Taste characteristics of Chinese bayberry juice characterized by sensory evaluation, chromatography analysis, and an electronic tongue

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Abstract To evaluate the taste characteristics of Chinese bayberry juice, four types of bayberry juice sourced from different origins and varieties were analysed using sensory evaluation, chromatography, spectroscopy analysis and an electronic tongue (E-tongue). Nine organic acids and three sugars were assessed using high performance liquid chromatography. Total polyphenols were measured by spectrophotometry. The overall taste profile was collected using the E-tongue. The four types of bayberry juice differed in the sensory attributes of sour, sweet, bitter, and astringent. The E-tongue responses combined with discriminant analysis were able to characterise the taste profiles of the juices. The relationships between the taste compounds and the sensory panel scores established by partial least squares showed that total polyphenols, quinic acid, maleic acid, fructose, citric acid, lactic acid, succinic acid and sucrose made significant contributions to the taste characteristics of the Chinese bayberry juice.

Keywords Chinese bayberry juice · Sensory evaluation · Electronic tongue · Chromatography · Spectroscopy

Introduction

Chinese bayberries (*Myrica rubra*) are subtropical fruits that have been cultivated in China for more than 2000 years (Xie et al. 2009). Due to their crimson to dark

purple-red colour, unique taste and healthy effects (Sun et al. 2013), they are popular with local people. Because bayberries are highly perishable, they are not available to consumers between harvests. They have been processed into juices and wines in recent years. Bayberry juice is becoming popular in China.

The flavour characteristics of fruit juice are influenced by the raw materials. The variety and origin of the raw materials are the prominent factors that influence the taste of fruit juice. Cheng et al. (2015) showed that bayberries of different cultivars had different flavour characteristics. Stinco et al. (2015) reported that the variety of orange significantly influenced the flavour characteristics of orange juice. Dorta et al. (2014) obtained similar results in a study of mangoes.

The taste characteristics of fruit juices are often measured by sensory evaluation. Sensory evaluation can provide integrated information about taste quality (Bleibauma et al. 2002). To investigate taste characteristics more deeply, chromatography or spectrometry methods are used to analyse the taste compounds of fruit juices. High performance liquid chromatography (HPLC) is often used in the analysis of sugars and organic acids in fruits. Using HPLC, Chinnici et al. (2005) determined the organic acids and sugars in apple, pear, apricot, and peach juices, Kelebek et al. (2009) determined the organic acids in orange juice, Stinco et al. (2015) determined ascorbic acid in different orange juices.

As a supplement to human judgement (Bleibauma et al. 2002), electronic tongues (E-tongues) are extensively used in the assessment of wine (Cetóá et al. 2015), other beverages (Peres et al. 2009) and fruit juice (Qiu et al. 2015). The E-tongue is simple, objective and reliable (Dias et al. 2011). An E-tongue was used by Peres et al. (2009) to discriminate between beverages, and similar research was

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conducted by Dias et al. (2011). Qiu et al. (2015) established a partial least squares (PLS) model to analyse the quality parameters of strawberry juices.

This study was conducted to characterise the taste attributes of bayberry juice using sensory evaluation, chromatography, spectroscopy and an E-tongue. Nine organic acids and three sugars were quantitatively analysed by HPLC. The concentration of total polyphenols was measured by spectrophotometry. The taste profile was analysed by the E-tongue combined with linear discriminate analysis (LDA). The relations between the taste compounds and sensory attributes were established by PLS to find out which compounds make significant contributions to the taste characteristics of bayberry juice. Determining the characteristic taste compounds will help manufacturers to improve the formula and stability of bayberry juice products.

Materials and methods

Samples and reagents

Chinese bayberry is mainly planted in Zhejiang province, Jiangsu province and Fujian province, China. Cixi city, Yuyao city and Xianju city are the three most famous production areas in Zhejiang province. The fruits of the ‘Biqi’ and ‘Dongkui’ cultivars are deep red. These two cultivars are usually used as the raw materials of bayberry juice.

Bayberry fruits were obtained from Haitong Co., Ltd (Zhejiang province, China) in June 2014. ‘Biqi’ fruits were sourced from Cixi city (CB), Yuyao city (YB) and Xianju city (XB), and ‘Dongkui’ fruits were sourced from Xianju city (XD). Juice was extracted from the fruits and was screened. It was homogenised, sterilised and preserved in a freezer at -80°C . The soluble solids portion of the juice was higher than 7 degrees Brix. Seven samples of each type of bayberry juice were obtained.

The acid and sugar standards were of chromatographic grade. Citric acid, succinic acid, lactic acid, malic acid, acetic acid, quinic acid, maleic acid, tartaric acid and ascorbic acid were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Glucose, fructose, fucose, gallic acid and rutin were sourced from Sigma-Aldrich (St. Louis, MO, USA). Folin-Ciocalteu was purchased from Lida Biotechnology Co., Ltd, Shanghai, China. Methanol and acetonitrile of chromatographic grade were purchased from Sinopharm Chemical Reagent Co., Ltd, Shanghai, China.

Sensory evaluation

The sensory evaluation was conducted according to the National Standard of China (GB/T 15038-2006) and ISO 4121. Twenty-four panellists participated in a sensory evaluation session in a sensory laboratory at room temperature. Eight panellists (four male and four female, average age of 28) were selected due to their high level of discrimination ability. The procedure was conducted in three parts. In the first part, the panellists tasted the bayberry juices to recognise and record all the sensory attributes. In the second part, the panellists discussed and determined the attributes, and then they established a final lexicon according to the provided standards. In the final part, the bayberry juices were evaluated using a quantitative descriptive analysis method on a scale from 0 (undetected) to 9 (very intense).

Twenty-mL samples of ready to drink bayberry juice (about 7 degrees Brix and 0.6% acidity) were placed in glass cups. Each sample was marked with a three-digit number. All of the samples were delivered to the sensory panel in Latin squares order. The panellists scored the perceived intensity of each attribute for each sample. For each sample, sensory evaluations were performed in triplicate. Before each tasting, the panellists rinsed their mouths with purified water.

HPLC analysis of the organic acids

Nine organic acids in the bayberry juices were analysed using an Agilent 1260 HPLC system (Agilent, CA, USA) equipped with an ultra-violet detector. The detection wavelength was set at 210 nm. The organic acids were separated by an Agilent C_{18} column (250 mm $\times$ 4.5 mm $\times$ 5 μm) at 30°C . The mobile phase was 0.1 mol/L $\text{KH}_2\text{PO}_4\text{--H}_3\text{PO}_4$ solution. The flow speed was set to 0.7 mL/min. The identification and the concentrations of the organic acids were determined by the external standards method. The average recoveries for the nine organic acids ranged from 76.38 to 103.04%. The relative standard deviation (RSD) of the standards ranged from 0.08 to 10.89%.

HPLC analysis of sugars

The bayberry juice samples were pre-separated by solid phase extraction. The pre-processing method was as follows: first, a Sep-Pak C_{18} solid phase extraction micro-column (Waters, Milford, MA, USA) was activated with 2 mL of methanol, and then eluted by 2 mL of water. The initial 2 mL of filtrate was discarded, and the subsequent 3 mL of filtrate was collected for sugar analysis.

The sugars were analysed using an Agilent 1260 HPLC system and a refractive index detector. The isocratic separation of sugars was performed by an Agilent Zorbax Carbohydrate column (250 mm × 4.6 mm, 5 µm). The column temperature was 30 °C. The mobile phase was acetonitrile in deionised water (8:2, v/v). The flow speed was 1 mL/min. The identification and concentrations of the sugars were determined by the external standards method. The average recoveries for the three sugars ranged from 86.94 to 95.26%. The RSD of the standards ranged from 2.14 to 9.60%.

Physicochemical indexes analysis

Total soluble solids content and total titratable acidity

The total soluble solids content in the bayberry juices was determined by a WYT- saccharimeter (Shanghai, China) with 0–32% Brix measurement range and no temperature compensation following ISO 2173-78. Total titratable acidity was determined by titrimetry according to the National Standard of China (GB/T12456-2008). The sugar to acids ratio, the ratio of total soluble solids content and titratable acids were calculated.

Total polyphenols

The total polyphenol content in the bayberry juice was calculated as the sum of anthocyanins, flavonoids and phenolic acids.

The anthocyanin content was measured in accordance with the method of Hosseinian et al. (2008). The flavonoid content was determined following the method of Verzelloni et al. (2007) with slight modifications. Briefly, 2 mL of the prepared supernatant and 0.3 mL of 0.5% sodium nitrite solution were added to a 10-mL measuring flask. After 6 min, 0.3 mL of 10% aluminium chloride was added and held for 6 min. Then, 4 mL of 1 mol/L NaOH solution was added. The solution was adjusted to 10 mL using 60% ethanol solution. After resting for 15 min, the absorbance at 510 nm was measured. The flavonoid content in the bayberry juice was calculated as the rutin equivalent.

The phenolic content was determined by the Foline-Ciocalteu method according to Canadanovic-Brunet et al. (2006) with slight modifications. One millilitre of the prepared supernatant, 30 mL of deionised water, 7.5 mL 10% aqueous sodium carbonate, and 2.5 mL of Foline-Ciocalteu reagents were put into a 50-mL brown volumetric flask. The solution was adjusted to 50 mL using 60% ethanol solution. The mixture reacted for 10 min at 75 °C. Absorbance at 765 nm was determined. The

phenolic content of the bayberry juice was expressed as gallic acid equivalent.

E-tongue analysis

The overall taste profile of the bayberry juice was measured by a potentiometric E-tongue (Alpha M.O.S., Toulouse, France). Seven silicon-based potentiometric sensors were used as working electrodes. The reference electrode was an Ag/AgCl electrode. The potentiometric difference between the working electrode and the reference electrode was recorded as the E-tongue response. Twenty-five millilitres of the ready to drink bayberry juice samples (about 7 degrees Brix and 0.6% acidity) was analysed at room temperature. Before each measurement, the sensor array was rinsed with a 0.012 mol/L HCl solution. Six replicate measurements were performed for each bayberry juice sample. The average of the six replicates was used for further analysis.

Statistical analysis

Analyses of variance (ANOVA) were used to assess the data on organic acids, sugars, physicochemical indexes and sensory attributes using XLSTAT software (v.2010, Addinsoft, New York, USA). When the ANOVA reached a 95% confidence level, the data were processed by Duncan's multiple range tests to account for the differences among the four different types of bayberry juice.

Discriminant analysis has often been used to establish qualitative models based on E-tongue response data. It was performed using Alphasoft v.11 (Alpha M.O.S., Toulouse, France).

The PLS method was used to explore the correlation between the taste compounds, physicochemical indexes and sensory panel scores to discover which compounds make an important contribution to the taste of bayberry juice. PLS was also used to establish the relationship between the E-tongue responses and the sensory attributes. All variables were standardised so that it could be ascertained if they made unbiased contributions. PLS was conducted using the XLSTAT software.

Results and discussion

Sensory analysis

The statistics results for the sensory panel scores evaluated by the eight sensory panellists for the four sensory attributes (sour, sweet, bitter, and astringent) are shown in Table 1. The four types of bayberry juice were significantly different in their sour, sweet, bitter, and astringent sensory

Table 1 Statistics results for the sensory panel scores evaluated by the 8 sensory panelists for the 4 sensory attributes (sour, sweet, bitter, and astringent)

	CB	YB	XB	XD
Sour	5.411 ± 0.336 ^c	4.804 ± 0.269 ^a	5.054 ± 0.238 ^{ab}	5.268 ± 0.222 ^{bc}
Sweet	4.429 ± 0.278 ^a	5.071 ± 0.525 ^b	5.768 ± 0.647 ^c	5.286 ± 0.247 ^{bc}
Bitter	5.393 ± 0.430 ^b	5.232 ± 0.301 ^b	4.625 ± 0.250 ^a	4.714 ± 0.304 ^a
Astringent	6.089 ± 0.393 ^c	5.393 ± 0.570 ^b	4.750 ± 0.375 ^a	5.036 ± 0.576 ^{ab}

^{a,b,c}Means within lines with different superscript letters are significantly different based

attributes. The samples from CB had the highest sensory scores for sour, bitter and astringent, and the lowest score for sweet, indicating that the bayberry juice from CB had a relatively sour and astringent mouth feel. The sour score for YB was the lowest. The sample from XB had the highest score for sweet, and the lowest scores for bitter and astringent, indicating that the bayberry juice from XB was relatively sweet. There were significant differences in the sweet and astringent attributes of the ‘Biqi’ samples from the three different cities (Cixi, Yuyao and Xianju). The sensory scores for ‘Biqi’ and ‘Dongkui’ bayberry juice collected from Xianju city were similar. The comparison results indicate that the place of production had a greater effect on the sensory attributes than the variety.

Physicochemical indexes data analysis

Sugars, organic acids and phenolic compounds make significant contributions to the nutritional and sensory attributes of fruit juice (Versari et al. 2008). The total soluble solids content, titratable acids, and total polyphenols were

analysed, and the statistics for the four types of bayberry juice are listed in Table 2. The total soluble solids content was highest in the ‘Dongkui’ variety from Xianju city and lowest in the ‘Biqi’ variety from Cixi city. There were no significant differences among the ‘Biqi’ bayberry juices from the three different places of production, indicating that the place of production had less influence than the variety on the soluble solids content. For the bayberry juice samples sourced from the same place of production (Xianju city), the total soluble solids content of the ‘Dongkui’ variety was higher than that of the ‘Biqi’ variety.

The titratable acid content was highest in the CB samples and lowest in the XB samples. In the ‘Biqi’ samples, the titratable acid content was significantly different among the three places of production. For the samples from Xianju city, the titratable acid content of the ‘Biqi’ variety was significantly different from that of ‘Dongkui’ variety. The comparison results show that the variety and place of production both influenced the titratable acid content. The sugar to acid ratio is an important index in the taste of fruit juice (Harker et al. 2002). The XB and XD samples had

Table 2 Analysis of physicochemical indexes, organic acids, and sugars in the bayberry juice samples

	CB	YB	XB	XD
Total soluble solid (%)	7.814 ± 0.227 ^a	7.862 ± 0.483 ^a	8.281 ± 0.801 ^a	9.914 ± 0.930 ^b
Titratable acid (%)	0.776 ± 0.011 ^c	0.657 ± 0.053 ^{ab}	0.618 ± 0.083 ^a	0.764 ± 0.040 ^{bc}
Total polyphenol (mg/L)	1.949 ± 0.204 ^c	1.455 ± 0.186 ^b	1.217 ± 0.237 ^a	1.083 ± 0.156 ^a
Tartaric acid (mg/L)	1.125 ± 0.316 ^a	21.382 ± 6.272 ^b	7.552 ± 1.004 ^a	ND ^a
Quinic acid (mg/L)	150.884 ± 15.825 ^b	142.656 ± 16.319 ^b	62.669 ± 15.213 ^a	132.029 ± 18.347 ^b
Malic acid (mg/L)	676.960 ± 56.707 ^a	770.533 ± 59.324 ^a	793.082 ± 98.099 ^a	749.802 ± 93.059 ^a
Ascorbic acid (mg/L)	4.390 ± 0.844 ^a	23.857 ± 6.406 ^d	18.862 ± 2.845 ^c	9.279 ± 1.323 ^b
Lactic acid (mg/L)	0.031 ± 0.007 ^b	0.021 ± 0.005 ^a	0.027 ± 0.004 ^{ab}	0.029 ± 0.009 ^b
Acetic acid (mg/L)	0.323 ± 0.086 ^a	0.184 ± 0.138 ^a	2.815 ± 0.443 ^b	0.184 ± 0.022 ^a
Maleic acid (mg/L)	2.134 ± 0.614 ^b	1.324 ± 0.832 ^{ab}	0.591 ± 0.036 ^a	1.871 ± 0.124 ^b
Citric acid (mg/L)	8914.286 ± 843.772 ^a	7519.877 ± 655.770 ^a	7764.293 ± 421.773 ^a	8478.266 ± 472.924 ^a
Succinic acid (mg/L)	528.440 ± 28.811 ^c	313.033 ± 40.072 ^b	437.660 ± 62.262 ^a	352.763 ± 89.841 ^a
Fructose (mg/L)	20.210 ± 2.210 ^b	19.370 ± 4.030 ^b	13.740 ± 2.630 ^a	11.600 ± 1.030 ^a
Glucose (mg/L)	16.810 ± 2.010 ^b	19.890 ± 3.090 ^b	10.950 ± 1.250 ^a	10.680 ± 1.090 ^a
Sucrose (mg/L)	8.080 ± 1.370 ^a	14.560 ± 3.510 ^a	26.830 ± 4.290 ^b	33.230 ± 9.840 ^b

^{a,b,c}Means within lines with different superscript letters are significantly different based

higher sugar to acid ratios than in YB and CB, indicating that the place of production had a greater effect on this attribute than did the variety. This was in accordance with the sensory evaluation results.

Bayberry juice has a bright colour and is rich in polyphenols, which have a bitter and astringent taste (Kallithraka et al. 1997; Lea and Arnold 1978). The overall taste profile of bayberry juice mainly depends on the balance between sourness, sweetness, and bitterness. The total polyphenol content of the ‘Biqi’ samples from Cixi city was the highest and that of the ‘Dongkui’ samples from Xianju city was the lowest. The total polyphenol content differed significantly among the ‘Biqi’ samples sourced from the three different places of production. However, the total polyphenol content was similar in the samples sourced from the same place of production. This indicates that the variety had a greater influence than the place of production on the total polyphenol content.

Organic acids data analysis

As Table 2 shows, tartaric acid, quinic acid, malic acid, ascorbic acid, lactic acid, acetic acid, maleic acid, citric acid and succinic acid were detected in the bayberry juices. The average concentrations, standard deviations (SD) and ANOVA results for the CB, YB, XB and XD samples are shown in Table 2. All the organic acids except tartaric acid were detected in the four types of bayberry juice. The concentration of citric acid was the highest, followed by malic acid and succinic acid. Lactic acid and acetic acid were found in trace amounts. The variety of organic acids has an important influence on the taste of the juice. For instance, succinic acid is of low acidity and has a fresh taste, acetic acid is stimulating, and lactic acid is mild. Citric acid is considered an ideal food acidity agent because it tastes soft. Tartaric acid is a characteristic acid in tamarind (Roopa and Kasiviswanatham 2013). In the ‘Biqi’ bayberry juice samples, the contents of tartaric acid, quinic acid, ascorbic acid, lactic acid, acetic acid, maleic acid and succinic acid were significantly different among the three places of production. For the bayberry juice samples sourced from the same place of production (Xianju city), the contents of quinic acid, ascorbic acid, acetic acid, and maleic acid were significantly different.

Sugars data analysis

The type and concentration of sugars and organic acids determines the acid–base balance, which affects the overall taste of fruit juice. The statistics for the fructose, glucose and sucrose content in the CB, YB, XB and XD samples are shown in Table 2. The glucose and fructose contents were close and synchronised in the same variety of

bayberry juice. Glucose and fructose are the principal simple sugars in fruits (Arena et al. 2013). Sucrose is found in trace amounts in grapes (Boss and Davies 2001).

The fructose, glucose and sucrose contents in the ‘Biqi’ samples from Yuyao city and Cixi city were significantly different from those from Xianju city. The sucrose content of the samples sourced from Yuyao city and Cixi city was lower, whereas the fructose and glucose contents were relatively higher. For the samples sourced from Xianju city, the sum of the three sugars in the ‘Biqi’ variety and ‘Dongkui’ variety were similar, and there were no significant differences in the fructose, glucose or sucrose contents. The content of sucrose in the ‘Dongkui’ variety was higher than that in the ‘Biqi’ variety. In general, the fructose and glucose contents were similar in the different bayberry juices, whereas the sucrose content differed. A possible reason is that sucrose was transformed into fructose and glucose in the transport process (Toldam-Andersen and Hansen 1997).

E-tongue analysis

The average radar map for the CB, YB, XB and XD bayberry juice samples is shown in Fig. 1. The BRS and SWS signal intensities were significantly different for the four types of bayberry juice, whereas the SRS, GPS, STS, UMS and SPS signal intensities were similar. Significant differences in sweet and bitter tastes were observed among the four types of bayberry juice. The results were in accordance with the sensory analysis results. In Fig. 1, it can be seen that the average response data for the CB, YB and XD samples were similar, indicating that the taste characteristics of XB were different from those of CB, YB and XD. Due to the influences of origin, variety, and climate, the taste of the bayberry juices differed.

Fig. 1 Average radar map of the bayberry juice samples sourced from CB, YB, XB, and XD based on the E-tongue response data

Relationship between sensory attributes, taste compounds and physicochemical indexes

To determine the contribution of the taste compounds and physicochemical indexes to the taste of the bayberry juices, a PLS model was established. The load diagram of the relationships between the bayberry juice samples is shown in Fig. 2. The 12 taste compounds and the 3 physicochemical indexes were regarded as the X-matrix in the PLS analysis, and the sensory attributes were regarded as the Y-matrix. The first two factors (t1 and t2) explained 99.15% of the cross-validated variance (data not shown). The XB and XD samples are upper-left corner of Fig. 2, with the sensory attribute of sweet, indicating that XB and XD had a strong correlation with the sweet taste. This observation is in accordance with that obtained in the sensory evaluation. Having the highest sensory score in sour, bitter and astringent attributes, CB had a strong correlation with sour, bitter and astringent. As can also be observed in Fig. 2, total polyphenols, quinic acid, maleic acid, and fructose significantly contributed to the bitter and astringent attributes. The taste compounds citric acid, lactic acid and succinic acid showed a good correlation with the sour attribute. Sucrose significantly contributed to the sweet taste. These results indicate that total polyphenols, quinic acid, maleic acid, fructose, citric acid, lactic acid, succinic acid and sucrose make significant contributions to the taste of bayberry juice. Total polyphenols, quinic acid, citric acid, succinic acid and lactic acid were strongly

associated with CB. YB was located in the bottom-left corner, associated with tartaric acid, ascorbic acid, and glucose. XB and XD had strong correlations with sucrose.

Relationship between the sensory panel scores and the E-tongue response data

To study the relationship between the sensory panel scores and the E-tongue response data, a PLS model was established. The results are shown in Fig. 3. The sensory panel scores and the response data of the seven E-tongue sensors were regarded as the X-matrix and the Y-matrix, respectively. The first two factors (t1 and t2) explained 83.14% of the cross-validated variance (data not shown). As can be observed in Fig. 3, the sweet attribute had a good correlation with SWS, and the bitter attribute was correlated with BRS. The sour attribute had good correlations with SRS, GPS and UMS. However, the astringent taste had no strong correlations with any taste sensors. This might be because SRS, SWS, BRS, STS, and UMS represent sour, sweet, bitter, salt, and fresh, respectively. Astringency is a composite feeling produced by the wrinkling and shrinkage of epithelial cells. There is no specific taste sensor to represent it.

Fig. 2 An overview of the relation between the taste compounds and physicochemical indexes (X-matrix) with the sensory attributes (Y-matrix). Obs represent juice samples of CB, YB, XB, and XD

Fig. 3 An overview of the PLS model for the sensory panel scores (Xmatrix) and the E-tongue response data (Y-matrix). Obs represent juice samples of CB, YB, XB, and XD

Conclusion

The sensory evaluation of the juice samples showed that the source of production had a greater effect on the sensory attributes than did the variety. Additionally, the E-tongue response data combined with DA were able to identify the taste profile of the four types of bayberry juice. The correlations between the taste compounds, physicochemical indexes and sensory attributes revealed that total polyphenols, quinic acid, maleic acid, fructose, citric acid, lactic acid, succinic acid and sucrose made significant contributions to the taste characteristics of the bayberry juice. The PLS model for the sensory panel scores and the E-tongue response data indicated that four sensory attributes can be evaluated by the E-tongue.

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