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Quality of Sour Cherry Juice of Different Clones and Cultivars (*Prunus cerasus* L.) Determined by a Combined Sensory and NMR Spectroscopic Approach

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ABSTRACT: Juice was manufactured from seven different sour cherry clones/cultivars and evaluated by quantitative descriptive sensory analysis and ^{1}H NMR spectroscopy. The sensory evaluation showed a large variation in several sensory attributes between the sour cherry clones/cultivars, which could be divided into two groups on the basis of both the sensory data and the NMR spectroscopic data. These groups were closely related to the genetic background of the clones. Kelleris clones were distinctly different from Stevnsberry and Fanal clones. Hence, ^{1}H NMR spectroscopic data seem to correlate with sensory quality of different sour cherry clones. In addition, malic acid was the most important metabolite for modeling the two highly correlated sensory attributes sweetness and sourness, whereas the glucose content had a slight effect and the fructose content had no impact on sweetness/sourness. Other metabolites (ethyl acetate, asparagine, ethanol) could be correlated with sensory attributes; however, a direct causal connection could not be established.

KEYWORDS: sour cherry, juice, sensory analysis, NMR

INTRODUCTION

In Denmark, there is a rather large production of sour cherries (*Prunus cerasus* L.). The fruits of sour cherries are not consumed raw due to their characteristic and sour taste; the fruits are mainly processed industrially to juice, concentrates, or purees. Sour cherry juice or concentrates are used in the production of fruit nectars and alcoholic beverages as well as nonalcoholic beverages. Purees are used in pastries, confectionary, or dairy products such as yogurt. In contrast to countries such as Germany, pure sour cherry juice is not offered in the Danish market, probably due to the unique and specific taste that is very unfamiliar to Danish consumers. In Denmark, only three types of sour cherry clones are used for commercial production: 'Stevnsbær', 'Kelleris 16', and 'Skyggemoreller'. However, selection of other cultivars or clones might contribute to products that differ significantly in sensory quality. In contrast to sweet cherries, only a few studies have focused on the sensory quality of sour cherry or sour cherry juices of different cultivars. Sensory evaluation of sweet cherry berries has been performed.[1−3] The sensory profile of sweet cherry includes color, appearance, and texture attributes (firmness and juiciness) as well as sweetness, sourness, and flavor intensity.[3] Apart from the sensory quality, yield and susceptibility to the most important diseases as well as firmness of the berries at harvest are very important quality parameters in the selection of sour berry cultivars for juice production.

Differences in the sensory quality between different sour cherry juices can be obtained by sensory methods such as quantitative descriptive methods using a trained sensory panel. However, sensory analysis is a time-consuming and quite expensive analytical method.[4] Hence, instrumentally based methods to predict the sensory quality could be beneficial for the industry and in scientific work. High-resolution proton (^{1}H) NMR spectroscopy has been used in studies of wine grapes and wines to describe varietal, seasonal, and growth conditional effects on grape and wine composition. The content of the major components in different wines was predicted using ^{1}H NMR spectroscopy and interval PLS,[5] and in two studies "wine body" was predicted using GC-MS and/or NMR.[6,7] Other methods have also been used, and, for instance, sensory characteristics were described and predicted in bread by GC-MS[8] and a model based on proton transfer reaction mass spectrometry for the prediction of coffee sensory characteristics was made.[9]

The aim of the present study was to screen juices from different sour cherry clones and cultivars for their sensory properties. The composition of sour cherries is dependent on cultivar, year of harvest, and storage time of the berries,[10,11] but the wide range of cultivars has not yet been investigated using sensory analysis. Furthermore, the aim was to elucidate the potential use of high-resolution ^{1}H NMR spectroscopy to classify juices from different sour cherry cultivars/clones and to investigate the relationship between the NMR metabolite profile and the sensory profile.

MATERIALS AND METHODS

Plant Materials. In August 2008, seven different sour cherry cultivars and clones were harvested at Research Centre Aarslev at Funen in Denmark. The seven cultivars and clones were grown on a sandy loam in a randomized block with three trees per plot in four replicates. The trees were spaced 3 m apart within a row and 5 m apart between rows, with grassed alleys between rows. By early spring or summer pruning the trees were trained as spindles with one central leader. The trees were

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Table 1. Characteristics of the Berries of Different Sour Cherry Clones and Cultivars at Harvest

type[a]	name of clone/cultivar and site of origin	fruit size (mm)	crash test (%)	firmness (g)	taste of berries
S	Stevnsberry clone LS	21.2	92.0	108.0	5.7
S	Stevnsberry clone PH	26.2	86.7	146.4	5.5
S	Stevnsberry clone JJ	21.4	84.9	124.1	3.8
SF	Tiki	26.3	41.3	165.1	2.7
F	Fanal clone Skælskør	26.3	60.0	120.3	4.5
K	Debrecini	26.3	81.3	134.1	7.3
K	Sumadinka	26.3	48.0	106.4	5.0
mean		24.9	70.6	129.2	4.9
significance level[b]		***	**	***	NS

[a] S, Stevnsberry; SF, breeding between Stevnsberry and Fanal; F, Fanal; K, Kelleris. [b] Significance level: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; NS, not significant.

fertilized with 100 kg/ha nitrogen (N) and 100 kg/ha potassium (K) per annum. The trees were protected against diseases according to good agricultural practice.

The berries were harvested by hand at three different time points. The first was 3 days before the calculated date of harvest,[12] the second at the day of harvest, and third 3 days after the calculated day of harvest. Fruit size as grams measured on 50 berries was registered, the content of soluble solids was measured on a refractometer RFM 330 (Bellingham + Stanley Inc., England), and fruit firmness was measured on a FirmTech-2 (Umweltanalytische Producte GmbH, Ibbendüren, Germany). Furthermore, a crash test was carried out to measure how shock-resistant the berries would be during mechanical harvest.

Juice Processing. The berries were kept at −24 °C until they were processed in October 2008. Before processing, the stones were removed using a stone remover device. The berries were placed in a pneumatic Enerpac press equipment (Leonhard Mohr K.-G., Karlsruhe-Durlach, Germany) to produce juice. The juice samples were kept at −24 °C until sensory analysis in January 2009 and NMR analysis in September 2009.

Sensory Analysis. A trained sensory panel of 10 assessors evaluated the juice samples of the seven sour cherry cultivars/clones in three replicates. The juice samples were thawed at room temperature overnight and diluted with tap water 1:1. Because some of the sour cherry juices were rather astringent and sour, 10% sucrose was added and dissolved in the juice samples before serving. Addition of sucrose and water to the samples made it much easier for the assessors to evaluate the samples properly. Ten percent sucrose was selected on the basis of a sensory evaluation by two experts of sour cherry juice with increasing amounts of sucrose. Ten percent sucrose was the lowest amount that gave an acceptable taste of the sour cherry juice. The samples were served at 20 ± 2 °C.

The sensory analysis was carried out as a quantitative descriptive analysis (QDA). The assessors developed a sensory profile for sour cherry juice, which included seven aroma descriptors, two descriptors related to the appearance and viscosity, eight flavor descriptors, and three taste descriptors as well as astringency. The assessors evaluated the aroma descriptors using their nose; astringency, viscosity, and taste and flavor descriptors using their mouth and nose after ingestion; and the intensity of the red color using their eyes. Before the sensory evaluation of the juice samples, the sensory panel trained the different attributes by evaluating four juice samples that differed with regard to the attributes. For some of the attributes, reference samples were used if possible during the training session, for example, almond essence, marzipan, fruit drinks, and almonds.

The attributes were evaluated on a 15 cm nonstructured continuous scale. The left side of the scale (= 0) corresponded to the lowest intensity and the right side of the scale (= 15) corresponded to the highest intensity of the attributes except for the red color. The color was evaluated as light red (= 0) to dark red (= 15).

^{1}H NMR Spectroscopy. The NMR measurements were performed at 25 °C on a Bruker Avance III 600 spectrometer, operating at a ^{1}H frequency of 600.13 MHz and equipped with a 5 mm ^{1}H TXI probe (Bruker BioSpin, Rheinstetten, Germany). Prior to the measurements, juice samples were thawed. Five replicates of each cherry juice (500 μL) were mixed with 100 μL OF D_2O containing 0.05% w/w sodium trimethylsilyl-[2,2,3,3-$^2H_4$]-1-propionate (TMSP). Standard one-dimensional (1D) ^{1}H NMR spectra were acquired using a single 90° pulse experiment with 64 scans and a relaxation decay of 5 s. Water suppression was achieved by irradiating the water peak during the relaxation delay, and 32K data points spanning a spectral width of 12.15 ppm were collected. Spectra were manually phased and baseline corrected.

The obtained ^{1}H NMR spectra were assigned using pure standards and two-dimensional (2D) ^{1}H−^{1}H correlation spectroscopy (COSY) and ^{1}H−^{13}C heteronuclear single-quantum correlation (HSQC). The COSY spectra were acquired with a spectral width of 6130 Hz in both dimensions, 8000 data points, and 512 increments with 32 transients per increment. The HSQC spectrum was acquired with spectral widths of 8000 Hz in the F2 dimension and 25000 Hz in the F1 dimension and a data matrix with a size of 1K × 256 data points and 64 transients per increment.

Multivariate Data Analysis and Statistical Analysis. The sensory data were analyzed by multivariate data analysis and classical statistical analysis, respectively. For classical statistical analysis the general linear model (GLM) procedure of the Statistical Analysis System (SAS Institute, Cary, NC) was used for analysis of variances and investigation of correlations. The data were checked for outliers. The source of variance was clone/cultivar. Duncan's test at $P \leq 0.05$ was used to assess significant differences in the sensory attributes.

Multivariate data analyses of both sensory and chemical data were carried out using SIMCA-P+ v. 12.0 (SIMCA-P+ software, Umetrics AB, Umeå, Sweden). The multivariate data analysis of the sensory data was conducted by principal component analysis (PCA). For PCA, all data were mean-centered and scaled to unit variance.

NMR spectra were aligned prior to multivariate data analysis, and this was performed using the icoshift procedure[13] on 22 manually selected intervals, and subsequently aligned spectra were divided into 0.0052 ppm bins. NMR data from 9.0 to 0.15 ppm were used for multivariate data analysis, leaving out signals from residual water at δ = 5.0−4.7 ppm. NMR data were not normalized prior to multivariate data analysis because the standard deviation of the total spectrum area within samples was between 1.7 and 7.0% and that between samples 4.2%. Likewise, normalization according to the internal standard, TMSP, could not be performed because this signal did not correlate with the intensity of other peaks within replicates. Thus, for multivariate data analysis absolute intensities from the NMR spectra were used. Cross-validation

Table 2. Sensory Attributes of the Tested Sour Cherry Juices[a]

	sour cherry clone/cultivar[b]								
sensory attribute	S-LS	S-PH	S-JJ	SF-Tiki	F-Skaelskor	K-Debrecini	K-Sumadinka	mean	significance level[c]
almond aroma	11.0 a	7.6 b	7.7 b	5.8 b	5.8 b	3.5 c	6.9 b	6.9	***
sour cherry aroma	7.5 a	6.7 a	7.7 a	7.1 a	6.5 ab	3.5 c	4.9 bc	6.3	***
raisin aroma	2.5 b	2.1 b	1.8 b	1.8 b	1.8 b	5.1 a	1.6 b	2.4	**
fruity aroma	2.5	2.1	3.2	2.7	2.9	3.6	3.8	3.0	NS
spicy aroma	4.8	4.4	3.9	3.9	3.7	4.0	3.4	4.0	NS
fermented aroma	2.8	3.0	3.8	5.0	3.8	4.8	3.8	3.9	NS
sour aroma	2.4	3.4	3.5	3.1	3.0	2.4	2.6	2.9	NS
almond flavor	8.1 a	7.7 ab	5.8 bc	4.5 c	5.4 c	4.1 c	4.3 c	5.7	***
sweetness	7.4 bc	6.8 bc	6.1 c	6.4 c	6.4 c	12.4 a	8.2 b	7.7	***
sourness	6.8 ab	6.3 bc	7.4 abv	8.0 a	6.7 ab	1.1 d	4.9 c	5.9	***
bitterness	2.7 ab	2.3 ab	2.5 ab	3.2 a	2.5 ab	0.5 c	1.7 bc	2.2	**
astringency	7.4 a	7.5 a	7.7 a	7.6 a	6.6 a	1.3 c	3.2 b	6.8	***
pungent flavor	4.1 a	3.2 ab	3.6 ab	4.4 a	2.4 b	0.8 c	1.2 c	2.8	***
sour cherry flavor	10.2 a	9.3 ab	9.7 ab	8.5 ab	8.1 b	3.0 d	5.1 c	7.7	***
fruity drink flavor	2.9 c	3.2 c	2.4 c	2.9 c	4.1 c	11.6 a	6.7 b	4.8	***
fresh berry flavor	4.9	4.7	5.2	6.8	5.7	4.9	4.9	5.3	NS
raisin flavor	2.0	2.6	2.1	2.1	1.8	4.8	0.9	2.3	NS
spicy flavor	4.5	5.1	3.9	4.5	3.0	3.4	2.2	3.8	NS
fermented flavor	2.4	2.3	3.3	3.3	2.5	3.5	2.8	2.9	NS
red color	12.6 a	12.7 a	11.0 b	13.0 a	9.6 c	0.7 e	3.0 d	8.9	***
viscosity	11.8 a	12.7 a	11.2 a	11.7 a	7.7 b	1.1 d	3.6 c	8.5	***

[a] Means within the same row with different letters are significantly different. [b] S, Stevnsberry; SF, breeding between Stevnsberry and Fanal; F, Fanal; K, Kelleris. [c] Significance level: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; NS, not significant.

of all multivariate models was performed using group-wise cross-validation, leaving out one replicate of each sample and using the remaining six samples for modeling. For inspection of loadings in PLS models autoscaled loadings were multiplied by their standard deviation, and spectra and loading plots were evaluated together for assignment of loadings to the corresponding peaks in the spectra.

For some multivariate analyses integrated peak areas were used. Integrals were determined using Topspin 2.1 (Bruker Biospin, Faellanden, Switzerland) and manual integration.

RESULTS AND DISCUSSION

Fruit Quality at Harvest. Type, names of clones and cultivars, fruit size, and characteristics of the berries are shown in Table 1. The seven clones/cultivars were selected from more than 50 different clones and cultivars. The preliminary screening was carried out on the basis of performance in the field, yield, and resistance to diseases. As shown in Table 1, the seven selected clones and cultivars varied with respect to fruit size, shock resistance during mechanical harvest, and fruit firmness. The Stevnsberry clones LS and JJ have the smallest fruit size. Tiki, Sumadinka, and Skelskor have the highest percentage of damaged berries after the crash test. However, Tiki was the firmest berry in contrast to Skelskor and Sumadinka (Table 1).

Sensory Evaluation of Juice from Different Sour Cherry Clones. The sensory evaluation showed a rather large variation in several of the sensory attributes between the juices of the seven sour cherry clones and cultivars. The only sensory attributes that did not differ significantly between the sour cherry clones/cultivars were fruity aroma, spicy aroma and flavor, fermented aroma and flavor, sour aroma, and raisin flavor as well as fresh berry flavor (Table 2). A PCA showed that the juices were grouped into two distinct groups along principal component 1 (PC1), explaining 63% of the variation. Sumadinka and Debrecini, which are both Kelleris cultivars, were distinctly different from the other five juice samples. The Stevnsberry clones PH and LS grouped together, whereas the Stevnsberry clone JJ and the Fanal clones Skaelskor and Tiki formed another group along PC2, explaining 17% of the variation (Figure 1). The juices of Tiki and especially the Stevnsberry clone JJ and the Fanal clone Skaelskor were very sour, bitter, and astringent. In addition, the juices were very dark red and had a pungent flavor. The juice of the two Stevnsberry clones PH and LS was characterized by high intensity of almond aroma and flavor, which is related to the content of benzaldehyde.[14] In contrast, the juice of Debrecini was very sweet, and it was characterized by a high intensity of fruity drink flavor, raisin aroma, and fruity drink aroma, although the latter attribute was not significant (Table 2). Sumadinka was rather sweet and had an intense fruity drink flavor, whereas other attributes were less dominant (Figure 1 and Table 2). As seen from Figure 1, there was a close correlation between several of the flavor attributes and the corresponding aroma attributes. Which of the sensory attributes having a significant impact on the liking of sour cherry juice has not, to our knowledge, been studied. However, this is very important in relation to innovation of sour cherry juices. In a study of the prediction of sweet cherry liking, flavor intensity and sweetness were identified as the two most important attributes for flavor/texture.[3] However, this might not be true for sour cherry juice.

^{1}H NMR Spectroscopy of Juices from Different Sour Cherry Clones. ^{1}H NMR spectra were acquired for the same juice samples that were used for sensory evaluation. However, the

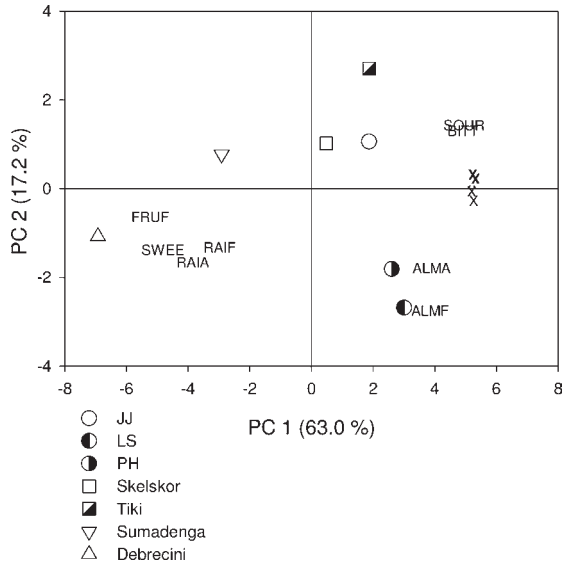

Figure 1. PCA biplot of sensory attributes and sour cherry varieties. Stevnsberry clones are shown with circles, Fanal clones are shown with squares, and Kelleris cultivars are shown with triangles. For clarity, only statistically significant sensory attributes are shown. Abbreviations: ALMA, almond aroma; RAIA, raisin aroma; RAIF, raisin flavor; FRUF, fruity drink flavor; ALMF, almond flavor; SWEE, sweetness; SOUR, sourness; BITT, bitterness. Some attributes (marked with X) are not described in the plot for clarity; they are viscosity, sour cherry aroma and flavor, pungent flavor, color, and astringency.

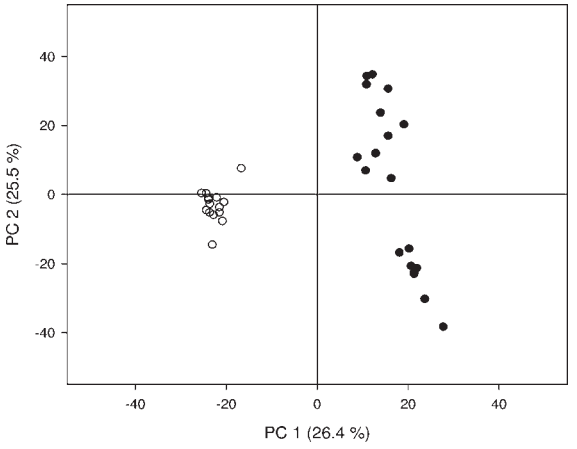

Figure 2. PLS-DA scoreplot for NMR data after grouping of cherry varieties into the two groups observed in the PCA model. Solid symbols, group 1 contains the Stevnsbaer clones JJ, LS, and PH and the Stevnsbaer/Fanal cultivar Tiki; open symbols, group 2 contains the Fanal clone Skaelskor and the Kelleris cultivars Sumadinka and Debrecini.

samples were taken prior to dilution and sugar addition. A preliminary PCA of the NMR data showed that juices grouped into two distinct groups along PC1, although with very poor predictive ability. One group contained the Stevnsberry clones JJ, LS, and PH as well as Tiki (referred to as group 1), which were also found to be related in the sensory analysis. The other group comprised the Fanal clone Skaelskor as well as Sumadinka and Debrecini (group 2). A PLS-DA model was made using the groups from the PCA to model the variation that was related to these two groups. The resulting model had a high predictive ability, with *X*-variables describing 82.0% of the variation in the *Y*-matrix (Figure 2). Mean spectra of the two groups with assignments (Table 3) are shown in Figure 3. Compounds being most abundant in group 1 included amino acids (Ala, 1.49 ppm; Thr, 1.33 ppm; Ile, 0.94 ppm; Gln, 2.14 and 2.45 ppm; Asn, 2.96 ppm), ethyl acetate (1.23 ppm), and γ-aminobutyric acid (GABA) (1.94 ppm), and two peaks were assigned to polyphenolic compounds (6.94 and 7.64 ppm). Only Pro (2.01 and 2.08 ppm) was identified as more abundant in group 2. A model, which contained only these spectral regions, improved the cross-validated predictive ability (91.2% *Y*-variation described by *X*), confirming their importance in the original model. The most abundant compounds, glucose (3.24, 4.64, and 5.24 ppm), fructose (4.11, 4.03, and 4.00 ppm), and malic acid (4.47, 2.87, and 2.73 ppm), did not, on the other hand, contribute to the model.

Table 3. Assignments of Sour Cherry NMR Spectra

compound	chemical shift (ppm)	assigment, type[a]
isoleucine	0.94 (t, C-5, CH_3), 1.01 (d, C-3′, CH_3)	A
leucine	0.96 (t, C-5,5′, CH_3)	A
valine	0.99 (d, C-4, CH_3), 1.04 (d, C-4′, CH_3), 2.26 (m, C-3, CH)	A
α-ketobutyric acid	1.07 (t, C-4, CH_3)	A
ethanol	1.18 (t, C-2, CH_3)	C
ethyl acetate	1.23 (t, C-4, CH_3)	A
threonine	1.31/22.2 (d, C-4, CH_3)	B
alanine	1.48 (d, C-3, CH_3)	B
GABA	1.90 (m, C-3, CH_2), 2.30 (t, C-2, CH_2), 3.02 (t, C-4, CH_2)	B
proline	1.98 (m, C-5, CH_2), 2.07; 2.34 (m, C-6, CH_2)	B
glutamine	2.14/29.1 (m, C-3, CH_2), 2.46/33.9 (m, C-4, CH_2)	D
glutamate	2.53 (m, C-3, CH_2)	B
succinic acid	2.42/36.9 (s, C- 2/3, CH_2)	C
malic acid	2.87/45.2 (dd, C-3, CH), 2.73/45.4 (dd, C-3, CH′), 4.47/73.1(dd, C-2, CH)	D
asparagine	2.86/37.5, 2.96/37.4	D
choline	3.18 (s, N-$(CH_3)_3$)	A
glucose	3.24/77.1(dd, C-2, βCH), 4.64/98.8 (d, C-1, βCH), 5.23/94.9 (d, C-1, αCH)	D
fructose	4.00/72.1 (m, C-5, CH)	D
phenylalanine	7.33 (m, C5/C9-ring CH), 7.39 (m, C7-ring CH), 7.43 (d, C6/C8-ring CH)	B

[a] A, tentative assignment through comparison with published data; B, comparison with pure standard; C, 2-D experiments (either COSY, HSQC, or both); D, both B and C.

Description of Sensory Attributes by ^{1}H NMR Specotroscopy. The close resemblance in grouping observed after analysis of the sensory and ^{1}H NMR spectroscopic data suggests that the NMR spectral data may be used for modeling and predicting sensory attributes. The clustering of sour cherry clones and cultivars into two groups closely resembles their genetic relationship. Both the sensory and NMR spectral data showed a distinct clustering between Kelleris and Stevnsberry clones and Kelleris and Fanal clones, although the cross between

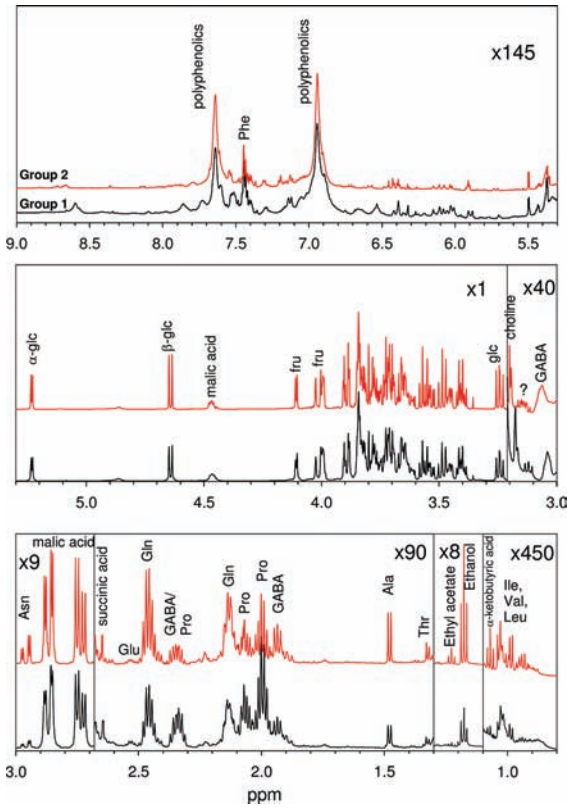

Figure 3. Mean NMR spectra of cherry juices belonging to the two groups observed in the PCA model. Black spectrum, group 1; red spectrum, group 2.

Fanal and Stevnsberry (Tiki) clustered closer to the Kelleris clones in the NMR analysis. However, due to the low number of samples, we did not further investigate the possibility of using NMR as a tool to model sensory attributes.

The correlation between NMR data and sensory attributes was investigated using PLS2. To ensure sufficient variation in the *Y*-matrix, only sensory attributes that were significantly different between juices (Figure 1 and Table 2) were included in the analysis. The PLS2 biplot of the correlation between sensory attributes and sour cherry cultivars/clones (Figure 4A) closely resembles the result presented in Figure 1 and shows that many of the sensory attributes were highly correlated (e.g., cherry, astringent, and pungent flavor and viscosity, color and cherry aroma); thus, these sensory attributes will share loadings. This fact will inevitably hamper the analysis and blur the significance of any causative relationship between NMR and sensory data.

Figure 4B shows the loadings that correlate the NMR data with the sensory attributes. The loadings show that signals related to carbohydrates (glucose, fructose) contribute both positively and negatively to the loadings of PC1 and PC2. This can be interpreted as an effect of shifts in peak positions,[15] but inspection of the spectra after alignment showed that the alignment algorithm removed spectral shifts. Therefore, we interpret these loadings as noise. Likewise, some of the malic acid resonances are noisy (4.47 and 2.87 ppm), whereas the resonance at 2.73 ppm indicates positive correlation between malic acid content and sourness.

Because sucrose was added to the juices prior to the sensory evaluation, an effect of endogenous carbohydrate content on

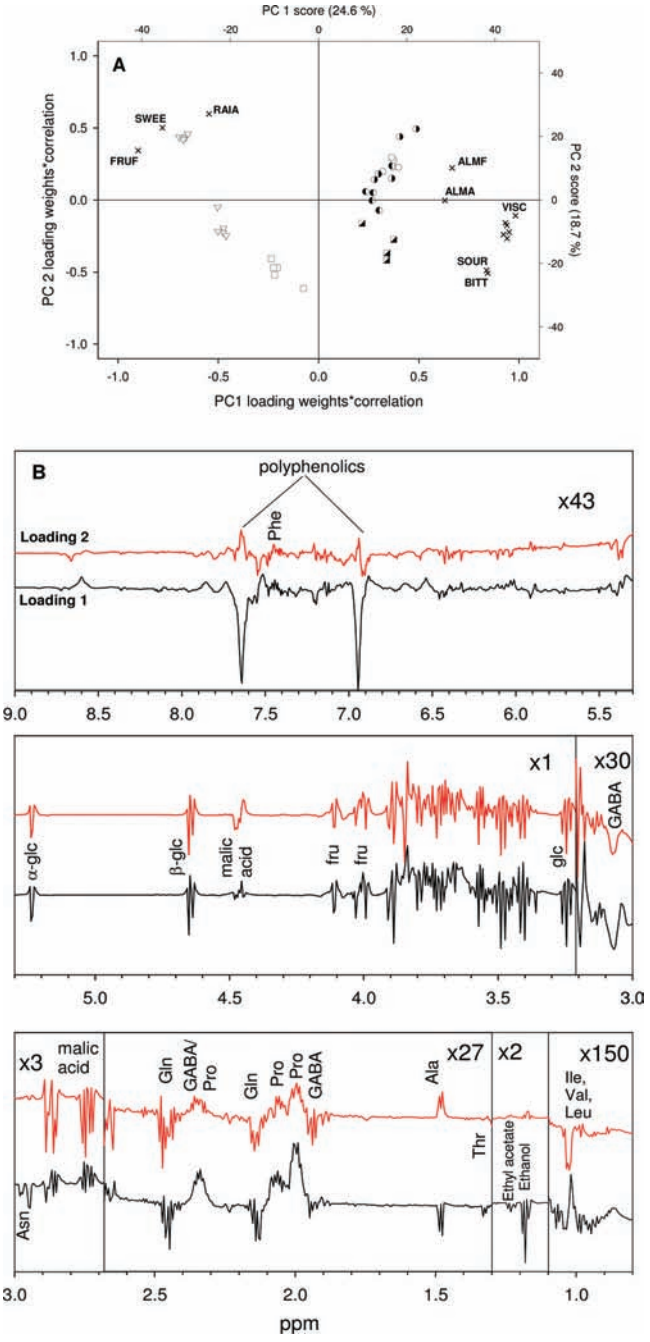

Figure 4. PLS biplot (A) showing sensory attributes of the cherry clones/cultivars. The corresponding loading plot (B) shows the correlations between NMR measurements and sensory evaluations. Sensory attributes are marked with a cross in the biplot. Some attributes (marked with X) are not described in the plot for clarity; they are viscosity, sour cherry aroma and flavor, pungent flavor, color, and astringency. Legend and abbreviations are as in Figure 1.

sweetness and sourness was not expected, and the effect of malic acid was also expected to be masked. To reveal if this was true, the relative amounts of all metabolites were determined by integration of the NMR signals, and another PLS model was constructed (not shown). This model indicated that malic acid affected the assessment of juices as sour and sweet, whereas glucose had a slight impact and fructose no impact on these attributes.

Table 4. Pearson Correlations (r) between Sensory Attributes (Sweetness and Sourness) and the Carbohydrate and Malic Acid Contents

	sensory attribute	
	sweetness	sourness
malic acid[a]	−0.83*	0.90**
glucose[a]	0.57	−0.45
fructose[a]	0.48	−0.34
glucose/malic acid[b]	0.97***	−0.95**
fructose/malic acid[b]	0.87	−087*
sweetness	---	−0.97***
sourness	−0.97***	---

[a] Carbohydrate and malic acid content determined as integrals in ^{1}H NMR spectra. [b] Ratios between carbohydrate and malic acid content.

Correlations between sensory attributes and carbohydrate and malic acid content are summarized in Table 4 and confirm that malic acid alone or the ratio between the glucose and malic acid contents is an important determinant of the perception of sourness and sweetness. In contrast, fructose content alone or the fructose/malic acid ratio did not contribute to the perception of sourness and sweetness. As pointed out above, this is unexpected because of the high amount of added sucrose in the samples, and the result should be considered with care, also taking into consideration the relatively small number of samples. Furthermore, it is in contradiction with a recent investigation of white wines. In this study fructose had the highest impact on the sensory attribute sweetness.[16] The surprising effect of malic acid content, irrespective of sucrose addition, may be, on the other hand, supported by a previous study on mango pulp.[17] In this study, taste and flavor attributes of mango pulp were evaluated with added sugar (sucrose/fructose, 2:1) concentrations from 0 to 18%, and no effect on perception of acidity was detected.[17]

Further inspection of Figure 4 shows that sweetness, fruity drink flavor, and raisin aroma are all correlated (Figure 4A). Loadings (Figure 4B) show that this correlated with the NMR signals from asparagine, ethanol, ethyl acetate, alanine, polyphenols, and, to some degree, threonine. Except for sweetness, these sensory attributes probably result from complex chemical interactions in both the juices and mouth.

In wine, ethyl acetate is present in relatively high concentrations, originating from ethanol metabolism under anaerobic conditions.[18] Ethyl acetate was also detected in fresh juices in the present study. Generally, esters, including ethyl acetate, are associated with pleasant and fruity flavor.[19] However, in mandarins, ethyl acetate and ethanol have been associated with an increase in off-flavor development during storage.[20] In wine high concentrations of ethyl acetate have been associated with a solvent/chemical aroma.[21]

On the positive side of PC1 (Figure 4A) the sensory attributes are dominated by taste attributes (astringent flavor, pungent flavor, almond aroma and flavor) and an attribute related to mouthfeel (viscosity). These attributes correlate mainly with the proline content, which in earlier studies has been correlated with the mouthfeel of wine.[6,7] In wine, sweetness and viscosity are also correlated due to the effect of residual sugar on viscosity.[6,22] However, in the present study sweetness and viscosity are not related, which might be explained by the added sugar. Almond flavor and aroma were expected to be directly related to the content of benzaldehyde. We expected to detect benzaldehyde in the NMR spectra, because the phenolic protons and especially the aldehyde group should appear in an uncrowded region of the spectra ($\delta_{aldehyde}$ = 9.93). However, no signals were detected in this region. Because the cherry stone is known to contain benzaldehyde, some additional juice samples, containing 1−5% cherry stone, were prepared. These samples were not included in the sensory analysis, but spectra of these juices contained weak signals at δ = 9.93, indicating the presence of benzaldehyde. This result clearly shows that NMR spectroscopy has some limitations, especially with respect to sensitivity, which in our case is below the sensory threshold value of benzaldehyde.

Sourness and bitterness are inversely correlated to sweetness, which is in accordance with a previous study showing that high sugar content could mask bitterness in carrots.[23] The sensory attributes sourness and bitterness are mainly described by the malic acid and GABA contents, of which the latter, to our knowledge, has not previously been described. Consequently, this finding must be considered of interest but further studies are needed to confirm this. Furthermore, this approach is limited by the number of samples that may be presented to a sensory panel (in our case, seven). This weakens the validity of the models and makes it difficult to generalize the findings.

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