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COMPOSITION AND CONTENT ANALYSIS OF SUGARS AND ORGANIC ACIDS FOR 45 GRAPE CULTIVARS FROM NORTHEAST REGION OF CHINA

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Abstract

The qualitative and quantitative analysis of sugars and acids of grape cultivars from northeast region of China was carried out for quality evaluation and variety improvement of grape. Analysis of major sugars and organic acids for 45 grape berries was carried out using High Performance Liquid Chromatography (HPLC). The result showed that glucose and fructose were the major sugars, beside that, some grape cultivars also contained sucrose. The quantity of glucose and fructose was almost equal in most of grape berries. A significant positive correlation existed between them, glucose content ranged from 53.24 mg/ml to124.18mg/ml and fructose content ranged from 48.39 mg/ml to118.84 mg/ml. Tartaric acid, malic acid, citric acid and oxalic acid were organic acids and tartaric acid was the main constituent in most grape berries and its concentration was higher than the other organic acids. However, in some grape cultivars, malic acid and citric acid were two highest organic acids while oxalic acid content was the lowest and even in some cultivars it could not be detected. Tartaric acid ranged from 1.28mg/ml to 6.82 mg/ml, malic acid ranged from 0.09mg/ml to 3.95 mg/ml, citric acid ranged from 0.08mg/ml to 4.43 mg/ml, oxalic acid ranged from0 mg/ml to 0.370 mg/ml. Thirty-four grape cultivars out of 45 cultivars accounted more than 50% tartic acid of the total organic acid contents. However, in cultivars Bixiang Wuhe and Shennong Jinhuanghou citric acid was the main organic acid. Malic acid and citric acid were significantly positively related with total acid. In 43 grape cultivars, the soluble sugars were glucose and fructose. Besides glucose and fructose, sucrose was also observed in cultivars of LN33 and Cayuga white.

Key words: Grape; Cultivar; Sugars; Acids.

Introduction

As an important fruit, grapes are cultivated all over the world, until 2013, the grape cultivation area has reached 7.15 million hectares with the production of 77.18 million tons (http://faostat.fao.org). Grapes are eaten fresh as resins or juice but the major use of grapes is in wine industry. The general appearance along with sugars, organic acids and aroma substance (aroma quality) are the major traits for the selection of wine industry. They can influence the taste and wine quality considerably (Robert *et al*., 1980; Wen *et al*., 2014). Sugars and organic acids composition play an important role in berries flavor quality and the research on this aspect may be helpful in the quality evaluation and variety improvement. Soulis & Avgerions (1984) reported that the major sugars in most grape berries were glucose and fructose while the other sugars were lower. High sucrose content varieties are reported in *V. labrusca* and *V. Rotundifolia* (Shiraishi, 1993). Tartaric acid and malic acid are major organic acids in grape berry, and also contains a little of succinic acid, citric acid and oxalic acid (Coombe, 1992; Lamikanra *et al*.,1995). Eyduran *et al*. (2015) investigated 9 Turkish grape varieties for sugars and organic acids contents. and identified some special varieties. Topalovic & Petkovsek (2010) reported variation in the sugars and organic acids contents during the ripening process of Cardinal grape. Sabir *et al*. (2010) also recorded the variation in main sugars and organic acids contents during fruit development process in 5 grape varieties.

In this study, 45 grape varieties cultivated in northeast of China were investigated including *V. labrusca* , *V. vinifera, V. amurensis* , hybrids of *V. vinifera* ×*V. labrusca* and hybrids of *V. amurensis* ×*V. vinifera* to measure the composition and content of soluble sugars and organic acids using HPLC in order to get a fair idea for quality evaluation and preservation of grape germplasm also.

Meterials and Methods

45 grape varieties were used (Table 1), including 27 genotypes of *V. vinifera* and 12 hybrid of *V. vinifera* × *V. labrusca*, 2 genotypes of *V. amurensis* , 1 genotype of *Vitis labrusca*, 2 filial generation of *V. amurensis* × *V. vinifera*, 1 filial generation of *V. labrusca* × *V.riparia* ×*V. vinifera*. They were all cultivated in grape germplasm nursery of Shenyang Agricultural University (northern latitude 41°49′, eastern longitude 123°34′, altitude 72m). Grape plants were cultivated in 2010, using trellis culture and dragon-shaped pruning. Row spacing was 4m, plant spacing was 1m. During the maturity period of these grape cultivars (August the 10th to September the 30th in 2014), we picked 3-5 clusters from 3 plants of every grape variety, 20 grape berries were randomly collected from the head, middle and bottom of grape clusters. The juice of the berries was extracted and used for qualitative and quantitative analysis of sugars and organic acids.

Determination of sugars and organic acids

Sugars and organic acids extraction: Sugars and acids extraction was done following the method of Liu *et al*. (2007) with a slight modification. The juice was diluted for 5 times using ultra pure water and centrifuged at 8000 r/min. for 5 minutes. Density was determined using 5 ml supernant.

Table 1. The correlation analysis of different components of sugar and acid.

	Glucose	Fructose	Total sugar	Tartaric acid	Malic acid	Citrate	Oxalate	Total acid
Glucose	-							
Fructose	0.790**	-						
Total sugar	0.902**	0.919**	-					
Tartaric acid	0.228	0.029	0.184	-				
Malic acid	-0.043	-0.090	-0.123	0.034	-			
Citrate	0.235	0.379*	0.309*	0.155	0.066	-		
Oxalate	0.375*	0.397*	0.422**	0.450**	0.112	0.452**	-	
Total acid	0.257	0.152	0.225	0.817**	0.446**	0.542**	0.611**	-

**Means significant correlation in the level of 0.01.
*Means significant correlation in the level of 0.05

Sugars extraction: Accurately transferred 0.75ml upper solution and mixed with acetonitrile in a ratio of 1:1, filtered with 0.45 μm millipore filters, and then kept for detecting.

Organic acids extraction: Accurately transferred 0.75ml upper solution and mixed with phosphate buffer (20mM KH_2PO_4 solution, PH=2.6) in a ratio of 1:1, filtered with 0.45 μm millipore filters, and then kept for detecting.

HPLC System

HPLC system of sugars: Sugars were detected by a Agilent RID G1362A refractive index detector, chromatographic column was Agilent Zorbax NH2 (4.6mm×250mm i.d., 5μm particle size),column temperature was 30°C, as a mobile phase, a acetonitrile: water (75:25) was used, 10μL injection volume with the flow rate of 1 ml/min.

HPLC system of organic acids: Organic acids were detected by a Agilent VWD G1314B ultraviolet detector, chromatographic column used was Agilent SB-AQ (4.6mm×250mm i.d., 5μm particle size), column temperature was 25°C, as a mobile phase, a methanol: KH_2PO_4 (5:95) was used, using phosphoric acid to adjust solution PH to 2.6, 0.7 ml/min flow rate, and 10μL injection volume. In the system, Agilent VWD G1314B ultraviolet detector was adjusted to the wavelength of 210 nm.

Quality and content analysis of sugars and organic acids: The sugar standard i.e. glucose, fructose and sucrose were mixed to make standard solution. The concentration was set as 20 mg/ml, 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml; the organic acids standard i.e. tartaric acid, malic acid, citric acid and oxalic acid, were mixed to make the mix standard solution, the concentration was set as 1 mg/ml, 1 mg/ml, 1 mg/ml, 1mg/ml, and then diluted the mix standard solution to 2 times, 4 times, 8 times, 16 times and 32 times. HPLC was used to detect the standard substance content of sugars and organic acids. Single standard solution was used to detect the quality of sugars and organic acids, mix standard solution was used to detect the content of sugars and organic acids.

Data statistics

Data were analyzed by Excel 2007 and SPSS 13.0. Range, median and distribution of the sugars and organic acids content in 45 grape varieties were analyzed by Origin7.5 software.

Result and Analysis

Analysis of sugars content: Sugars content range and distribution of 45 grape varieties are shown in figures 1, 2 and table 1. The result showed that glucose and fructose were the main components in grape berries, there content ratio was 1:1. Sucrose was not observed in most grape varieties except in two varieties having were very small sucrose.

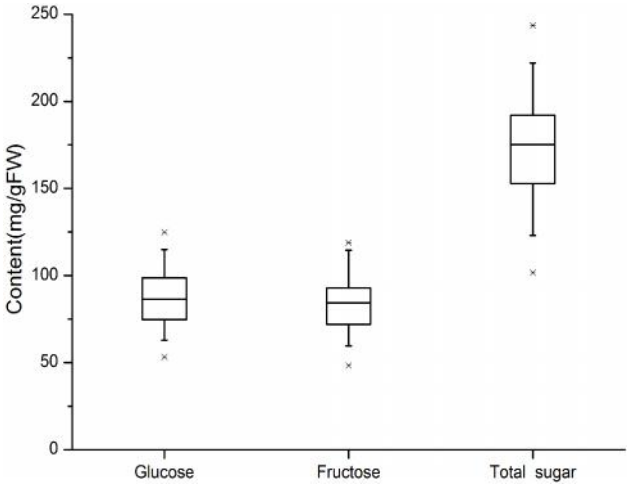


Fig. 1. Range and distribution of total sugar content in 45 grape cultivars.

Glucose range was between 53.24 mg/ml-124.96 mg/ml. cultivar Beibinghong had the highest glucose content (124.96 mg/ml), having 51.30% of the total sugars content, Tenzan had the lowest glucose content (53.24 mg/ml), having 52.38% of the total sugars content.

Fructose range was between 48.39 mg/ml -118.84 mg/ml. Canadice Seedless had the highest fructose content (118.84 mg/ml), with 53.53% of the total sugars content, Tenzan had the lowest fructose content (48.39 mg/ml), having 47.61% of the total sugars content.

Only in LN33 and Cayuga white that sucrose was observed. 51.48 mg/ml sucrose content was observed in LN33 and 31.68 mg/ml sucrose content was observed in cultivar Cayuga white. Total sugar content range was between 101.63 mg/ml-243.57 mg/ml. Beibinghong was the highest total sugar content variety while was the lowest.

The horizontal lines in the interior of the box are the median values. The height in the box is equal to the interquartile distance, indicating the distribution for 50% of the data. Approximately 99% of cultivars falls inside the whiskers and the cultivars outside these whiskers are indicated by horizontal lines.

Analysis of organic acids content: Organic acids content range and distribution of 45 grape varieties are shown in figures 3 and 4. The result showed that tartaric acid was the main composition in most grape cultivars, malic acid and citric acid were also observed in some grape cultivars, oxalic acid content was the lowest even could not be detected in some grape cultivars.

Tartaric acid range was between 1.28mg/ml -6.82 mg/ml, accounted for 33.61%-90.63% of the total acid content, the highest tartaric acid was found in Emerald Seedless variety while Jingyu had the lowest.

Malic acid range was between 0.09-3.95 mg/ml, Tamina had the highest malic acid content 3.95 mg/ml, while Red Balad had the lowest, only 0.09 mg/ml.

Citric acid range was between 0.08 mg/ml-4.43 mg/ml, citric acid content of Flame Seedless was significantly lower than the other grape cultivars, only 0.08 mg/ml, while Zhuosexiang was 4.43 mg/ml, as the highest citric acid in grape cultivar.

Oxalic acid range was 0-0.370 mg/ml, oxalic acid was not observed in most grape cultivars, for example, Zaoheibao, Jingyu, Shine Muscat, Tenzan and so on. The total organic acids range was 2.460 mg/ml -10.516 mg/ml, Zhuosexiang ranged the highest total organic acids content cultivar, 10.516 mg/ml, while Jingyu was the lowest, 2.460 mg/ml.

Tartaric acid content accounted for more than 50% in 34 grape cultivars, higher malic acid content were observed in some cultivars, for example: Crimson Seedless (57.7%), Italia (52.38%), Zaoheibao (44.42%), Black Muscat (44.02%) and Muscat Hamburg (41.08%). Higher citric acid content was also observed in some cultivars, for example: Zhuosexiang (42.11%), Shennong Jinhuanghou (40.71%) and Bixiang Wuhe(40.03%). Besides the sugars and organic acids content, the content ratio of sugars and organic acids can also influence the flavor of grape cultivars. The content ratio of 45 grape cultivars ranged between 12.73-66.3, higher content ratio were observed in Shine Muscat, Horizon, Lakemont Seedless with more than 60 whereas Tamina was the lowest, only 12.73. Big variance existed between different grape cultivars (Fig. 5).

Content correlation analysis between sugars and organic acids: We conducted the content association between sugars and organic acids for the 45 grape cultivars (Table 2). The result showed that content of glucose and fructose had significantly positive correlation with the total sugar content. Positive correlation was also observed between the content of glucose and fructose. Tartaric acid, Malic acid and Citric acid were significantly positive correlation with the total organic acids content. Content of fructose and total sugars were significantly positive correlation with citric acid.

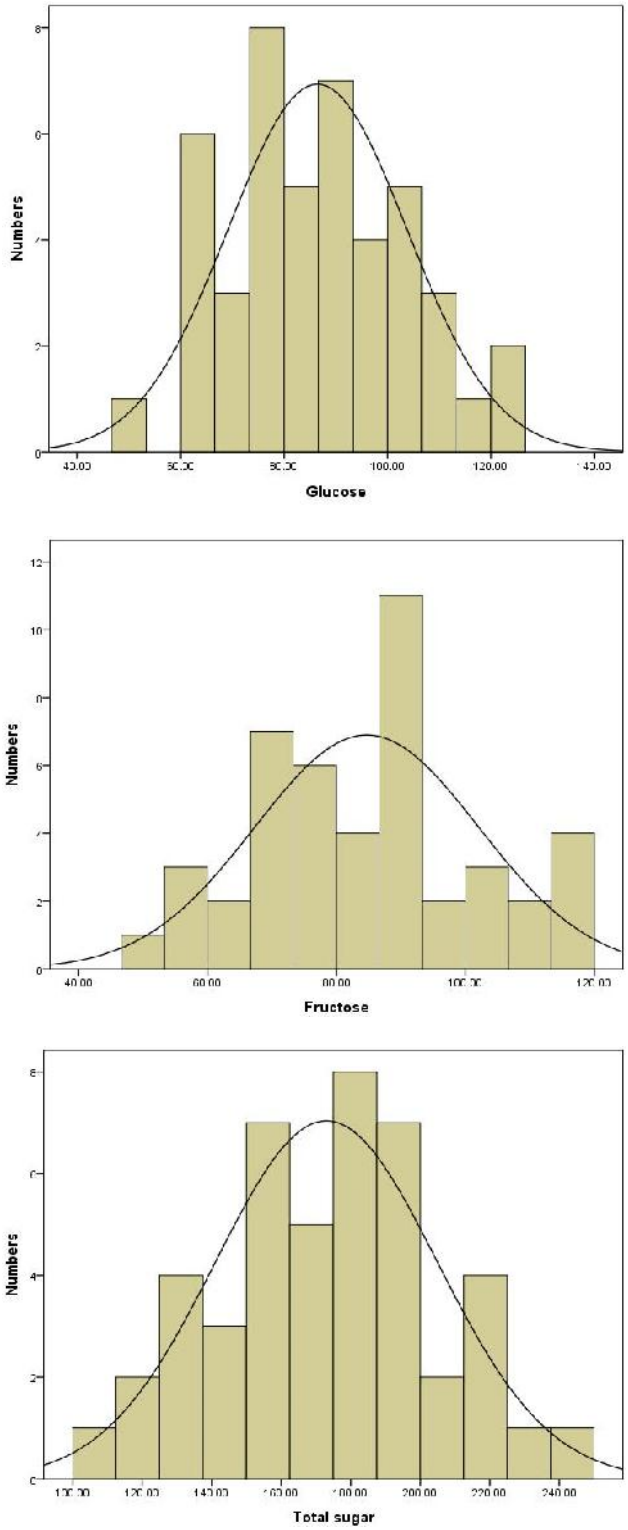


Fig. 2. The probability distribution of total sugar and soluble sugars in 45 cultivars of grape.

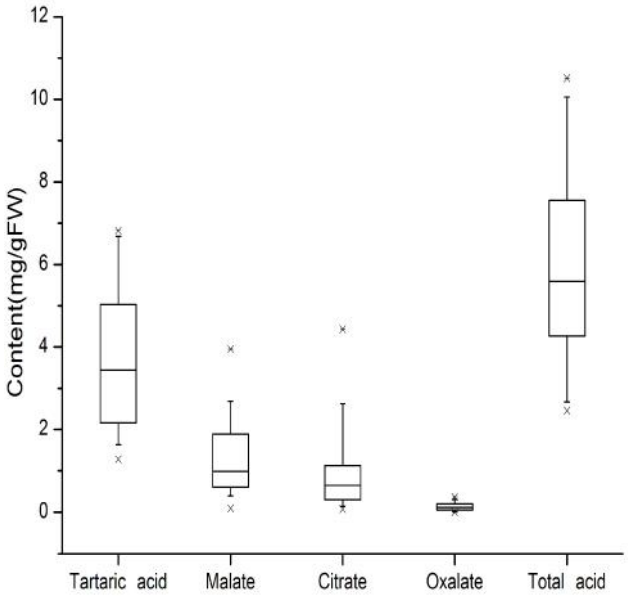
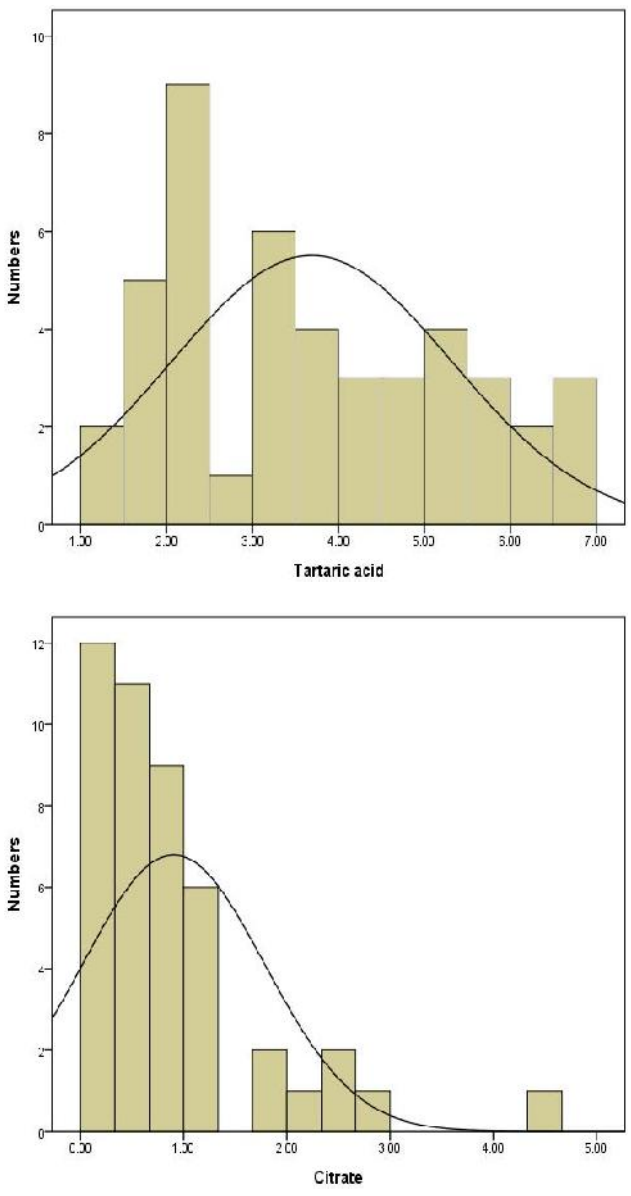


Fig. 3. Range and distribution of organic acid in 45 cultivars of grape.



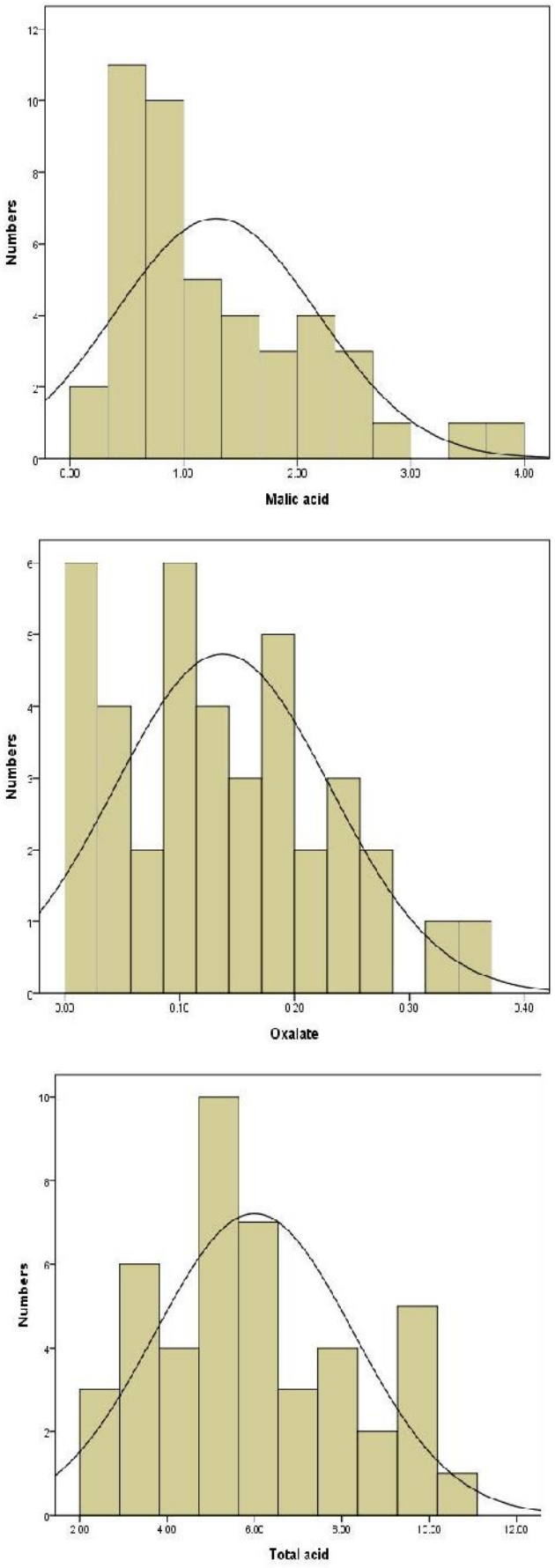


Fig. 4. Probability distribution of each organic acid and total acid in 45 cultivars of grape

Note: x-axis was the content(mg/ml), y-axis was the number of cultivars.

Table 2. Content of sugars and organic acids in 45 grape cultivars.

Cultivars	Species	Glucose mg/ml	Fructose mg/ml	Sucrose mg/ml	Total sugar mg/ml	Tartaric acid mg/ml	Malatemg /ml	Citrate mg/ml	Oxalate mg/ml	Total acid mg/ml
87--1	*V. vinifera* L.	61.68	57.97	-	119.65	5.18	0.90	0.31	0.048	6.438
Exotic	*V. vinifera* L.	87.36	88.00	-	175.36	4.38	0.18	0.52	0.138	5.218
Bixiang Wuhe	*V. vinifera* L.	100.59	99.65	-	200.24	1.99	1.55	2.37	0.014	5.924
Tamina	*V. vinifera* L.	63.25	59.69	-	122.94	5.29	3.95	0.35	0.069	9.659
Yatomi Rosa	*V. vinifera* L.	88.34	92.84	-	181.18	3.62	1.38	2.98	0.217	8.197
Emerald Seedless	*V. vinifera* L.	86.41	78.81	-	165.22	6.82	1.70	1.17	0.118	9.808
Ruby Seedless	*V. vinifera* L.	75.03	75.73	-	150.76	2.16	1.05	0.49	0.053	3.753
Jingzaojing	*V. vinifera* L.	98.05	95.79	-	193.84	3.39	0.47	0.22	0.092	4.172
Shennong Jinhuanghou	*V. vinifera* L.	76.85	80.70	-	157.55	1.92	1.31	2.28	0.086	5.596
Jingyu	*V. vinifera* L.	73.94	69.26	-	143.20	1.28	0.73	0.45	-	2.460
Autumn Black	*V. vinifera* L.	91.79	103.00	-	194.79	2.09	1.16	0.31	0.096	3.656
Christmas Rose	*V. vinifera* L.	83.00	91.84	-	174.84	2.33	1.40	0.90	0.229	4.859
Riesling	*V. vinifera* L.	71.47	71.94	-	143.41	3.19	2.39	0.68	0.116	6.376
Autumn Seedless	*V. vinifera* L.	124.18	65.18	-	189.36	5.72	0.89	0.28	0.170	7.060
Crimson Seedless	*V. vinifera* L.	92.74	92.80	-	185.54	2.15	3.56	0.30	0.157	6.167
Muscat Hamburg	*V. vinifera* L.	77.40	75.44	-	152.84	2.28	2.21	0.75	0.138	5.378
Thompson Seedless	*V. vinifera* L.	92.75	89.14	-	181.89	4.77	1.89	0.84	0.176	7.676
Tenzan	*V. vinifera* L.	53.24	48.39	-	101.63	1.77	0.76	0.14	-	2.670
Victoria	*V. vinifera* L.	65.63	59.70	-	125.33	2.25	0.45	0.43	0.006	3.136
Centennial Seedless	*V. vinifera* L.	85.98	89.24	-	175.22	3.66	0.72	1.95	-	6.330
Chardonnay	*V. vinifera* L.	96.36	91.53	-	187.89	4.81	2.69	2.63	0.004	10.134
Italia	*V. vinifera* L.	68.08	64.89	-	132.97	1.63	2.31	0.47	-	4.410
Zaoheibao	*V. vinifera* L.	81.58	76.16	-	157.74	2.58	2.35	0.36	-	5.290
Zaoxiameigui	*V. vinifera* L.	76.02	66.83	-	142.85	2.02	0.98	0.25	0.009	3.259
Red Balad	*V. vinifera* L.	66.36	68.85	-	135.21	3.87	0.09	0.26	0.047	4.267
Flame Seedless	*V. vinifera* L.	75.48	77.29	-	152.77	3.44	0.40	0.08	0.027	3.947
Shennong Cuifeng	*V. vinifera* L.	74.81	79.01	-	153.82	3.60	0.47	0.58	0.195	4.845
LN333	*V. labrusca* × *V. riparia* × *V. vinifera*	66.00	67.14	51.48	184.62	4.19	0.50	0.44	0.111	5.241
Cayuga white	*V. vinifera* L. × *V. labrusca* L.	67.01	72.45	31.68	171.14	6.14	0.57	0.68	0.167	7.557
Schuyler	*V. vinifera* L.× *V. labrusca L.*	105.94	113.36	-	219.30	4.17	0.60	1.84	0.270	6.880
Horizon	*V. vinifera* L.× *V. labrusca L.*	107.05	113.21	-	220.26	1.81	0.74	0.75	0.110	3.410
Brown Seedless	*V. vinifera* L.× *V. labrusca L.*	98.77	91.90	-	190.67	3.19	1.23	0.65	0.109	5.179
Black Muscat	*V. vinifera* L.× *V. labrusca L.*	88.16	84.31	-	172.47	2.01	2.32	0.89	0.047	5.267
Mars Seedless	*V. vinifera* L.× *V. labrusca L.*	95.08	100.03	-	195.11	5.07	0.53	1.13	0.198	6.928
Venus Seedless	*V. vinifera* L.× *V. labrusca L.*	74.77	81.26	-	156.03	6.14	1.17	1.26	0.244	8.814
Lakemont Seedless	*V. vinifera* L.× *V. labrusca L.*	86.79	89.30	-	176.09	2.32	0.49	0.10	0.006	2.916
Remaily Seedless	*V. vinifera* L. × *V. labrusca* L.	114.93	114.52	-	229.45	4.75	0.77	0.54	0.059	6.119
CanadiceSeedless	*V. vinifera* L.× *V. labrusca* L.	103.14	118.84	-	221.98	3.20	0.51	1.18	0.196	5.086
Shine Muscat	*V. vinifera* L.× *V. labrusca* L.	84.50	87.35	-	171.85	1.46	0.96	0.17	-	2.590
Zhuosexiang	*V. vinifera* L.× *V. labrusca* L.	101.69	104.99	-	206.68	5.03	0.74	4.43	0.316	10.516
Concord	*V. labrusca* L.	109.73	108.27	-	218.00	3.38	1.49	1.11	0.370	6.350
Zuoyouhong	*V. amurensis* Rupr. × *V. vinifera* L.	106.68	80.45	-	187.13	6.74	0.60	0.31	0.191	7.841
Beibinghong	*V. amurensis* Rupr. × *V. vinifera* L.	124.96	118.61	-	243.57	5.61	2.22	0.69	0.227	8.747
Shuanghong	*V. amurensis* Rupr.	62.85	68.07	-	130.92	5.91	2.63	1.27	0.251	10.061
Shuangyou	*V. amurensis* Rupr.	104.56	87.46	-	192.03	6.68	1.90	0.82	0.278	9.678

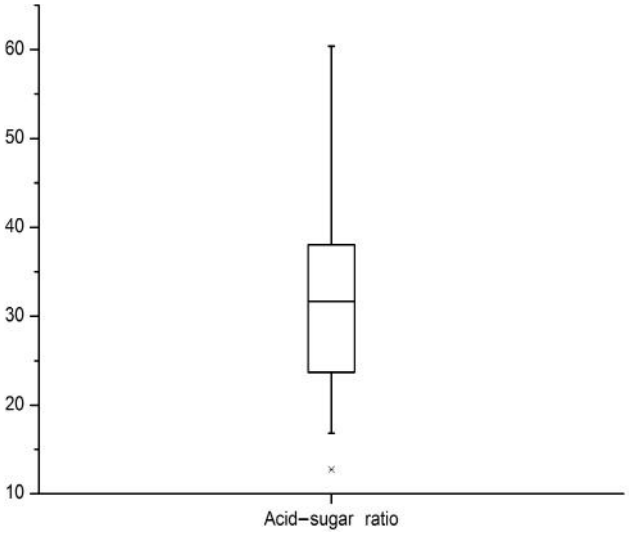


Fig. 5. Distribution of acid-sugar ratio in 45 grape cultivars

Discussion

Fortyfive grape cultivars from the northeast region of China, including *V. vinifera*, *V. labrusca*, *V. amurensis*, hybrids of *V. vinifera* × *V. labrusca*, hybrids of *V. Amurensis* × *V. vinifera*, for the composition and content analysis of sugars and organic acids were investigated using HPLC (High Performance Liquid Chromatography) method, this study can provide the reference for parents choice in grape breeding and metabolism of sugars and organic acids.

Sweetness differed in different sugars, it was higher in fructose than glucose and sucrose (Pangborn,1963; Doty,1976), hence the sugar variety and content may influence the sweetness of grape berries. Fructose content was lower in *V. vinifera* than the other grape cultivars, while hybrids of *V. Vinifera* × *V. labrusca* had higher fructose, especially in the cultivar of Canadice Seedless, fructose content could be as high as 118.84 mg/ml.Fructose content was the highest in Beibinghong which came from the hybrids of *V. amurensis* × *V. vinifera*, fructose content could reach to 243.57 mg/ml. Because of the high cold resistance descent of *V. amurensis*, Beibinghong was suitable for cultivation in cold region. High fructose content grape cultivar can be used as the parents to breed table grape varieties which have sweeter taste. Organic acids content was lower than sugars in grape berries, but they have significant influence in the quality of grape wine (Ryan & Dupan, 1973; Robert *et al*., 1980; Wen *et al.*, 2014). Tartaric acid and malic acid were the main organic acids in grape cultivars, citric acid , succinic acid and other organic acids were also observed, but the content were very low, organic acids variance exited in different grape cultivars (Kliewer,1966; Philip & Nelson,1973; Carroll &

Marcy, 1982; Coombe, 1992). Higher or lower organic contents do not contribute to the grape flavor. Acidity variance exhibited in different organic acids, tartaric acid was stronger than malic acid (Amerine *et al*., 1965). Higher ratio of tartaric acid to malic acid is required for wine grape, while moderate content of malic acid and lower tartaric acid are required for table grape (Liu *et al*., 2007). Tartaric acid was the main organic acid in most grape cultivars, higher malic acid content grape cultivars were Crimson Seedless and Italia, higher citric acid content grape cultivars were Zhuosexiang, Bixiang Wuhe and Shennong Jinhuanghou. *V. amurensis* and *V. vinifera* were richness for tartaric acid and the content were higher than in other grape cultivars. Large tartaric acid content range was found in *V. vinifera*, some grape cultivars had very high and others had very low.

Low total sugar content was observed in 27 grape cultivars of *V. vinifera*, the average total sugar content was 159.18 mg/ml. Higher total sugar content was observed in 15 interspecific hybrids, the average content was 196.42 mg/ml. He (1999) reported that cross-fertilized having long geographic origin grape cultivars, the heterosis of sugar content was very significant in progeny. The average organic acids content of interspecific hybrids was a little higher than *V. vinifera*.

V. amurensis originated in China, as an important grape variety, has high cold resistance and can survive upto -40^{o}C, it can also resist many diseases. Because of its high organic acids and low sugar content, it is not suitable for table grape and making wine. Progenies of cross-fertilized by *V. amurensis* and other grape varieties, high sugar content and moderate organic acids content cultivars were found (Liu *et al*., 2014). This is the first time qualitative and quantitative analysis of sugars and organic acids for *V. amurensis* and filial generation of *V. amurensis* × *V. vinifera* were conducted. This will promote the development and utilization of *V. amurensis* resource.

Sugars and organic acids are easily influenced by culture technique, climate, soil and other environmental conditions. Large variance exists in different regions what we called interaction of genotype and environment. Eyduran *et al*. (2015) reported much higher sugar contents in Thompson seedless variety from Turkey as compared to our findings. However, the organic acids content was nearly the same and agrees with the results of Eyduran *et al*. (2015). It seems desirable to find out the variation in sugars and organic acids in different years in the varieties growing in the same region.

In this study, we got the preliminary data of sugars and organic acids content of 45 cultivars in northeast region of China, this can provide the reference of conservation, variety utilization and metabolism of sugars and organic acids for grape resources in future.

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