Analysis of flavour compounds and prediction of sensory properties in sea buckthorn (*Hippophaë rhamnoides L.*) berries

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Running title: Flavour compounds of sea buckthorn berry

Summary

The aim of this study was to investigate sugars, organic acids, flavonol glycosides (FGs), proanthocyanidins, and volatiles as flavour compounds in sea buckthorn (SB) berries of five cultivars and to predict the sensory properties of berries. The profiles of flavour compounds in SB berries varied significantly among the cultivars. Total proanthocyanidins and FGs were highest in 'Pertsik' and 'Raisa', respectively. Total volatiles was highest in 'Vorobyevskaya' and lowest in 'Raisa'. A previously established PLS model was used to predict the sensory properties of SB berries based on the non-volatile flavour compounds. The mouth-drying astringency can be predicted the most reliably, which has the highest regression coefficients with quinic acid, isorhamnetin-3-*O*-sophoroside-7-*O*-rhamnoside and total proanthocyanidins. Bitterness cannot be predicted using the model. 'Pertsik' berries were predicted to be more mouth-drying astringency and sour than those of 'Raisa'. The research supports the cultivar selection in cultivation and industry of SB berries.

Keywords

Flavour compounds; *Hippophaë rhamnoides*; Predicted sensory properties; Sea buckthorn

Introduction

Berries are rich in dietary fiber, micronutrients and different functional bioactive compounds, such as phenolic compounds, but also a challenge explained by the perceived sensory quality of the berry (Jimenez-Garcia *et al*,. 2013; Laaksonen *et al*,. 2016). Sea buckthorn (SB, *Hippophaë rhamnoides* L.) berry is regarded as a food raw material of high value and a source of many essential nutrients and bioactive compounds that have been linked to various health benefits, such as reducing the risk of type 2 diabetes and coronary heart diseases (Bal *et al*,. 2011; Olas 2018). Despite these nutritional and health-promoting properties, the sensory quality of SB berries limited their consumption, due to their intense sourness, perceived astringency and bitterness, coupled with a very low degree of sweetness (Laaksonen *et al*,. 2016).

Chemical constituents of the berries have strong impacts on the sensory quality, thus affecting the consumer liking and acceptance of berries and berry products. The ratio between sweetness (sugars) and sourness (acids) has been regarded as a critical factor affecting the sensory quality of berries (Tiitinen *et al*,. 2005). In SB berry, intense sourness induced mainly by abundance of malic acid would have a negative influence on the pleasantness (Laaksonen *et al*,. 2016; Ma *et al*,. 2017a). The astringent and bitter of SB berries have been reported to have a correlation with the contents of flavonols, proanthocyannins (PAs) and ethyl β-D-glucopyranoside (EG) (Ma *et al*,. 2017a; Ma *et al*,. 2017b). Besides these non-volatile compounds, odour-active volatiles have crucial influence on the sensory quality of SB berries (Lundén *et al*,. 2010). The amount of ethanol correlated with the intensity of pungent odour, and the concentration of propyl 2-methylbutanoate is related to the fermented odour (Lundén *et al*,. 2010; Tiitinen *et al*,. 2007). Previous researches has revealed that olfactory stimuli accompanying with sweet or sour-tasting foods may induce the enhancement of the associated taste quality in fruit (Schwieterman *et al*,. 2014; Sung *et al*,. 2019). Moreover, aroma is also a good indicator of freshness, quality and authenticity of SB products (Caprioli *et al*,. 2016; Tiitinen *et al*,. 2006).

Extensive variations of SB berries in chemical composition have been revealed among subspecies or cultivars. Among the cultivars of *H. rhamnoides* ssp. *mongolica,* 'Chuiskaya' berries had the most abundant fructose, and level of malic acid was the highest in 'Pertsik' (Zheng *et al*,. 2012). The total content of flavonol glycosides (FGs) varied from 23 to 250 mg/100 g fresh berries of *H. rhamnoides* ssp. *sinensis* and ssp. *mongolica* (Ma *et al*,. 2016). The origin brought the variance in the volatile profile of SB, e.g. the content of ethyl hexanoate varied from 50 to 1692 μg/kg fresh berries among 4 cultivars, and the concentration of ethyl butanoate ranged between 1.2–450 μg/kg among 13 cultivars (Lundén *et al*,. 2010; Vítová *et al*,. 2015). These aforementioned compounds have been well documented in previous research, however, most of the studies on chemical composition of SB berries have been focused on identifying new compounds or only quantifying specific components. Currently, there is limited information on the content of compounds contributing to the flavour of SB berries of specific subspecies and cultivars. Obtaining a comprehensive and systematic quantitative compositional data is needed in order to improve the current understanding of the flavour chemistry of SB berries.

The present study was focused on investigation and comparison of the profiles and contents of sensory-related non-volatile compounds (sugars, organic acids and phenolic compounds) and volatile compounds in berries of five selected SB cultivars cultivated in Finland and Estonia, as well as the prediction of sensory properties of those berries. The ultimate goal of the study is to produce comprehensive and systemic knowledge on composition of flavour compounds in sea buckthorn, and to investigate the correlation of the key components to the sensory properties in prediction model.

Materials and Methods

Berry samples

The berries of sea buckthorn cultivars ‘Pertsik’ and ‘Raisa’ were harvested on August, 2015, at Turku, Finland. The berries of cultivars ‘Botanitsheskaja ljubitelskaja (Bot-lju)’, ‘Askola’, and ‘Vorobyevskaya’ were harvested on September, 2015, at Röhu Experimental Station of Estonia. Berries were hand-picked when they were optimally ripe as defined by local experienced horticulturists, frozen immediately at −20 °C, and stored until analyses.

Quantification of sugars, ethyl *β*-D-glucopyranoside, and organic acids

Juices were manually pressed from thawed sea buckthorn berries as described before (Ma *et al*,. 2017a). Individual major sugars and organic acids as well as ethyl *β*-D-glucopyranoside (EG) were analyzed with gas chromatography (Shimadzu GC-2010 Plus, Kyoto, Japan) as trimethylsilyl (TMS) derivatives. Briefly, a sample of 1 μL was injected via an AOC-20 auto sampler, then detected with a flame ionization detector (FID). The parameters were the same as described earlier (Ma *et al*,. 2017a). The sugars, EG, and acids were identified by analysis of samples spiked with the reference compounds (Zheng *et al*,. 2009). The internal standards sorbitol (Fluka, Buchs, Switzerland) and tartaric acid (Merck, Darmstadt, Germany) were used for quantification of sugars and acids, respectively. EG was quantified as glucose (Fluka, Buchs, Switzerland).

Quantification of phenolic compounds in sea buckthorn berry

The method of extracting flavonol glycosides (FGs) sea buckthorn berries was performed according to our previous method (Ma *et al*,. 2016). FGs were analysed using a Shimadzu Nexera ultrahigh performance liquid chromatograph UHPLC system (Shimadzu Corporation, Kyoto, Japan). The analysis procedure was controlled by Shimadzu workstation LabSolutions (version 5.42SP3). The samples were separated on a Phenomenex Aeris peptide XB-C18 (3.6 µm, 150 × 4.60 mm) column. The chromatographic conditions were the same as described previously (Ma *et al*,. 2017b). The peaks were monitored at 360 nm. Identification of FGs was based on reference compounds and the results described earlier (Ma *et al*,. 2016; Zheng *et al*,. 2016). Quantification of all the FGs was carried out as applied earlier, using calibration curves constructed with quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside and isorhamnetin-3-*O*-rutinoside (≥ 99%, Extrasynthese, Genay, France) as the external standards (Ma *et al*,. 2016).

Extraction and purification of proanthocyanidins from the SB berries were performed as described previously (Yang *et al*,. 2016b). The quantification of PA oligomers (dimers, trimers and tetramers) was conducted with HILIC-ESI mass spectrometry in positive ion mode using single ion recording (SIR) method, whereas the content of total PAs was determined according to the Brunswick Laboratories 4-dimethylaminocinnamaldehyde (BL-DMAC, Sigma-Aldrich, St. Louis, MO) assay (Yang *et al*,. 2016b). The contents of oligomeric PAs and total PAs were detected using the standard curves of procyanidin B2 (≥ 99%, Extrasynthese, Genay, France), and quantified as procyanidin B2 equivalents.

HS-SPME-GC-MS profiling of volatile compounds

Extraction of the volatiles from the SB berries by HS–SPME and GC–MS analyses were carried out with a method previously employed with some modifications (Marsol-Vall *et al*,. 2018). Weighted (20 g) SB puree mixed with an equal amount of 10% NaCl aqueous solution, then 1.0 g of the slurry was transferred to a 20-mL headspace vial and spiked with 10 µL of the internal standard solution (neryl acetate at 100 µg/mL in methanol, Sigma-Aldrich, St. Louis, MO). The internal standard fulfilled the criteria as described in our previous study (Marsol-

Vall *et al*,. 2018). The samples were incubated and the collection of the volatiles by HS-SPME was carried out with a 1-cm SPME fiber as described in Marsol-Vall *et al*,. (2018) for 20 min.

After the extraction, GC-MS analyses were carried out with a HP 6890 Series (HP Hewlett-Packard, Little Falls, DE) gas chromatograph system coupled to a 5973 Mass Selective detector (HP Hewlett-Packard). The SPME fiber was desorbed into the injection port equipped with an SPME liner at 220 °C for 20 min. Volatile compounds were separated with a DB-WAX column (60 m x 0.25 mm i.d.; 0.25 µm film thickness) from Agilent Technologies (Palo Alto, CA) using helium as carrier gas (1.4 mL/min). The oven was temperature programmed from 50 °C (hold for 3 min) to 190 °C at a rate of 5 °C/min, hold at 190 °C for 10 min. Mass spectra were recorded in electron impact (EI) mode at 70 eV within the mass range *m/z* 40–300. The transfer line and the ionization source were thermostated at 200 and 220 °C, respectively. The system was operated with HP ChemStation software (B.01.00).

Identification of volatile compounds was carried out as detailed in previous works by mass spectra match (direct match > 800) and comparison of linear retention indexes (RI) calculated with an homologous series of *n*-alkanes (C_9-C_{30} from Supelco) with those available in the Nist WebBook (Lindstrom and Mallard 1998; Marsol-Vall *et al*,. 2017; Marsol-Vall *et al*,. 2018). Total ion current (TIC) was used for peak area integration. Quantitation of volatiles was carried out using neryl acetate assuming no differences in response factors among the quantified volatiles.

The prediction of sensory properties

In previously established PLS model, sensory characteristics of the SB purees were evaluated using a generic descriptive analysis, and the intensities of the attributes were rated from 0 (none) to 10 (very strong) with the help of anchored reference samples as described in the Ma *et al*,. (2017b). The PLS model and the compositional data of the non-volatile compounds (n=41, X-data) were used to predict the sensory properties (Y-data) of SB berries in this study. The predictions were made for the sweetness, sourness, bitterness, mouth-drying and puckering astringency, as well as total intensity of flavour of berries.

Statistical analysis

All samples were prepared and analysed in triplicates. The SPSS statistical package (version 22.0, SPSS, Inc., Chicago, IL, U.S.A.) was used. All the data were expressed as the means ± SD (standard deviations). One-way analysis of variance (ANOVA) with Tukey's honest significant difference (HSD) post hoc tests was carried out for multiple comparisons.

Unsupervised classification with principal component analysis (PCA) was used to investigate variations in the non-volatile sensory-related compounds (n = 41) and volatiles (n = 50) of the SB berries of different cultivars. Partial Least Squares (PLS) regression model from the earlier study was applied to predict the sensory qualities of the five cultivars in this study with all non-volatile compounds as X-data (n = 41) and sensory variables as Y-dat. An additional PLS regression model was created using only the most significant X-variables (n = 15) in first model. Full cross-validation was used to estimate a number of factors for statistically reliable models. Root mean square error (RMSE) of cross-validation was used to examine the error in the predicted models. Multivariate models were created with Unscrambler 10.3 (Camo Process AS, Oslo, Norway).

Results and Discussion

The contents of individual sugars, organic acids and ethyl *β*-D-glucopyranoside

Glucose appeared the most numerous sugar among all cultivars studied, the content of which ranged from 0.5 to 4.6 g/100 mL (Table 1). Followed by fructose and L-quebrachitol, the concentrations of these compounds varied from 0.2 to 1.7 g/100 mL and 0.2 to 0.4 g/100 mL, respectively. ‘Raisa’ berries had the highest content of EG (0.7 g/100 mL), which indicated that EG might play a more important role in the sensory quality of ‘Raisa’ than in other cultivars, due to the contribution of EG to bitterness (Ma *et al,*. 2017a). Malic acid was as the most abundant acid with the content varying from 3.0 to 6.9 g/100 mL. Quinic acid had the highest content in the berries of ‘Raisa’ (3.9 g/100 mL). Citric acid was present at lower levels (0.03−0.08 g/100 mL) than malic and quinic acids. The highest sugar to acid ratio was found in ‘Vorobyevskaya’ (1.3). Thus, the berries of ‘Vorobyevskaya’ were expected to be perceived sweeter than the berries of other cultivars studied (Tiitinen *et al,*. 2005).

Phenolic profiles in sea buckthorn berries

Ten major flavonol glycosides (FGs) were detected and quantified in this study as described previously (Ma *et al,*. 2017b), of which the sum was taken as total FGs (Table 1). Only the glycosides of isorhamnetin (Is, 85.7−95.2 %) and quercetin (Qu, 4.8−14.3 %) were detected in studied samples. Isorhamnetin-3-*O*-rutinoside (I-3-R, 21.4−35.6 % of total FGs) and isorhamnetin-3-*O*-glucoside-7-*O*-rhamnoside (I-3-G-7-Rh, 28.5−35.2 % of total FGs) were the two most abundant FGs in all the samples (Table 1). The latter compound had been reported to have a close association with the astringent attributes of SB berries (Ma *et al,*. 2017b). The content of total FGs varied significantly from 35.0 to 158.4 mg/100g fresh berry among all the cultivars (p < 0.05, Table 1), nearly 5-fold. ‘Raisa’ had the highest content of most FGs, whereas, the lowest contents of FGs existed in ‘Bot-lju’ (p < 0.05).

Besides flavonol glycosides in SB berries, compounds belonging to other groups of polyphenols, i.e. oligomeric proanthocyanidins (PAs) and total PAs were also determined in the samples studied.

As reported previously, only B-type PAs were found in the SB berries, PA dimers, trimers and tetramers were the principal constituents of PA oligomers (Kallio *et al,*. 2014; Yang *et al,*. 2016b). The contents of PA dimers, trimers, tetramers and total PAs were shown in the Table

1. Among the cultivars, 'Pertsik' presented the lowest contents of oligomeric PAs, but the highest content of total PAs ($p < 0.05$). The highest contents of oligomeric PAs and the lowest content of total PAs were found in 'Vorobyevskaya' and 'Bot-lju', respectively ($p < 0.05$). In all the samples, the contents of prodelphinidins (PDs), such as Dim-3 and PD-based Tri-3 dominated in oligomeric PAs. It was worth noting that quantified as procyanidin B2 equivalents, oligomeric PAs only accounted for a small portion (4–23%) of total PAs, since the content of total PAs based on the BL-DMAC method not only covered the oligomeric PAs, but also covered both monomeric PAs and polymeric PAs (degree of polymerization > 4).

Volatile profiles in sea buckthorn berries

The 45 volatile compounds were found in the SB berries under study (Table 2), most of which have already been reported previously in SB (Leung and Marriott 2016; Socaci *et al*,. 2013; Tiitinen *et al*,. 2006). However, it is likely that the few compounds from the ester chemical group were the major volatile compounds responsible for the particular aroma of SB as alcohols, aldehydes, hydrocarbons and ketones were all found below their reported odor threshold (George 2009). It is, however, important to recognize that the analysis method used in this study is semi-quantitative and the juice matrix has significant impact on the odor thresholds of aroma compounds. It has to be noted that esters represented at least 88% of the total volatile profile in all the cultivars. 'Vorobyevskaya' was strongly linked to the esters (41187 μg/kg) involved compounds 21, 6 and 16 (Table 2). 'Raisa' presented the lowest content of esters (12915 μg/kg). Considering their contents in the samples and the odor thresholds reported in the literature (George 2009; Lundén *et al*,. 2010), compounds 2, 5, 6, 16, 18, 19, 21, 31, 33 and 41 were considered to be the key volatile components (bold in Table 2), which were expected to have a contribution on the aroma of the studied cultivars.

Compounds 5 and 6 both presented a fruity and apple like odorant, which were found at higher contents in 'Vorobyevskaya', 1147 and 2290 μg/kg, respectively (Table 2). Compound

16, a fruity berry-like odorant, was found in a heterogeneous distribution, being the lowest in 'Askola' (2871 µg/kg) and the highest in 'Vorobyevskaya' (7216 µg/kg). Compound 21 has been descripted as fermented, spoiled and compost in SB berries by GC-O (Lundén *et al,*. 2010) and was found to be particularly high in 'Bot-lju' (16257 µg/kg). Compound 31 had been reported to have a coconut, synthetic aroma (Lundén *et al,*. 2010) and was found to be especially high in 'Vorobyevskaya' (2379 µg/kg). This compound is reported to have an odor threshold of 92 µg/kg indicating its contribution to the SB aroma (George 2009). Moreover, it has been found that terpene flavour induced by terpene compounds, such as compounds 9 and 13, was related to the consumer negative perception of fruit (Sung et al 2019).

Importantly, internal-standard mixture was employed to correct any possible analytical deviation caused by variations in the performance of the fiber and instrumentation, considering negligible difference in the affinity of the fiber with the volatiles. Hence, quantitation of volatiles was based on this approach. This limitation of volatile analysis should be considered when the results are interpreted.

Comparison of the sea buckthorn cultivars

PCA models were applied as a multivariate data analysis technique to display more detailed information of the differences and similarities among the cultivars (Figure 1). In the PCA model of non-volatile sensory-related compounds, the first two validated principal components shown in Figure 1A explained 73% of the variance of the data (n = 41). 'Raisa' was located on the right side of the plot with a higher content of FGs and organic acids, such as I-3-R and quinic acid. 'Askola' was located in the middle of the plot with the least abundance of glucose and total sugars, but the highest abundance citric acid. Whereas 'Vorobyevskaya' berries had the closest association with the content of oligomers of PAs and sugars locating on the left of the plot. The second component (PC2) discriminated 'Bot-lju' and 'Pertsik' from the other cultivars. The ‘Bot-lju’ berries had the lowest content of most phenolic compounds, while the berries of ‘Pertsik’ contained the highest content of total acids and total PAs. This highlights the importance of the non-volatile composition, which could be most influential in discriminating among the different cultivars of sea buckthorn.

In the PCA model of volatile compounds, the first two validated principal components shown in Figure 1B explained 74% of the variance of the data (n = 50). ‘Raisa’ was located on the right side of the plot with the lowest content of total volatiles and compound 33, but the highest content of compound 41 (Table 2). The ‘Bot-lju’ was located on the left side with the least abundance of compounds 5, 6, 31, 41, but the highest content of compounds 21 and 33. The compound 21 was descripted as fermented and spoiled odor by GC-O (Lundén *et al*,. 2010). The cultivars ‘Pertsik’ and ‘Askola’ were located in the middle of the plot. ‘Pertsik’ was rich in compound 17 and the berries of ‘Askola’ had the highest content of compounds 28 and 30. The second component (PC2) discriminated ‘Vorobyevskaya’ from all other 4 cultivars correlating with the most abundant compounds 2, 5, 6, 19 and 31.

Importantly, besides cultivars, other climatic and environmental conditions affected by the location of the growth sites may also have contributed to the contents and compositions of compounds in the berries, which have to be considered when interpreting the results.

The prediction of sensory properties

The chemical composition has strong correlation with the intensities of sensory attributes in the SB berries (Ma *et al*,. 2017b; Ma *et al*,. 2017a; Tiitinen *et al*,. 2005). The predicted attributes of the berries of five cultivars commonly were associated with sugars, acids and phenolic compounds (Table 3). The attribute sourness had the highest Q^2 value (0.808, with 4 factors), indicating the reliable prediction among attributes, regardless of the high RSME value. The lowest predictive value was shown in bitterness (Q^2 0.023, with 4 factors) with higher error

(RMSE 0.460). The original regression model published in Ma *et al,*. (2017b) showed only weak interaction between the chemical variables and bitterness, thus indicating that the bitterness of the five cultivars could not be explained well by the non-volatile chemical variables studied. Moreover, the variations in the predictions varied notably among the cultivars (model deviations in Table 3). Variations are the lowest with the cultivar 'Pertsik' and 'Raisa', whereas the attributes for other cultivars were not predicted with equal level of confidence.

Table 4 summarized the regression coefficients of the PLS-model of non-volatile chemical components (n=41) for each attribute. In general, organic acids and phenolic compounds had positive association with the all predicted attributes, except sweetness. On the other hand, the pH and sugars showed positive correlation with the sweetness, negative with all the other attributes. These results were in accordance with previous findings showing that sugars contributed to the sweetness, phenolic compounds contributed to the astringency, and organic acids and pH influenced not only sourness but also astringency (Ma *et al,*. 2017a; Peleg and Noble 1999; Tiitinen *et al,*. 2005). In particular, isorhamnetin-3-*O*-sophoroside-7-*O*-rhamnoside (I-3-S-7-Rh) was a strong factor for all attributes except bitterness (Table 4). Previously, the content of I-3-S-7-Rh had been found to be closely related to the astringent attributes of SB purees (Ma *et al,*. 2017b). Besides I-3-S-7-Rh, the contents of I-3-R, Dimer-2 and total PAs were correlated strongly to the predicted mouth-drying astringency. Only one phenolic compound, isorhamnetin-glucoside, was closely related to the puckering astringency. EG and quinic acid had close correlation with bitterness (Table 4).

In order to improve prediction of the sensory attributes, another model was created based on the factors showing the highest regression coefficients (n=15, X-data) in the first PLS-model (Tables 3 and 4). The prediction of the attributes sweetness, sourness and mouth-drying astringency had notably higher Q^2 values (0.801, 0.866 and 0.900, respectively, with 4 factors) and with lower errors (RMSE values 0.206, 0.450 and 0.114, respectively), indicating the better explanation compared with other attributes. Especially, mouth-drying astringency can be predicted the highest reliability. With the more efficient model, the cultivars ‘Raisa’ and ‘Bot-lju’ were predicted to be less mouth-drying astringent and sour than ‘Pertsik’ and ‘Askola’. Similarly to the first model, however, model variations were the highest for ‘Askola’ and ‘Vorobyevskaya’, indicating their significant compositional difference from the original cultivars in the previous study (Ma *et al*,. 2017b). Again, bitterness was not predicted by these key components (Table 3).

It is important to note here that the prediction in this study was made based on only the non-volatile compounds, although the volatile compounds will definitely influence the flavour of SB berries. The prediction of sensory properties of food is a challenging task because of the influence of complex food matrices. The taste-taste interaction and the taste-aroma interaction should be taken into account, which may suppress or enhance the perception of individual compounds in food matrices (Breslin 1996; Keast and Breslin 2003).

Conclusions

The profiles of flavour compounds varied significantly in SB berries of 5 cultivars correlating with the distinct sensory qualities. Total volatiles was highest in ‘Vorobyevskaya’ and lowest in ‘Raisa’. In ‘Pertsik’ the highest total PAs and in ‘Raisa’ the highest FGs were noted. The mouth-drying astringency was predicted with high level of reliability using the new model created with 15 variables of the highest regression coefficients of the first PLS-model. Bitterness of SB cultivars studied could not be predicted by those variables. Based on the predictive models, the berries of ‘Pertsik’ were predicted to be more mouth-drying astringency and sour than those of ‘Raisa’. The composition of flavour compounds can be correlated with the predicted sensory qualities of sea buckthorn berries, which could be used in the future selection and breeding of superior sea buckthorn cultivars.

Acknowledgements

The authors thank Mr. Hannu Lappalainen, Mr. Bärtil Lappalainen, Mr. Seppo Lappalainen, and Ms. Hedi Kaldmäe for providing the sea buckthorn berries.

Declarations of interest

The authors declare no competing financial interest.

Statements

Research data are not shared.

Ethics approval was not required for this research.

Funding sources

This work was supported by the Finnish Food Research Foundation (the Finnish Food and Drink Industries' Federation) and Turku University Foundation.

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