ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume 63 20 Number 1, 2015
http://dx.doi.org/10.11118/actaun201563010147

COMPARISON OF FLAVOUR AND VOLATILE FLAVOUR COMPOUNDS OF MIXED ELDERBERRY JUICES

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Abstract

VÍTOVÁ EVA, SŮKALOVÁ KATEŘINA, MAHDALOVÁ MARTINA, BUTOROVÁ LENKA, BABÁK LIBOR, MATĚJÍČEK ALEŠ. 2015.Comparison of Flavour and Volatile Flavour Compounds of Mixed Elderberry Juices. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* 63(1): 147–152.

The aim of this work was to find the best composition for fruit drink based on elderberries with optimal flavour characteristics. For this purpose elderberry juice was mixed with various fruit juices (grape, black currant, apple, orange, carrot) in various ratios, flavour was evaluated sensorially and instrumentally as the content of aroma compounds.
Five flavour characteristics (sweet, acid/sour, bitter, astringent, characteristic elderberry), off-flavour, odour, texture (mouth-feel), colour and overall acceptability were evaluated sensorially using scale. Aroma compounds were extracted by solid phase microextraction and assessed by gas chromatography with flame ionization detection and gas chromatography-mass spectrometry. The significant differences ($P < 0.05$) in flavour were found between samples, which could be explained by differences in their volatile profiles. In total 57 compounds were identified in fruit juices and included 20 alcohols, 10 aldehydes, 8 ketones, 7 acids, 7 esters and 5 other compounds. Alcohols were quantitatively the most important group of all juices. The grape-elderberry juice, in optimum ratio 7:3 (70% v/v of elderberry), was proposed for practical use owing to the pleasant sweetish, elderberry flavour, and excellent other sensory characteristics.

Keywords: elderberry, flavour, aroma compounds, GC, SPME, sensory analysis

INTRODUCTION

The popularity of functional beverages led to the search for other sources of raw materials that provide great taste and functionality to consumers. Black elder (*Sambucus nigra* L.) is a plant with miscellaneous use, for its therapeutic effects in medicine, for its aroma and taste in the cuisine. Wild elderberry fruits and flowers are used mainly for the home made production of marmalades, juices, syrups, teas, liqueurs and wines. Fruits are known by high content of anthocyanine pigments and they are used for production of natural colorants (Karovicova *et al.*, 1990). The production of cultivated elderberries in Czech Republic is only at the beginning, Czech food industry uses only imported frozen elderberries as fruit component of yoghurts. However, elderberry contains many health promoting substances (Christensen *et al.*, 2008; Veberic *et al.*, 2009), which, in the form of appropriate modern food products, could enrich the consumer market. Successful commercialization of elderberry fruits depends especially on good flavour, which is related to the content of sugars and acids, and aroma, which is strongly associated to the content of volatile aroma active substances (Kaack *et al.*, 2005).

The aroma of elder flowers (Velisek *et al.*, 1981; Jørgensen *et al.*, 2000; Kaack and Christensen, 2008) and elder berries (Davidek *et al.*, 1982; Jensen *et al.*, 2000) have been characterized before in detail by several authors and more than 100 volatiles have been identified. Most of them are well known aroma constituents of fruit products. Several authors also dealt with sensory evaluation of flavour of elderberries. They mostly evaluated selected descriptors, e.g. sweetness, sourness, fruitness, freshness, flowerness and bitterness using scale: 1-the lowest intensity ⇒ 5-the highest intensity (e.g. Kaack, 2008a; Kaack, 2008b). The others used preference test using scale: 0-without elderberry flavour to 10-intensive elderberry flavour (e.g. Kaack *et al.*, 2005; Kaack *et al.*, 2006).

The aim of this work was to identify and quantify the volatiles in elderberry juice mixed with various types of fruit juices in various ratios in order to find the best composition for attractive and healthy fruit drink based on elderberries with optimal flavour. Aroma compounds were extracted by solid phase microextraction (SPME) and assessed by GC-FID and GC-MS, the flavour was evaluated sensorially. SPME is very useful alternative to other tedious or expensive extraction methods and have been used by many authors to measure the volatile compounds of food samples (reviewed by Kataoka *et al.*, 2000) including various fruit and vegetable juices (Riu-Aumatell *et al.*, 2004; Soria *et al.*, 2008; Aprea *et al.*, 2009; Li *et al.*, 2010; Schmarr and Bernhardt, 2010) and wines (Verzera *et al.*, 2008; Olivero and Trujillo, 2010). Many works about sensory evaluation of various types of fruit and fruit juices were also published (Jain *et al.*, 2003; Cliffe-Byrnes and O'Beirne, 2007; Perez-Cacho *et al.*, 2008; Altisent *et al.*, 2011; Baroň and Kumšta, 2012).

MATERIALS AND METHODS

Samples

Six types of fruit juices were analysed: grape, black currant, apple, orange, carrot and elderberry (standard). The various mixtures of elderberry with other fruit juices were prepared in a ratio 3:7 (30% v/v of elderberry). Then the mixture elderberry-grape was tested in various proportions (50–90% v/v of elderberry). Sample labeling: E: pure elderberry, G: pure grape, E-gr: elderberry-grape, E-bc: elderberry-black currant, E-ap: elderberry-apple, E-or: elderberry-orange, E-ca: elderberry-carrot. E-gr 50 to E-gr 90: elderberry-grape in various proportions.

Elderberry juice was acquired by pressing of fresh berries (Breeding institute of Faculty of Agriculture, Mendel University in Brno, Lednice, Czech Republic, picked in September 2012), pasteurized (78 °C 30 s) (Pasteur EHA 18, Germany) and immediately cooled to 6 °C until analysis. Grape, black currant, apple, orange and carrot juices were bought on the Czech market.

SPME-GC-FID/MS Conditions

Volatile compounds were extracted by solid phase microextraction (SPME), identified by gas chromatography-mass spectrometry (GC-MS) and quantified using standards by GC-FID. For analysis 1 ml of juice was placed into vial for SPME extraction, three samples of every juice was taken, every sample was analysed three times.

The SPME conditions were: SPME fiber CAR™/PDMS 85 μm (Supelco). Sample volume 1 ml, extraction temperature 35 °C, equilibrium time 30 min., extraction time 20 min., desorption temperature 250 °C, desorption time 5 min.

Gas chromatograph used was TRACE™ GC (ThermoQuest, I), capillary column DB-WAX (30 m × 0.32 mm × 0.5 μm). GC conditions: injector 250 °C, splitless desorption 5 min., carrier gas N_2 0.9 ml.min^{-1}, flame ionization detector (FID) at 220 °C, H_2 35 ml.min^{-1}, air 350 ml.min^{-1}, make up N_2 30 ml.min^{-1}. The oven temperature was 40 °C for 1 min, 40–200 °C at 5 °C/min, 200 °C for 7 min. GC-MS analyses were performed on GC 8000 (Carlo Erba, I) with MS TRIO 1000 (Fisons Instruments, USA). Carrier gas He, GC column and conditions were the same as described above. The validation parameters of SPME-GC-FID/MS method were published previously (Vítová *et al.*, 2006; Vítová *et al.*, 2007).

Sensory Analysis

The sensory analysis was carried out in a sensory laboratory (ISO 8589: 2008). The assessors were selected, then trained (including sensory profiling) and monitored for six months in accordance with (ISO 8586-1: 2002). Fifteen assessors (9 women and 6 men) were then used for evaluation of the samples in different sessions. 30 ml of the samples were served in 50 ml glass beakers, marked with 4-digit codes, in random order. Tap water was used between the samples.

The sensory attributes were evaluated using unstructured 10 cm line scale, anchored from each end to identify the direction. The list of attributes comprised one term for colour (characteristic elderberry, ranging from atypical to typical deep purple), odour (characteristic elderberry, from imperceptible to very strong), five flavour characteristics (sweet, acid/sour, bitter, astringent, characteristic elderberry, from weak to very strong), off-flavour (from imperceptible to very strong), texture and mouth-feel attribute encompassing viscosity (from thin to viscous), and overall acceptability (from unacceptable to delicious) (ISO 13299: 2003).

Statistical Evaluation

The results of sensory analyses were statistically evaluated by means of Kruskall-Wallis test and then by Nemenyi multiple comparison test; they are expressed as mean (n = 15). The results of instrumental analyses were treated using parametric one way analysis of variance and subsequently by Duncan test; they are expressed as mean ± SD (n = 9). All these analyses were performed at $p < 0.05$ using Unistat version 5.5 (Unistat, London, United Kingdom).

RESULTS AND DISCUSSION

Sensory Evaluation of the Fruit Juices

The main intention of this work was to find the best composition of fruit juices for fruit drink based on elderberries with optimal flavour. The flavour should be pleasant-tasting and simultaneously retaining noticeable characteristic elderberry note. Kaack (2008a; 2008b) dealt with similar idea of new elderberry products in his works. Recent research found that elderberries are concentrated sources of anthocyanins that appear to benefit health in several ways owing to their powerful antioxidant capacity (Christensen *et al*., 2008). So the processing of elderberry to new food products and their increased consumption is highly advisable. However, the pure elderberry juice has bitter, sour, astringent taste, not suitable for direct consumption. It is possible to assume that these negative properties could be improved by mixing of elderberry with other fruit juice. For this reason the several partial aims were set:

i) the samples of selected types of fruit juices, in various ratios mixed with elderberry juice, were prepared;
ii) the samples were sensorially evaluated to find the optimum sensory quality;
iii) the content of aroma compounds was determined in these samples to compare the aroma profiles;
iv) the OAVs (odour activity values) were calculated for compounds identified to estimate their contribution to aroma and flavour quality.

At first various types of juices, in various ratios combined with elderberry, were preliminary evaluated by panel of 6 experts (results are not included). On the base of sensory properties, and also market price taking into consideration, grape, black currant, apple, orange and carrot juices were chosen and were further tested in recommended ratio 30% elderberry : 70% other fruit (v/v) (i.e. combined mixed fruit juices). The pure elderberry juice was evaluated simultaneously as comparative standard. The results are given in Tab. I.

The emphasis was put on preservation of characteristic deep purple colour, odour and flavour of elderberry. The mixture black currant-elderberry had the highest ($P < 0.05$) sensory ratings in colour, odour and characteristic flavour, similar to pure elderberry. The colour of carrot- and orange-elderberry juices was evaluated as unsatisfactory owing to their atypical, brownish shade. The texture (viscosity) of all juices was well evaluated, the mixtures with black currant and grape were the nearest to standard. Flavour and odour were considered as the most important characteristics. The mixtures with black currant and grape were evaluated as tastiest ($P < 0.05$), with the highest ratings of overall acceptability. Currant with more acid/sour (piquant) taste, grape was sweeter, very pleasant. Both of them maintained characteristic elderberry flavour in sufficient intensity, bitterness and astringency were suppressed and almost imperceptible. Carrot-elderberry was found as the worst ($P < 0.05$) with strong unpleasant earthy off-flavour.

Finally the grape-elderberry juice was chosen for practical use owing to the pleasant sweetish taste; good price and accessibility of grapes in the market were also taken into consideration. This mixture was further investigated; the others were excluded from this study because of low practical utility. So the various blends grape-elderberry with various contents (50–90% v/v) of elderberry juice were prepared to find the optimum ratio with the best flavour (Tab. I). All of these samples (including the most diluted by grape juice) kept the deep purple colour and good texture. Significant ($P < 0.05$) differences were found in odour and flavour. As expected, juices with higher addition of grape had higher ratings in sweet flavour and overall acceptability, juices with predominance of elderberry had stronger elderberry flavour. Finally the compromise was selected, the mixture with 70% (v/v) of elderberry, which was evaluated as very good, sweet with adequately strong characteristic elderberry aroma.

Comparison of Aroma Profiles of Juices

The content of volatile aroma compounds and their contribution to flavour is one of the important characteristics of fruits. The flavour of fruits is made up of a great number of volatile compounds, among which may be a number of alcohols, esters, acids, terpenes, carbonyl compounds, phenols and lactones (Rosillo *et al*., 1999). These aroma compounds are produced during ripening, harvest, post-harvest and storage depending on many factors related to the species, variety and type of technological treatment. Their concentrations are very low and vary between varieties (Kataoka *et al*., 2000). Despite the developments in flavour research, most biochemical pathways determining this quality trait are still unknown (Song and Forney, 2008).

In total 57 compounds were identified in samples including 20 alcohols: ethanol, propan-1-ol, propan-2-ol, butan-1-ol, butan-2-ol, pentan-2-ol, pentan-1-ol, hexan-1-ol, heptan-2-ol, octan-1-ol, octan-2-ol, decan-1-ol, decan-2-ol, (E)-3-hexenol, oct-1-en-3-ol, 2-methylpropanol, 2-methylbutan-1-ol, 3-methylbutan-1-ol, phenylmethanol, phenylethanol, 10 aldehydes: pentanal, hexanal, heptanal, octanal, nonanal, (E)-2-hexenal, (E)-2-octenal, 3-methylbutanal, benzaldehyde, phenylethanal, 8 ketones: butan-2-one, pentan-2-one, heptan-2-one, nonan-2-one, decan-2-one, undecan-2-one, 4-methylpentan-2-one, 3-hydroxybutan-2-one, 7 acids: acetic, propanoic, octanoic, decanoic, 2-methylpropanoic, 3-methylbutanoic, 2-hydroxypropanoic, 7 esters: methyl acetate, ethyl acetate, propyl acetate, butyl acetate, ethyl butanoate, ethyl octanoate, ethyl decanoate and 5 other compounds: limonene, β-damascenon, α-terpineol, I: *Colour, odour, flavour, texture and mouthfeel and overall acceptability ratings (in %) of mixed elderberry juices*

Sensory characteristics		**Type of fruit juice**										
		E	**E-gr**	**E-bc**	**E-ap**	**E-or**	**E-ca**	**E-gr 50**	**E-gr 60**	**E-gr 70**	**E-gr 80**	**E-gr 90**
Colour	Characteristic elderberry	92.1^{aAC}	63.1^{bc}	87.9^{a}	73.2^{bc}	44.2^{c}	31.1^{c}	75.5^{B}	78.9^{B}	81.3^{BC}	87.6^{C}	89.4^{AC}
Odour	Characteristic elderberry	93.4^{aA}	69.5^{ab}	82.4^{ab}	64.3^{bc}	47.3^{cd}	38.4^{d}	74.8^{B}	74.9^{B}	81.2^{BC}	88.2^{AC}	90.3^{AC}
Flavour	Sweet	21.5^{aA}	84.8^{bc}	51.3^{ac}	72.8^{bc}	69.3^{c}	55.4^{ac}	79.6^{B}	69.5^{BC}	61.5^{BCD}	42.4^{AC}	35.3^{AD}
	Acid/sour	84.4^{aA}	32.4^{bc}	78.6^{ac}	44.3^{bc}	42.4^{c}	42.8^{c}	40.1^{B}	45.6^{BC}	59.4^{BCD}	62.7^{AC}	79.3^{AD}
	Bitter	89.3^{aA}	41.5^{bc}	54.6^{c}	42.2^{bc}	43.4^{c}	44.1^{c}	50.2^{B}	59.1^{BC}	64.3^{CD}	77.9^{D}	86.4^{A}
	Astringent	78.1^{aA}	37.2^{bc}	53.8^{c}	36.9^{b}	38.3^{bc}	41.8^{c}	44.5^{B}	58.6^{BC}	62.4^{CD}	71.4^{D}	75.8^{A}
	Characteristic elderberry	95.4^{aA}	61.2^{b}	84.4^{ab}	59.3^{b}	42.3^{bc}	39.4^{c}	72.5^{B}	76.5^{BC}	82.4^{CD}	88.2^{DE}	91.6^{AE}
	Off-flavour	11.8^{aA}	36.4^{bc}	33.2^{b}	41.5^{bc}	43.2^{c}	87.6^{d}	32.2^{B}	25.8^{BC}	15.4^{CD}	13.8^{AD}	12.6^{A}
Texture and mouthfeel	Viscosity	81.3^{aA}	71.0^{b}	74.3^{c}	68.9^{b}	66.4^{c}	69.6^{bc}	77.3^{B}	78.1^{BC}	78.3^{BC}	79.2^{AC}	81.2^{A}
Overal acceptability		72.3^{acA}	86.2^{b}	85.6^{b}	81.5^{ac}	64.0^{ac}	35.1^{c}	87.4^{B}	87.2^{B}	83.7^{BC}	79.8^{CD}	75.4^{AD}

The results are expressed in % as mean ($n = 15$). Different small letters in the same row indicate significant differences ($p < 0.05$) among combined mixed fruit juices, different capital letters in the same row indicate significant differences ($p < 0.05$) among elderberry-grape juices. Sample labeling: E: pure elderberry, E-gr: elderberry-grape, E-bc: elderberry-black currant, E-ap: elderberry-apple, E-or: elderberry-orange, E-ca: elderberry-carrot. E-gr 50 to E-gr 90: elderberry-grape in various proportions (50–90% v/v of elderberry).

II: *Comparison of chemical groups of compounds identified in mixed elderberry juices*

Aroma compounds ($\mu g.ml^{-1}$)	**Type of fruit juice**					
	E	**E-gr**	**E-bc**	**E-ap**	**E-or**	**E-ca**
Alcohols	1450.6 ± 13.93^{aA}	1006.2 ± 19.91^{b}	760.5 ± 21.69^{c}	1255.5 ± 14.42^{ab}	842.2 ± 17.59^{c}	1169.3 ± 17.83^{ab}
Aldehydes	0.6 ± 0.22^{aA}	0.1 ± 0.01^{b}	0.2 ± 0.02^{c}	0.5 ± 0.08^{a}	0.1 ± 0.01^{c}	1.5 ± 0.32^{d}
Ketones	1.2 ± 0.04^{aA}	2.4 ± 0.05^{b}	2.4 ± 0.09^{b}	2.3 ± 0.15^{b}	4.3 ± 0.85^{c}	3.0 ± 0.16^{bc}
Acids	230.1 ± 8.69^{aA}	87.2 ± 11.09^{b}	139.2 ± 13.88^{c}	96.3 ± 34.09^{b}	189.4 ± 6.68^{ac}	31.7 ± 1.72^{d}
Esters	11.8 ± 2.27^{aA}	7.1 ± 0.54^{b}	7.4 ± 0.41^{b}	7.0 ± 0.29^{b}	3.6 ± 0.11^{c}	6.1 ± 0.33^{b}
Others	0.7 ± 0.02^{aA}	1.0 ± 0.01^{b}	nd	0.5 ± 0.01^{c}	nd	nd
In total AC	1694.4 ± 16.58^{aA}	1102.8 ± 21.59^{b}	909.1 ± 32.09^{c}	1361.7 ± 49.03^{ab}	1039.2 ± 24.80^{bc}	1211.4 ± 11.36^{b}
	E-gr 50	**E-gr 60**	**E-gr 70**	**E-gr 80**	**E-gr 90**	**G**
Alcohols	1118.1 ± 12.32^{B}	1202.9 ± 14.54^{B}	1326.5 ± 21.34^{AB}	1396.4 ± 13.34^{A}	1351.0 ± 18.48^{AB}	661.0 ± 4.06^{C}
Aldehydes	0.6 ± 0.03^{A}	0.3 ± 0.02^{B}	0.2 ± 0.02^{B}	0.4 ± 0.08^{B}	0.2 ± 0.04^{BC}	0.1 ± 0.02^{C}
Ketones	1.1 ± 0.01^{A}	1.1 ± 0.01^{A}	1.0 ± 0.01^{A}	1.0 ± 0.01^{A}	1.0 ± 0.01^{A}	0.1 ± 0.01^{B}
Acids	200.2 ± 5.36^{A}	132.7 ± 5.36^{B}	150.5 ± 10.12^{B}	105.0 ± 1.59^{C}	114.2 ± 1.80^{C}	166.1 ± 2.84^{AC}
Esters	7.1 ± 1.04^{B}	9.2 ± 0.32^{C}	10.8 ± 0.18^{AC}	12.9 ± 0.42^{A}	12.1 ± 0.86^{AC}	0.5 ± 0.01^{D}
Others	0.4 ± 0.02^{B}	1.1 ± 0.05^{C}	1.6 ± 0.03^{D}	1.4 ± 0.07^{D}	1.3 ± 0.04^{CD}	1.5 ± 0.12^{D}
In total AC	1327.3 ± 14.89^{B}	1347.4 ± 5.47^{B}	1490.3 ± 21.34^{AB}	1517.3 ± 9.59^{A}	1480.2 ± 13.03^{AB}	828.9 ± 4.96^{C}

The results are expressed as mean ± SD ($n = 9$). Different small letters in the same row indicate significant differences ($p < 0.05$) among combined mixed fruit juices, different capital letters in the same row indicate significant differences ($p < 0.05$) among elderberry-grape juices. nd – not detected. AC – aroma compounds. Sample labeling: E: pure elderberry, G: pure grape, E-gr: elderberry-grape, E-bc: elderberry-black currant, E-ap: elderberry-apple, E-or: elderberry-orange, E-ca: elderberry-carrot. E-gr 50 to E-gr 90: elderberry-grape in various proportions (50–90% v/v of elderberry).

(Z)-rose oxide, linalool. Most of them are known as important aroma components and were found by many authors in various types of fruits (e.g. Rocha *et al.*, 2007; Soni *et al.*, 2008; Fraternale *et al.*, 2011; Selli and Kelebek, 2011).

The comparison of chemical groups of compounds is given in Tab. II. Alcohols were the most important group of all juices forming about 80–90% of all compounds quantified. Aldehydes and ketones were quantitatively the least ($P < 0.05$) important; their concentrations were minimal in all fruit juices. The highest total content of compounds was found in pure elderberry. In spite of good sensory rating, the grape juice contained the lowest total content of aroma compounds, especially caused by low concentration of alcohols. It gradually increased in grape-elderberry mixtures with increasing portion of elderberry (Tab. II). In spite of significant ($P < 0.05$) differences in sensory evaluation of flavour, the total content of aroma compounds in samples E-gr 50 to E-gr 90 was similar and was created by combination of aroma compounds in pure grapy and elderberry juices.

Both sensory studies and instrumental analysis confirm the importance of volatiles production in fruit and their contribution to eating quality. The calculation of OAV, which is defined as "the ratio of concentration in food to threshold concentration in the same matrix", is the possibility how to predict which compound could contribute to aroma. Odour threshold concentrations were acquired from the literature (Rocha *et al*., 2007; Verzera *et al*., 2008; Sanchez-Palomo *et al*., 2010); the calculated OAVs suggest that ethanol (mild alcoholic), propan-1-ol (sweet alcoholic), propan-2-ol (buttery), 2-methylbutan-1-ol (fruity), hexanal (fruity, grassy), heptanal (fruity), phenylethanal (flowery), nonan-2-one (fruity, flowery), undecan-2-one (fruity, flowery), 4-methylpentan-2-one (fruity), 3-methylbutanoic acid (oily), β-damascenon (woody, elderberry) and α-terpineol (flowery) could be the contributors to aroma of samples in this study, which is in accordance with other authors (Sanchez-Palomo *et al*., 2010; Selli and Kelebek, 2011). Theoretically, the remaining compounds did not directly contribute (OAVs < 1), they can only enhance some notes because of synergistic effects.

Acknowledgement

This work was supported by a project of Ministry of Agriculture of the Czech Republic [Grant No. QH92223]. We are grateful to prof. Řezníček from Mendel University in Brno and Breeding Institute of Pomology in Holovousy for providing the berries for the study.

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