Food Research International 44 (2011) 2018–2026

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Sugars, sugar alcohols, fruit acids, and ascorbic acid in wild Chinese sea buckthorn (*Hippophaë rhamnoides* ssp. *sinensis*) with special reference to influence of latitude and altitude

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ARTICLE INFO

Article history:
Received 3 September 2010
Accepted 5 October 2010

Keywords:
Sea buckthorn
Hippophaë rhamnoides ssp. *sinensis*
Sugars
Acids
Latitude
Altitude

ABSTRACT

Wild berries of *Hippophaë rhamnoides* ssp. *sinensis* were collected from nine natural growth sites in China in three consecutive years in order to get an overall profile of the sugars, sugar alcohols, fruit acids, and ascorbic acid, and especially of the influence of the latitude and altitude of the growth place on these components. The contents of fructose, glucose, and L-quebrachitol in the berry juice varied in the ranges of 0.01–7.17, 0.05–7.85 and 0.21–1.09 g/100 mL, respectively, those of malic, quinic, and ascorbic acids were 1.55–8.84, 0.07–2.94, and 0.25–1.66 g/100 mL, respectively. The berries from Hebei and Inner Mongolia were characterized by high contents of sugars and L-quebrachitol and low contents of malic acid and ascorbic acid. In contrast, the berries from Sichuan and Qinghai contained lower contents of sugars and higher contents of malic acid and ascorbic acid than the berries from other growth areas. The berries from Sichuan differed considerably from others by the remarkably low contents of sugars and the exceptionally high contents of acids. The contents of fructose, glucose, and total sugar decreased as the altitude increased and as the latitude decreased ($p<0.05$). In contrast, the contents of malic acid and ascorbic acid increased as the altitude increased and as the latitude decreased ($p<0.05$). The contents of quinic acid and L-quebrachitol correlated strongly and positively with the latitude ($p<0.01$).

1. Introduction

Hippophaë rhamnoides ssp. *sinensis* is an important natural resource in China. It is used as a pioneer plant with a significant value for water and soil conservation because of its strong roots with nitrogen fixing ability as well as its resistance to extreme conditions such as drought, cold, and salinity. Sea buckthorn berry has been applied as an ingredient in traditional Chinese medicine since the ancient times. The berry and berry fractions have beneficial effects on the skin (Upadhyay et al., 2009; Yang et al., 1999, 2000), mucosa (Xing et al., 2002), eyes (Larmo et al., 2010), and the cardiovascular system (Basu et al., 2007; Johansson, Korte, Yang, Stanley, & Kallio, 2000; Larmo, Alin, Salminen, Kallio, & Tahvonen, 2008). Antioxidative activities (Geetha, Ram, Singh, Ilavazhagan, & Sawhney, 2002; Shukla et al., 2006) and positive effects on sugar metabolism (Nemes-Nagy et al., 2008) have also been reported.

Sugars and fruit acids are important components contributing to the sensory properties and to the consumer acceptance of sea buckthorn berries (Tiitinen, Hakala, & Kallio, 2005; Tiitinen, Yang, Haraldsson, Jonsdottir, & Kallio, 2006), and the prevalence of these components in the major subspecies of *H. rhamnoides* berries is known (Raffo, Paoletti, & Antonelli, 2004; Tiitinen et al., 2005, 2006; Yang, 2009). The presence of inositols and methylinositols in *H. rhamnoides* has been reported by our laboratory (Kallio et al., 2009; Yang, 2009).

Genetic differentiation between sea buckthorn populations along latitudinal and altitudinal gradients has been observed (Chen, Wang, Zhao, Korpelainen, & Li, 2008; Sheng et al., 2006). Lian, Lu, Xue, and Chen (2000) reported the correlations between latitude, longitude, and altitude and the values of total sugar, total acid, and sugar/acid ratio of *H. rhamnoides* ssp. *sinensis*. However, there is a lack of detailed information on individual sugars and acids and there seems to be that no systematic knowledge on the variations of these compounds in wild sea buckthorn from different growth areas in China is available as yet.

In the present study, wild berries of *H. rhamnoides* ssp. *sinensis* were collected from nine natural growth sites in six provinces in China in order to create an overall profile of the sugars, sugar alcohols, fruit acids, and ascorbic acid in the berries. The growth locations covered the longitude range from 101°23′E to 127°06′E, and the latitude range from 31°01′N to 47°14′N. The altitudes varied from 210 to 3115 m. Special attention was paid to the effects of the latitudes

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doi:10.1016/j.foodres.2010.10.007

and altitudes of the growth locations on the content and composition of these compounds. The berries were collected in three consecutive years to cover the variation among the harvesting years.

2. Materials and methods

2.1. Samples

Wild berries of *H. rhamnoides* ssp. *sinensis* were collected from nine locations in six provinces in China from 2006 to 2008. The growth sites were Suiling Town, Suiling County, Heilongjiang (longitude 127°06′E, latitude 47°14′N, altitude 210 m); West Channel, Liu Jianfang, Dage Town, Fengning County, Hebei (116°34′E, 41°17′N, 818 m); La'erguan, Dongxia Village, Huangyuan County, Xi'ning, Qinghai (101°23′E, 36°45′N, 3115 m); Jiucheng Palace, Hantai Town, Dongsheng District, Ordos, Inner Mongolia (109°48′E, 39°47′N, 1480 m); Beizhe Channel, Li Jiahui, San Daochuan Forestry Centre, Guan Dishan, Lüliang, Shanxi (113°52′E, 37°05′N, 1512 m); Paoma Weir, Xihua Town, Qian'nian Forestry Centre, Guan Dishan, Lüliang, Shanxi (113°52′E, 37°05′N, 2182 m) and three altitudes in Natural Reserve, Wolong, Wenchuan County, Sichuan (106°54′E, 31°01′N, 2000, 2500, and 3000 m). The plants of sea buckthorn in each growth site were divided into two to four field blocks. Berries picked randomly from the plants in the same field block were treated as a single sample lot. Berry samples were harvested in duplicate in Heilongjiang, Inner Mongolia, and Shanxi and in quadruplicate in Hebei and Qinghai. The samples from Sichuan were collected in duplicate in 2007 and in quadruplicate in 2006 and 2008. Berries were picked as soon as they were optimally ripe as defined by experienced horticulturists based on color, flavor, and structure of the berries. Due to the natural variation in these parameters in sea buckthorn, the optimal ripeness of the berries used in the current study was determined by the local sea buckthorn experts, who have been investigating the local sea buckthorn for years and have collected the berry samples for the study. The berries from different growth sites were picked at the same stage of ripeness. The berries were loosely frozen immediately after picking and kept at −20 °C before being analyzed within one year after the collection. The abbreviations *sinensis*-HL, *sinensis*-HB, *sinensis*-QH, *sinensis*-IM, *sinensis*-SX, and *sinensis*-SC will be applied, henceforth, for the berry samples collected from Heilongjiang, Hebei, Qinghai, Inner Mongolia, Shanxi, and Sichuan, respectively.

2.2. Reagents

Reference compounds, D-fructose, quinic acid, and ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). D-glucose, *myo*-inositol, and the internal standard D-sorbitol (for sugars) were purchased from Fluka (Buchs, Switzerland). Malic acid and the internal standard tartaric acid (for acids) were purchased from Merck (Darmstedt, Germany). Sucrose and citric acid were purchased from J. T. Baker (Deventer, Holland). L-quebrachitol (1L-2-*O*-methyl-*chiro*-inositol) was purchased from Alexis Corporation (Läufelfingen, Switzerland).

2.3. Sample preparation

Quadruplicate extractions of sugars, sugar alcohols, fruit acids, and ascorbic acid of each sample were performed as described earlier (Zheng, Yang, Tuomasjukka, Ou, & Kallio, 2009). About 7 g of berries was weighed accurately in duplicate, thawed at room temperature for 15 min, and pressed manually 30 times with a potato masher. The slurry was centrifuged at 4360 ×*g* for 10 min. The juice was separated and the volume was determined. A portion of 0.25 mL of the juice was taken in duplicate, and 0.25 mL of internal standard sorbitol (0.5 g/100 mL) and 0.25 mL of internal standard tartaric acid (1.0 g/100 mL) were added. The juice was then diluted with distilled water to a final volume of 5 mL. The remnant of the juice was combined, and the pH (Inolab pH level 1 meter, Wissenschaftlich Technische Werkstätten, Weilheim, Germany) and soluble solids (0 to 32 °Brix refractometer, Atago, Tokyo, Japan) were determined. The diluted juice was filtered (0.45 μm). An aliquot of 300 μL of the filtrate was evaporated to dryness under nitrogen stream at 40 °C and kept in a desiccator over P_2O_5 overnight. Trimethylsilyl (TMS) derivatives of sugars, sugar alcohols, fruit acids, and ascorbic acid were prepared by adding 600 μL of Tri-Sil (Pierce, Rockford, IL, USA) reagent, shaking vigorously with a Vortex (Vortex-Genie, Springfield, MA, USA) for 5 min, and incubating at 60 °C for 30 min. The sample was then cooled down to room temperature.

2.4. Quantification of sugars, sugar alcohols, fruit acids, and ascorbic acid

TMS derivatives of the dried juice samples were analyzed with a Hewlett Packard 5890 Series II GC (Hewlett Packard Co., Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and a Hewlett Packard 7673 auto-sampler. The analyses were carried out with a methyl silicone Supelco Simplicity-1 fused silica column (30 m × 0.25 mm i.d. × 0.25 μm d_f) (Bellefonte, PA, USA). A sample of 1 μL was injected into a split/splitless injector. The flow rate of the carrier gas helium was 1.4 mL/min. The temperature of the injector was 210 °C and that of the detector 290 °C. The column temperature was programmed as 2 min at 150 °C, raised to 210 °C at a rate of 6 °C/min, and to a final temperature of 275 °C at a rate of 40 °C/min, and was held at 275 °C for 10 min. Quantification of the sugars and sugar alcohols was carried out by using sorbitol as an internal standard. The fruit acids and ascorbic acid were quantified by using tartaric acid as an internal standard. Correction factors were determined and used for quantification for each of the sugars, sugar alcohols, fruit acids, and ascorbic acid. The ethyl β-D-glucopyranoside (henceforth ethyl glucose) was quantified as glucose and methyl-*myo*-inositol as L-quebrachitol. The total sugar content was defined as the sum of sugars, sugar derivatives, and sugar alcohols.

2.5. Statistical analysis

Statistical analyses were carried out with SPSS 16.0.1 (SPSS, Inc., Chicago, IL, USA) and Unscrambler 9.8 (Camo Process AS, Oslo, Norway). Differences in the composition between samples from different growth areas and between samples from different altitudes in Sichuan Province, as well as between different harvesting years in each growth sites, were analyzed with a one-way analysis of variance (ANOVA). The Student–Newman–Keuls (SNK) test for population with equal variances and Tamhane's test for population with unequal variances were performed for multiple comparisons. Comparison of samples between two altitudes in Shanxi Province was conducted by an independent-sample *t*-test. Principal component analysis (PCA) and bivariate correlation analysis were combined to investigate the compositional profiles of sea buckthorn berries from different growth areas and the correlation between metabolites in sea buckthorn berries. Bivariate correlation analysis and partial correlation analysis were performed to study the correlation between the latitudes and altitudes of the growth places and the composition of sea buckthorn berries. Differences reaching a confidence level of 95% were considered as statistically significant.

3. Results and discussion

3.1. Compositional profile

Fig. 1 presents the GC–FID chromatograms of the TMS-derivatized berry juice of sea buckthorn collected from Inner Mongolia in 2007 and 2008 and from Sichuan in 2008. Fructose (three isomeric forms of fructose, α- and β-D-furanose and β-D-pyranose) and glucose (two isomeric forms of glucose, α- and β-D-pyranose) were the most abundant sugars in sea buckthorn juice. Malic acid and quinic acid were the two major acids in the berries. The content of ascorbic acid varied from 0.25 to 1.66 g/100 mL juice (Table 2).