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The Aroma of Some Hybrids Between High-bush Blueberry (Vaccinium corymbosum, L.) and Bog Blueberry (Vaccinium uliginosum, L.)

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Aroma einiger Kreuzungen zwischen Kulturheidelbeere (Vaccinium corymbosum, L.) und Moorheidelbeere (Vaccinium uliginosum, L.)

Zusammenfassung. Die wichtigsten flüchtigen Aromastoffe einiger Kulturheidelbeeren-Varietäten und Moorheidelbeeren sowie von sieben Kreuzungen zwischen Kulturheidelbeere (*Vaccinium corymbosum*, L.) und Moorheidelbeere (*Vaccinium uliginosum*, L.) wurden nach der massenfragmentophischen SIM (Selective Ion Monitoring)-Technik bestimmt.

Die Konzentrate von 19 Hauptkomponenten der Kulturheidelbeer-Varietät „Rancocas" und Moorheidelbeere wurden von allen Proben analysiert. Weiter wurden an einigen Heidelbeer-Kreuzungen vorläufige sensorische Analysen sowie Zucker- und Gesamtsäurebestimmungen durchgeführt. Es wurde festgestellt, daß viele Aromastoffe sowie der hohe Zucker-Gehalt sich zum größten Teil auf die Sprößlinge vererben.

Die bestimmten Aromastoff-Konzentrationen liegen jedoch unter den Schwellenkonzentrationen. Möglicherweise vermag der Synergismus eine wichtige Rolle bei der Gesamtbildung der Heidelbeeraromen zu spielen.

Summary. The most important volatile components of some cultivated high-bush blueberry varieties and of bog blueberry, together with those of seven hybrids between high-bush blueberry (*Vaccinium corymbosum*, L.) and bog blueberry (*Vaccinium uliginosum*, L.), were studied by a mass-fragmentographic SIM (Selective Ion Monitoring) technique.

The concentrations of 19 major components of the high-bush blueberry variety "Rancocas" and of bog blueberry were determined in each sample. In addition, tentative sensory analyses were performed and the contents of sugars and titratable acids in some blueberries were determined. The most aroma components and the high sugar content of high-bush blueberry varieties were found to be inherited to a large extend by the progenies.

The concentrations of most of the components determined were, however, present below their threshold values and it is possible that synergism may play an important role in the over-all impression of the odour of blueberries.

Introduction

High-bush blueberry (*Vaccinium corymbosum*, L.) is very extensively cultivated in the United States and nowadays also in Central Europe. High-bush blueberry varieties have very large berries and pleasant aromas, but the climatic conditions prevailing in Northern Europe make their cultivation unreliable and uneconomical. Over 20 different varieties have been tested in the Institute of Horticulture at Piikkiö, Finland, but even the most suitable varieties ("Rancocas" and "June") are too susceptible to frost damage and canker caused by the *Fusicoccum putrefaciens* Shear [1, 2]. Therefore attempts have been made to obtain more hardy varieties by crossing the high-bush blueberry with the bog blueberry (*Vaccinium uliginosum*, L.). The bog blueberry, which grows wild over the sub-polar region of Northern Europe, is the only European tetraploid ($2n = 48$ chromosomes) *Vaccinium* species, and thus it has been possible to cross this species with tetraploid high-bush blueberry [3–5].

Systematic breeding and selection work carried out in the Institute of Horticulture at Piikkiö has led to some markedly succesful crosses between bog blueberry plants obtained from wild population at Piikkiö and the high-bush blueberry varieties "Rancocas", "Pemberton" and "Bluecrop".

Viable seeds were obtained only when bog blueberry was used as the parent seed. Unfavourable gene combinations of the F_1-generation hybrid have been at least partly eliminated by backcrossing F_1-individuals with high-bush blueberry varieties. The winter hardiness and blueberry-canker resistance of these new hybrids have been found to be clearly better than those of the earlier American varieties. In addition, many desirable characteristics, such as bushy growth, fruit yield and berry size have also been inherited by the hybrids from the high-bush blueberry varieties. The aroma of these hybrids is much stronger than that of bog blueberry and resembles rather closely that of high-bush blueberry [2, 5]. One of the hybrids, "Rancocas" × (bog blueberry × "Rancocas"), variety "Aron", has reached the stage of commercial cultivation [6].

Table 1. Compounds determined in the blueberries and the corresponding monitored ions (m/e) and their relative intensities in the 70 eV

Compound	Monitored ions (m/e) and relative intensities
1-Hexanol	84 (15%), 69 (40%)
cis-3-Hexen-1-ol	82 (40%), 69 (25%)
trans-2-Hexen-1-ol	82 (25%), 41 (55%)
Linalool	93 (70%), 69 (55%)
α-Terpineol	59 (100%), 93 (55%)
Citronellol	69 (100%), 82 (60%)
Nerol	69 (100%), 41 (65%)
Geraniol	69 (100%), 41 (95%)
Hydroxycitronellol	59 (100%), 41 (30%)
Farnesol	69 (100%), 41 (40%)
Farnesyl acetate	69 (70%), 59 (50%)
trans-2-Hexenal	41 (100%), 69 (40%)
Benzaldehyde	106 (85%)
Benzylalcohol	107 (55%)
2-Phenylethanol	91 (100%)
Phenol	94 (100%)
Eugenol	164 (100%)
trans-Cinnamyl alcohol	91 (90%)
Vanillin	152 (100%)

Selected ion monitoring was performed with a GC/MS controller using the software program "Six Ion SIM"

The aim of this investigation was to study the inheritance of the characteristic aroma compounds of high-bush blueberry and bog blueberry by their hybrids. Using a mass-fragmentographic method the major aroma components of bog blueberry, three high-bush blueberry varieties and seven new hybrids were studied. The components determined were the most important aroma components of bog blueberry and high-bush blueberry variety "Rancocas", identified in a previous study [7]. Besides the aroma of bog blueberry and high-bush blueberry varieties "Rancocas", "Bluecrop" and "June", the aromas of the following seven hybrids were studied: F_1-generation hybrid between bog blueberry and "Rancocas" (F_1, code number 61007006), "Rancocas" × F_1 (variety "Aron"), F_1 × "Pemberton" (65017017), "Bluecrop" × F_1 (65011011), "Bluecrop" × F_1 (65011016), "Bluecrop" × F_1 (65011019) and "Bluecrop" × F_1 (65011021). The four last-mentioned hybrids are different individuals from the same crossing.

The aroma of high-bush blueberry has previously also been studied by the present authors [7] also by Parliment et al. [8]. The last authors, however, did not report the variety of high-bush blueberry investigated.

Experimental

The berries of different high-bush blueberry varieties and hybrids, cultivated at The Agricultural Research Centre Institute of Horticulture, Piikkiö, Finland, were harvested in August 1982. The berries of bog blueberry were collected from forest areas of Southern Finland in August 1982. All berries were deep-frozen and stored at −30 °C until used.

After thawing for five hours, the berries were pressed in a hydraulic press. Isolation and concentration of the volatiles were performed as described previously [9, 10]. From 20 to 100 grams of each berry sample were used in these experiments.

The quantitative determination of the major blueberry volatiles was carried out by an applied mass-fragmentographic (SIM, Selected-Ion-Monitoring) technique (Hewlett-Packard 5992). The apparatus was equipped with an OV-351 fused-silica capillary column (i.d. 0.25 mm, length 30 m). The operating conditions were as follows: linear temperature programming from 50 to 220 °C at 6 °C/min; carrier gas He 2.0 ml/min; electron current 300 μA; ionization voltage 70 eV; scanning speed 100 μsec/ion injection volume 1 μl. An on-column injection device was used.

The concentration of 19 compounds in each sample were determined using SIM-technique. The compounds selected and the corresponding ions (m/e) monitored are listed in Table 1.

The contents of sugars and titratable acids were determined from bog blueberry, high-bush blueberry variety "Rancocas", the F_1-generation hybrid, the variety "Aron" and the hybrid between "Bluecrop" × F_1 (65011019). The contents of sugars, glucose, fructose and saccharose were determined by enzymatic methods [11]. Titratable acids were titrated with 0.1 *N*-NaOH to pH 8.1 and calculated as a percentage of anhydrous citric acid.

The sensory evaluation of variety "Rancocas" and of three different hybrids (F_1, "Aron", and "Bluecrop" × F_1 65011019) was performed by twelve panelists with earlier experience in sensory evaluation. In the evaluations a graphical scaling method was used as described by Stone et al. [12]. The detailed description of the evaluations has been discussed in an earlier report [10]. The intensity of odour, over-all impression of odour, sweetness, over-all impression of taste, sourness, off-odours and off-tastes were evaluated.

Results and Discussion

The results of the mass-fragmentographic determinations of blueberry volatiles are presented in Table 2. It can be seen that the total amount of volatiles determined is clearly lower in bog blueberry than in both high-bush blueberries and the hybrids. Further, in the back-crossings the total concentration of volatiles increased in comparison with that of the F_1-hybrid. In the variety "Bluecrop" the concentrations of nearly all the compounds determined were the highest recorded amongst these blueberry samples. The aroma compounds of the variety "Bluecrop" were inherited in considerable amounts by all four back-crosses between "Bluecrop" and the F_1-generation hybrid. In one of these hybrids (65011019) the concentration of aroma compounds was found to be three times higher than that in the variety "Aron" (65011019 1.21 mg/kg and "Aron" 0.34 mg/kg). A very high total concentration (1.6 mg/kg) was also obtained in the back-cross F_1 × "Pemberton". In the last case the high value originated to a large extened from *trans*-2-hexenal (1.2 mg/kg), obviously inherited from "Pemberton". Unfortunately, berries of this variety were not available for the present study. The total amount of aroma compounds in the variety "June" was found to be equal to that in "Rancocas".

Table 2. Concentrations of the main aroma compounds determined in three high-bush blueberry varieties, bog blueberry and seven hybrids between bog blueberry and high-bush blueberry

Compound	Concentration mg/kg										
	High-bush blueberries			Bog blueberry	Hybrids between bog blueberry and high-bush blueberry						
	Rancocas	Bluecrop	June		Bog blueberry × Rancocas F_1	Rancocas × F_1 Aron	F_1 × Pembert 65017017	Bluecrop × F_1 65011011	Bluecrop × F_1 65011016	Bluecrop × F_1 65011019	Bluecrop × F_1 65011021
Hexan-1-ol	0.20	0.40	0.04	0.05	0.10	0.05	0.07	0.15	0.04	0.15	0.05
cis-3-Hexen-1-ol	0.03	0.06	0.02	0.06	0.01	0.01	0.05	0.01	0.04	0.02	0.01
trans-2-Hexen-1-ol	0.15	0.35	0.08	0.04	0.05	0.07	0.07	0.30	0.25	0.50	0.15
Linalool	0.03	–	0.15	–	–	0.01	+	–	+	+	+
α-Terpineol	0.01	0.01	0.03	+	+	+	+	+	+	+	+
Citronellol	0.01	0.01	0.03	–	–	+	+	+	+	0.01	0.01
Nerol	0.02	0.08	0.05	–	–	–	+	+	0.01	0.05	0.01
Geraniol	0.10	0.25	0.15	–	0.01	0.03	0.04	0.03	0.06	0.15	0.10
Hydroxy-citronellol	0.02	0.07	0.03	–	+	+	+	+	+	0.02	+
Farnesol	+	–	–	–	–	–	–	–	–	–	–
Farsnesyl acetate	0.01	–	–	–	–	–	–	–	+	+	+
trans-2-Hexenal	0.08	0.50	0.05	0.02	0.04	0.15	1.2	0.30	0.40	0.20	0.30
Benzaldehyd	+	–	+	+	+	+	+	+	+	+	+
Benzylalcohol	0.02	0.02	0.03	0.01	0.01	0.01	0.03	0.01	0.02	0.02	0.01
2-Phenylethanol	0.02	0.10	0.03	+	+	+	0.03	+	0.01	0.03	0.01
Phenol	0.01	0.01	0.01	0.01	0.01	+	0.01	+	0.01	0.01	+
Eugenol	+	0.02	+	–	–	+	+	+	+	+	+
trans-Cinnamyl-alcohol	0.01	+	+	–	–	–	–	+	0.01	0.05	+
Vanillin	0.01	0.05	0.01	+	0.01	0.01	0.06	+	0.01	+	+
Total amount	0.73	1.93	0.71	0.19	0.24	0.34	1.57	0.80	0.86	1.21	0.65

The influence of the individual aroma compounds on the odour of berry samples was rather difficult to verify with certainty, because the concentrations of most of the 19 components determined were below their threshold concentration values. According to the odour values obtained by dividing the concentration of a compound by its threshold concentration value [13], it would appear that *trans*-2-hexenal, geraniol, *cis*-3-hexen-1-ol, linalool, 2-phenylethanol and vanillin may contribute to the typical aroma of blueberries. The weak aroma of bog blueberry was in accordance with the low concentrations of the compounds determined. With the exception of *trans*-2-hexenal all the above compounds were found to be present below their threshold concentrations. No traces of geraniol, citronellol, nerol, farnesol, farnesyl acetate and hydroxycitronellol were found in the berries of bog blueberry. These compounds were, however, inherited in considerable amounts from high-bush blueberry by the back-crosses. The synergism of the individual components at low concentrations may play an important role in the overall impression of odour of blueberries.

The differences between the present and a previous [7] study in the recorded concentrations of the volatiles of bog blueberry and of variety „Rancocas" may be due partly to different methods of preparation of the samples and partly to different weather conditions prevailing during the growing season. In this study the volatiles of the berry juices (15–70 ml) were extracted in a separating funnel, whereas in the previous study a Kutscher-Steudel extraction apparatus (700 ml) was used. During the summer of 1982 the weather conditions were more favourable to the growth and ripening of the berries than in 1981.

The contents of sugars and titratable acids, and their ratios in five different blueberries, together with the corresponding values for bilberry (*V. myrtillus*, L.), cowberry (*V. vitis-idaea;* L.) and cultivated strawberry variety Senga Sengana, are summarized in Table 3. It can be seen that the total amounts of sugars and titratable acids of blueberry crosses were about equal to those of the variety „Rancocas". The ratio of these components was also more favourable in the crosses than in bog blueberry. In comparison with the variety

Table 3. Sugars, titratable acids and the ratio of sugars to titratable acids in some blueberry varieties, bilberry, cowberry and cultivated strawberry

Plant	Sugar g/kg			Titratable acids g/kg	Ratio of sugars to tritratable acids
	Fructose	Glucose	Saccharose		
Blueberries:					
Variety Rancocas	64	61	0	2.0	63
F_1-hybrid (61007006)	62	69	0.2	6.6	19
Variety Aron	71	69	0	3.2	44
Back-cross (65011019)	61	60	0.6	4.8	25
Bog Blueberry	29	22	2	14.1	3.6
Bilberry[14]	37.8–38.2	27.6–31.6	0	12.8–13.2	~ 5.2
Cowberry[14]	30.4	29.4	traces	33.8	1.8
Strawberry variety Senga Sengana	33	30	–	1.2	53

„Rancocas" and with the crosses with other *Vaccinium* berries, bilberry and cowberry, the contents of sugars were rather high, and those of titratable acids were particularly low.

It is interesting to note that the in „Rancocas" and in the crosses sugar contents were about twice those in the strawberry variety Senga Sengana, whereas the ratios of sugars to titratable acids were of the same magnitude, due to lower content of titratable acids in Senga Sengana. The concentration of saccharose was almost insignificant in all the blueberry samples investigated.

Because the available quantities of the berries of the crosses and of some high-bush blueberry varieties were limited, it was not possible to train the panelists of the sensory evaluation prior to the testing. The sensory evaluations are therefore based on relatively few trials and the analysis was tentative. The variety „Rancocas" and the crosses F_1, „Aron" and „Bluecrop" $\times F_1$ (65011019) were the only ones evaluated. In these restricted analyses only rather small differences were obtained between the sensory properties of the samples evaluated. In general, all four samples were considered as having very sweet and only slightly sour taste qualities, which confirms the results of the determinations of sugars and titratable acids. However, no significant relationships were found between sensory odour parameters and the sum of the concentrations of the volatiles determined, which could be due to the unfamiliarity of the panelists with the berries. Very possibly, the very low concentrations of the individual components may have made the sensory evaluations more difficult. No character-impact compounds could be found for blueberries.

According to the results obtained in this study the aroma components and the high sugar content of high-bush blueberry varieties are inherited to a large extent by the progenies of crossings with bog blueberries, whereas the contents of titratable acids are present in considerably lower concentrations in the crosses than in bog blueberry. This inheritance pattern results in an attractive flavour of the crosses. Some of the crosses in the breeding and selection were clearly more suitable for cultivation under the climatic conditions of Southern Finland than were the earlier blueberry varieties. According to Hiirsalmi [2] the productivities of „Rancocas", „Aron" and bog blueberry were in the period 1970–1974 842 g/bush, 497 g/bush and 92 g/bush, respectively. The berry size of „Aron" is comparable with that of „Rancocas" and this, together with the good quality of the berries, makes it suitable for cultivation in Finland.

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