temperature 30 °C. UV detection (210 nm); sugar mobile phase (UPW), column temperature 25 °C, differential detector. Isocratic elution, the flow rate of 0.5 ml/min; sample size (10 μL).

2.3.2. Preparation of standard solution

Preparation respectively 0.5, 0.4, 0.3, 0.4, 0.3 mg/ml of oxalic acid, tartaric acid, pyruvic acid, malic acid, citric acid, succinic acid, lactic acid, acetic acid standard solution.

2.3.3. Sample solution preparation

1.0 ml sample was diluted five times with UPW, through 0.22 μm nylon membrane, filtrate computer analysis.

3. Results and discussion

3.1. Standard curve equation and precision

The above the prototype sample 3 times in a row. Using concentration (X) and peak area (Y) for standard curve. And calculating the precision of method.

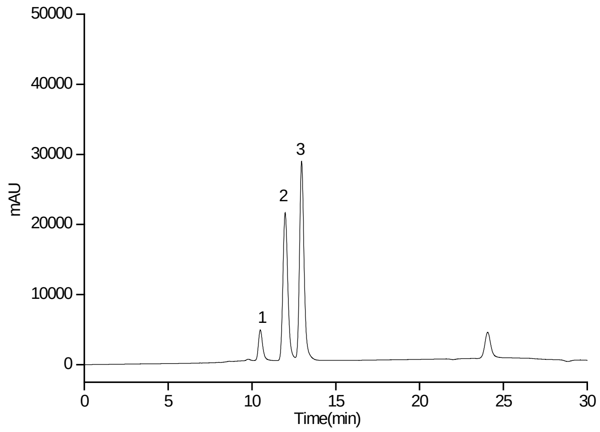

Fig.1 Chromatogram of Sugar mixed standard (1. Fructose; 2. Glucose; 3. Sucrose）

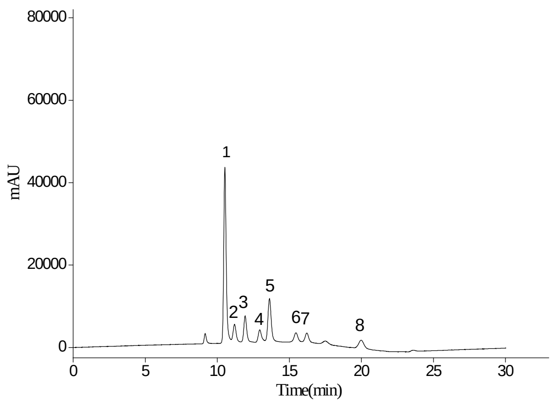

Fig.2 Chromatogram of organic acids profile (1. Oxalic acid; 2. Citric acid; 3. Tartaric acid; 4. Malic acid; 5. Pyruvic acid; 6. Succinic acid; 7. Lactic acid; 8. Acetic acid)

3.2. Dragon fruit wine organic acid content analysis

The organic acids and sugar of sample were determined according to chromatographic conditions. Table 2 showed that oxalic acid content is declining in the fermentation process, it can help absorb nutrients, Coinciding with low oxalic acid diet[13].Citric acid and malic acid was decreasing by 71.02% and 71.30%, respectively, at the end of the fermentation. This is consistent with the previous research results[14-15].Lactic acid content increased by 86.71%, this suggests that malic acid to acetic acid conversion to more completely and make more mellow feeling of the dragon fruit wine. Succinic acid, pyruvic acid and acetic acid are emerging in fermentation process. Succinic acid and acetic acid content increased, pyruvic acid rise after falling, which may be associated with mixed bacteria growth with for fermentation anaphase. Tartaric acid changed little in the fermentation process. Sucrose and glucose fell by 78.54% and 59.06%, respectively. fructose content increased by 95.23%.Because of the dragon fructose sugar content is less, so adding sugar provide energy for growth of yeast. As the fermentation, yeast converts sugar into glucose, sucrose, for their own production and breeding.

Table 1. Linear relation and recovery of organic acids and sugars.

	RT (min)	equation of linear regression	R^2	Resolution
Oxalic acid	10.580	y=169857x + 181840	0.9997	3.26
Citric acid	11.303	y = 32932x + 23899	0.9994	2.31
Tartaric acid	11.886	y = 48985x + 28545	0.9998	1.75
Malic acid	13.113	y = 101444x + 71434	0.9991	2.80
Pyruvic acid	13.776	y = 91944x + 96767	0.9993	1.71
Succinic acid	15.997	y = 11503x + 5699	0.9991	4.53
Lactic acid	16.511	y = 36000x + 14186	0.9990	1.42
Acetic acid	19.931	y = 23429x + 26695	0.9992	2.32
fructose	12.452	y = 598241x - 29732	0.9997	12.27
glucose	11.966	y = 410598x - 19978	0.9995	3.41
sucrose	13.061	y = 801205x - 36952	0.9992	2.22

Table2. Content of organic acids and sugars of sample.

Time (d)	1	2	3	4	5	6	7
Oxalic acid	0.062	0.045	0.049	0.043	0.035	0.051	0.026
Citric acid	0.176	0.105	0.123	0.139	0.148	0.069	0.051
Tartaric acid	0.398	0.417	0.425	0.556	0.592	0.473	0.406
Malic acid	3.502	2.471	2.038	1.765	1.539	1.201	1.005
Pyruvic acid	0.301	0.005	0.078	0.211	0.316	0.407	0.613
Succinic acid	0.152	5.625	5.969	6.667	8.239	8.952	9.067
Lactic acid	0.217	1.096	1.279	1.368	1.435	1.501	1.633
Acetic acid	0.298	0.183	0.269	0.311	0.398	0.459	0.502
glucose	4.651	4.082	3.586	2.998	2.439	1.872	1.904
fructose	0.032	0.082	0.106	0.118	0.196	0.379	0.671
sucrose	2.526	2.073	1.732	1.490	1.036	0.743	0.542

4. Conclusion

This method of measuring sugar and organic acid is easy to operate, mobile phase is simple and separation is fast, organic acids and sugar can be completely separated in 20 min and 15 min. Citric acid, malic acid, oxalic acid content of dragon fruit wine is reduced in the fermentation process, succinic acid, pyruvic acid, lactic acid, acetic acid is on the rise, tartaric acid change smaller, glucose, sucrose content reduce, fructose content increased.

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