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Diversity and dynamic of sensory related traits in different apple cultivars

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Summary

Unquestionably the sensory quality is one of the most important quality traits of apple. On globalised markets any newly bred apple cultivar must be characterised by excellent eating quality. But flavour generally is a very difficult criterion in the breeding process. To establish a quality assessment system including the sensory profile which is usable as selection tool altogether 15 cultivars, standard types and new bred, resistent varieties, were examinated to evaluate altogether 52 quality determining parameters. The results show the high diversity of sensory traits especially the aroma patterns in cultivars. Within the 15 samples cultivars can be distinguished with aroma profiles dominated by esters or alcohols (aroma types). The aroma types can alter between harvest time and optimal eating quality. Regarding the aroma profile a more powerful influence of the cultivar than those of the harvest year was measured.

Introduction

Apples are one of the fruits highly in demand world-wide. The world production of apple is around 41 Million tons per year (N.N., 2009). In Germany, they are considered to be the most important one with a consumption rate of about 30 kg per person and year (N.N., 2007). Like other fruits, apples are a constituent part of a healthy human diet (PODSEDEK, 2000). The outstanding flavour and mouthfeeling (e. g. crispiness and juiciness) is responsible for a high enjoyment value and at least high preference of some cultivars.

Demonstrably since the neolithik, about 10,000 years ago, human is using apple genotypes (BIENIEK and LITYNSKA-ZAJAC, 2001). The domestication and breeding of plants is still an ongoing process. Today exist more than 20,000 cultivars of apple, and still a lot of new ones are bred to improve traits like fruit quality, storability and/or resistance against certain plant diseases such as mildew or scab. Also flavour (texture, taste and aroma) is an important breeding aim for new varieties especially in view case of saturated markets (KADER, 2008; DUNEMANN et al., 2009). The high consumer acceptance of 'new' apple cultivars, such as 'Fuji', 'Braeburn', 'Gala' and 'Pinova' is usually attributed to their outstanding sensory quality in combination with other positive characteristics. Any newly released apple which seeks to replace or compete available cultivars on the market must be of comparable eating quality (HAMPSON et al., 2000).

The volatile profiles and biosynthesis of flavour constituents were examined extensively in the past (BERGER, 1991; FELLMAN et al., 2000; FUHRMANN and GROSCH, 2002). The aroma of apples was one of the earliest object in flavour analysis. More than 370 volatile compounds were reported in literature (FUHRMANN and GROSCH, 2002). Among others as character impact compounds (*E*)-β-damascenone, (*Z*)-2-nonenal, green compounds like (*Z*)-3-hexenal, several esters including ethyl 2-methylbutanoate and alcohols were identified (FUHRMANN and GROSCH, 2002). But apart from pre-, post-harvest and storage conditions the aroma patterns depend especially on the genotype. From the view point of consumers it is a well known fact that the apple cultivars available on the market differ significantly in their flavour characters. Most of the preferred apple varieties are characterized by a Cox-like intensive fruity aroma but also cultivars like 'Granny Smith' which are sweet and sour, high in crispiness and juiciness but low in aroma are successful in markets (SAFTNER et al., 2005). In analogy to other fruits like strawberries (ULRICH et al., 1997) also for apples aroma types were defined based on the relation between ester and alcohol contents (ULRICH et al., 2005; ZHAO et al. 2006; WANG et al., 2007).

The typical flavour of an apple cultivar develops during ripening and storage. The maximum volatile concentration occurs at the climacteric peak but until now there is no complete understanding how biosynthesis is induced during climacteric (DIXON and HEWETT, 2000). The fruits of all cultivars underly an alteration of metabolic patterns both on and off the tree initiated and/or co-ordinated by ethylene. Between harvest time (physiologically maturity or 'harvest quality') and eating time (ripeness or 'eating quality') physical and biochemical traits like skin colour, texture of skin and flesh, juiciness and characteristic flavour are changing dramatically. Which of the genes act during the postharvest period depend also on the preharvest conditions.

Regarding sensory quality at least for the breeders and also consumers only the resulting eating quality is of interest. The dependence of eating quality (including aroma profiles) from the whole history of the fruit life time is an important problem especially for plant breeders or breeding research. For established cultivars guidelines for harvest time and duration of storage until the optimal eating quality exists. During the breeding process of apples high numbers of genotypes are produced in the progeny of a crossing. Every new seedling represents a new genotype with unknown properties regarding inner and outer quality. Therefore the incomplete understanding regarding the dynamic of flavour active compounds from the tree to the eating time is a drawback not only for the plant breeder but also for studies of biochemical pathways and molecular research.

The topic of this work is a) is to establish a convenient methodology for accessing physical and sensory parameters which is usable especially in apple breeding programmes and quality research and b) to use this methodology for studying the dynamic of sensory related traits between harvest time and eating time by human sensory and instrumental analysis. The latter topic is from special importance in selection of new breeding lines regarding their sensory quality. As model systems 15 cultivars, standard types and new bred, resistant varieties, were examinated to evaluate altogether 52 quality determining parameters.

Experimental

Material. Harvest, storage and sample partition – The apples were grown in the experimental garden of the JKI (formerly BAZ – Federal Centre for Breeding Research for Cultivated Plants) in Dresden-Pillnitz. The fruits were sampled from three trees at the optimal ripening stage of each cultivar. The samples were shipped and stored at 3 - 5 °C under atmosphere until analysis. For analysis ten typical apples were used. Each apple was rinsed with water and dried with a towel before using for colour and firmness measurements. Afterwards

the fruits were sliced as shown in Fig. 1. By slicing the apples in 3 parts the use of identical fruit material for sugar, acid, dry matter and aroma analyses as well as human sensory was realized. The used cultivars are summarized in Tab. 1.

Colour and firmness. First of all non-destructive colour and firmness measurement were performed (Fig. 1). The technical details of the penetrometer test (TIRAtest 27025 materials testing machine) and the colour measurement (LAB system) were published in Quilitzsch and Hoberg (QUILITZSCH and HOBERG, 2003).

Tab. 1: List of apple cultivars.

no.	cultivar	optimal eating period*	resistance to scab	AE factor
1	Pirol	1 - 74		1.35
2	Rebella	1 - 105	x	1.16
3	Resi	14 - 143	x	4.33
4	Renora	32 - 166	x	137.28
5	Reanda	37 - 166	x	3.67
6	Piflora	37 - 166		6.84
7	Pinova	19 - 217		0.47
8	Pilot	130 - 260		213.19
9	Remo	cider apple	x	1.06
10	GD	32 - 211		6.84
11	Florina	62 - 187	x	0.49
12	Clivia	30 - 150		1.32
13	Undine	60 - 240		26.39
14	Alkmene	1 - 60		4.66
15	Cox	10 - 70		0.16

* days after harvest at cool storage

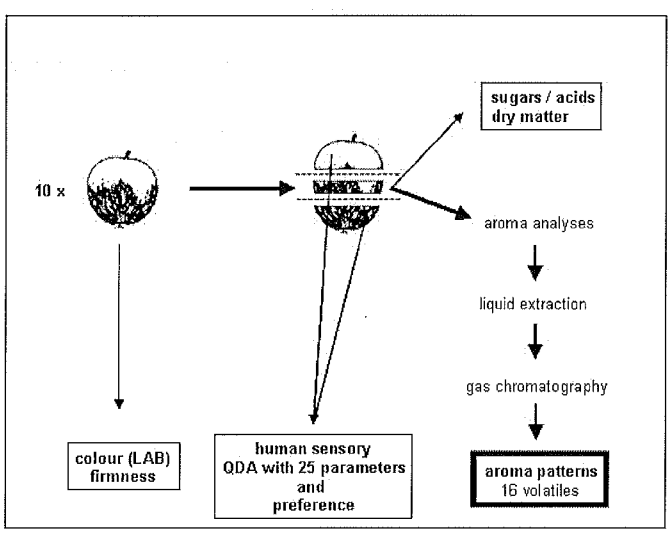

Fig. 1: Scheme of instrumental methods for complex quality control of apples in plant breeding. Ten typical apples were used for one repetition.

Sugar, acid and dry matter. Sugars (sucrose, fructose, glucose), acids (malic and citric acid) were analyzed by HPLC using a fraction of the equatorial disk separated from each apple. A part of the same sample fragment was used for dry matter estimation (Fig. 1). The details of the methods are summerized in Quilitzsch and Hoberg (QUILITZSCH and HOBERG, 2003).

Aroma analysis. After removing from the apple core the equatorial slices of ten apples (appr. 200 g) each sample was homogenized in NaCl solution (18.6 % w/v) for 1 min. The relation of fruit weight to the volume of NaCl solution was 1 : 1 (w/v). The homogenate was centrifuged at 4 °C and 3000 rpm for 30 min to give a supernatant. In order to obtain a clear juice, the supernatant was filtered using filter gauze. A part (250 ml) of the filtrate was subjected to the fluid-fluid extractor apparatus (RAPP et al., 1976) after adding an internal standard (IST = 2,6-dimethyl hept-5-en-2-ol in ethanol). The resulting concentration of IST in the supernatant was 0.1 ppm (v/v). The volatile compounds were extracted by approx. 40 ml methylenchloride from the aqueous solution at room temperature for 20 h. The solvent fraction was dried over sodium sulfate and concentrated to 100 µl by distilling the solvent using a vigreux column (1 cm diameter and 20 cm length) directly before the analyses. The analyses of aroma patterns were done in triplicate. The following volatile compounds were semi-quantified by relating the peak areas to those of the internal standard: propyl acetate (11), 2-methylpropyl acetate (12), butyl acetate (13), 2-methylbutyl acetate (14), butanol (15), pentanol (16), 2-methylbutanol (17), (*E*)-2-hexenal (18), pentanol (19), hexyl acetate (20), hexanol (21), (*E*)-2-hexenol (22), 2-methyl butanoic acid (23), 3-methyl thiopropanol (24), farnesene (25), β-damascenone (26).

Human sensory. After removing the equatorial disk the two remaining parts of each fruit were cut into pieces and mixed to get a representative pooled sample. The profile analysis was done in a sensory laboratory with red lighting by a trained panel consisting of 15 trained testers (HOBERG et al., 2003). The 25 profile parameters are: a) *odour* – aromatic (28), pear (29), fruity (30), green-grassy (31), solvent (32), flowery (33), almond (34), sweetish (35), musty (36), unpleasant (37). b) *taste and retronasal odour* – sweet (38), sour (39), bitter (40), aromatic (41), pear (42), fruity (43), green-grassy (44), solvent (45), flowery (46), almond (47), sweetish (48), musty (49), unpleasant (50). c) *mouth feeling* – crispiness (51), juiciness (52). d) *additional sensations* (54) – free choice parameter. After profile analysis a preference test followed. The preference value P was scored on a 5-point structured hedonic scale ranged from 1 (dislike extremely) to 5 (like extremely). This test was performed by the same panel and the same conditions mentioned above. Eight out of the 15 cutlivars were selected for sensory tests.

Time schedule and sample alignment. The measurements covered three harvest years. In the first year the development of the volatile patterns over the hole storage time up to 210 days were measured by at most 10 dates depending on the shelf life of the cultivars. In the second and third year the aroma analysis was done at four dates: the harvest time and 3 repetitions in the optimum eating time. The analysis of aroma patterns was completed by HPLC, colour and firmness measurements as well as the human sensory.

Statistical analysis. Analytical data were subjected to a multiway analysis of variance (ANOVA) and a principal component analysis (PCA) using the software Statistca 7.1 by StatSoft.

Discussion

Methodology for quality assessment. The complex of methods described in materials and methods is a convenient method to measure altogether 52 quality determining parameters: the aroma pattern consisting of 16 volatiles, the sensory profile with 26 characters, the preference, 5 non-volatiles, dry matter, firmness and 3 colour parameters. The most important advantage of the used methodology is the application of identical sample material for all methods at one analysis date. To develop this method to a more rapid one it is possible to replace the time consuming liquid-liquid extraction as sample preparation method by a more convinient one like headspace-SPME (SCHULZ et al., 2003; ULRICH et al., 1998) or immersion-SBSE (KOMES et al., 2005).

Dynamic of volatile patterns during storage. In the first year the dynamic of the volatile accumulation in the apples from harvest time to senescence was monitored by covering the storage time by up to ten analysis points. The dynamic of 16 volatile compounds is exemplified in Fig. 2. The selection of the 16 volatiles covers the most odour active compounds in apples (FUHRMANN and GROSCH, 2002). The concentration characteristics is strongly influenced by the genotype. The qualitative and quantitative proportions are unique for every cultivar. But in principle all cultivars are characterized by low concentration levels of volatiles at harvest time, a continous increase of the sum of volatiles to a maximum with a subsequent decrease of concentration in the phase of senescence. The interval of optimum eating quality of each cultivar (see Tab. 1) is located always in the increasing branch of the volatile development. Fig. 2 shows the patterns of two basic kinds of volatile patterns (ULRICH et al., 2005; ZHAO et al., 2006; WANG et al., 2007): an alcohol dominating cultivar ('Golden Delicious') and a more ester accentuated type ('Pinova'). The alcohol type is dominated by volatiles such as butanol, hexanol and 2-methylbutanol from early stages of storage until senescence. In the ester type butyl acetate and hexyl acetate occur in high concentration beside butanol. On the one hand the question of aroma type is important for consumers on the other hand is it an important problem in apple breeding in the step of selection in sensory driven breeding programmes. To compare the concentration pattern of the ten cultivars between harvest time and optimum of aroma production a rank correlation was performed (Tab. 2). The higher the Spearman rank coefficient (SRC) the more similar the aroma patterns are between harvest and aroma optimum. 'Pirol' shows the highest similarity with a SRC of 0.91 and 'Golden Delicious' the lowest with 0.61. The results show that some cultivars underly a dramatic change of volatile profiles during storage with very low similarity between harvest and senescence.

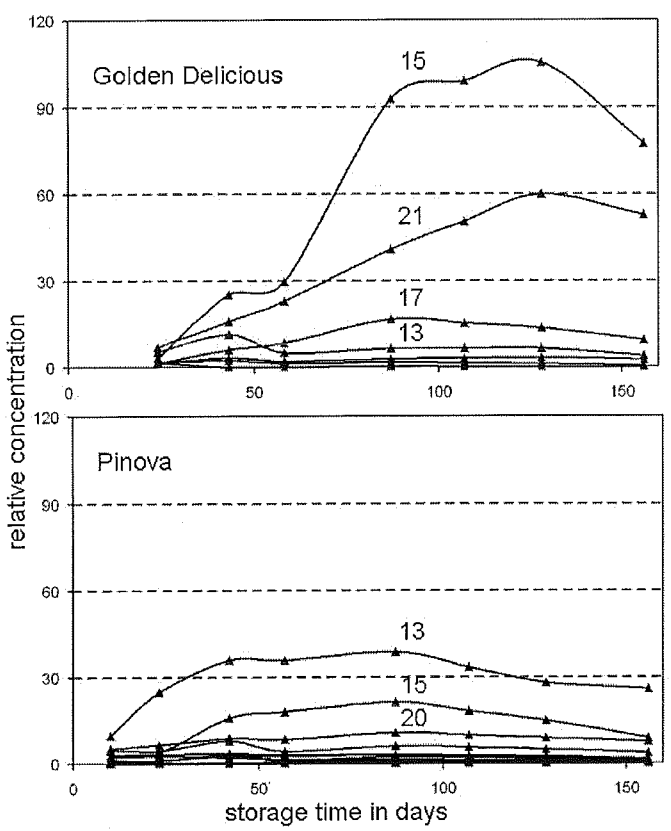

Fig. 2: Dynamic of volatile content of two typical apple cultivars. The nomenclature of aroma compounds refer those mentioned in materials and methods section and Tab. 3. Non-labelled curves of volatiles have very similar concentrations.

Tab. 2: Results of rank correlation of 16 volatiles between two analysis dates.

no.	cultivar	day of 1st analysis	day of maximum aroma content	rank coefficient (Spearman)
1	Pirol	8	108	0.9059***
2	Rebella	8	79	0.8059***
3	Resi	8	106	0.7913***
4	Renora	11	86	0.8609***
5	Reanda	8	149	0.6912***
6	Piflora	14	87	0.7941***
7	Pinova	10	87	0.8500***
8	Pilot	10	128	0.8403***
10	GD	24	128	0.6106***
11	Florina	11	128	0.8529***

Diversity of quality parameters at ripeness. To evaluate the possible diversity of aroma patterns altogether 15 different cultivars were included in the measurements. In Tab. 3 the concentrations of the volatiles are displayed as the mean of three harvest years with 3 repetitions each at the maximum of aroma development for 10 out of 15 cultivars. To separate the influence of cultivar and year a multiway ANOVA (Tukey HSD test) was performed (details are not shown). Using ten cultivars with 16 aroma compounds altogether 450 comparisons of means are computable for the influence of genotype. 374 (83 %) of them show significant differences. For evaluation of the influence of the 3 harvest years with 16 compounds altogether 48 comparisons of means are possible from which 23 (48 %) are significant in difference. The result of comparisons of means show that a higher influence results from the cultivar/genotype than from the influence of harvest year.

A group of 4 out of 16 analyzed volatiles belong to the compound group of alcohols (butanol, 1-pentanol, 2-methylbutanol, hexanol). Six more compounds are esters (propyl acetate, 1-butyl acetate, 2-butyl acetate, 1-pentyl acetate, 2-methylbutyl acetate, hexyl acetate). It is known that apple cultivars develop different types of aroma pattern – so called ester or alcohol types (ULRICH et al., 2005; ZHAO et al., 2006; WANG and Chen, 2007). To estimate the aroma type the AE factor was defined which is the quotient between the sum of alcohol content related to the sum of ester content. The numerical values (Tab. 1) are in the range from 0.16 to 213.19 for 15 cultivars, where low values advise of ester types and high values of alcohol types. In

Tab. 3: Aroma patterns of ten apple cultivars at maximum aroma content

	GD IOZ		Pilot		Rebella		Reanda		Pirella		Florina		Renora		Resi		Piflora		Pinova	
#	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
11	0.42	0.27	0.29	0.21	1.19	0.67	0.76	0.28	0.27	0.23	4.48	0.85	0.01	0.02	0.32	0.21	0.59	0.44	1.36	0.80
12	0.07	0.04	0.02	0.01	0.25	0.06	0.10	0.06	0.08	0.06	0.37	0.14	0.01	0.01	0.09	0.06	0.06	0.03	0.21	0.07
13	9.36	3.59	0.98	0.98	21.75	5.40	15.40	3.76	14.72	5.98	21.52	6.81	0.28	0.22	15.80	5.69	9.82	3.48	24.63	8.79
14	0.81	0.33	0.05	0.04	3.64	2.38	3.62	1.04	1.76	0.74	6.41	2.40	0.50	0.21	2.51	0.79	1.18	0.56	2.50	0.72
15	55.24	37.00	167.71	132.28	20.02	12.91	51.41	23.60	13.35	5.44	9.50	2.52	49.75	34.39	66.64	43.29	57.41	24.09	10.28	6.92
16	0.16	0.08	0.01	0.00	0.42	0.12	0.43	0.11	0.26	0.06	0.46	0.24	0.00	0.00	0.43	0.27	0.13	0.02	0.28	0.06
17	8.18	5.61	48.23	17.71	5.70	3.29	12.95	7.20	2.07	0.67	4.28	0.84	25.08	11.49	11.38	6.94	12.10	3.12	1.69	0.63
18	0.99	0.36	1.70	0.55	1.20	0.35	2.21	0.85	1.51	0.49	1.26	0.32	1.30	0.42	4.28	0.90	1.03	0.46	0.99	0.28
19	1.08	0.78	4.85	2.34	0.45	0.19	1.30	0.82	0.32	0.12	0.21	0.03	2.32	0.98	1.32	0.81	0.84	0.22	0.09	0.04
20	4.05	1.21	0.09	0.06	7.57	3.18	6.87	1.47	3.85	0.90	5.15	1.94	0.02	0.02	5.45	1.86	2.20	0.73	8.76	2.12
21	30.63	17.79	101.49	27.74	14.25	5.71	33.98	23.08	12.13	4.91	5.01	0.84	36.12	17.05	27.27	17.64	25.27	5.09	5.92	2.33
22	0.11	0.08	0.39	0.22	0.32	0.10	0.63	0.12	0.12	0.08	0.16	0.06	0.31	0.14	0.67	0.12	0.41	0.66	0.26	0.31
23	0.02	0.02	0.04	0.04	0.04	0.04	0.02	0.02	0.03	0.04	0.07	0.13	0.19	0.18	0.04	0.03	0.02	0.01	0.02	0.02
24	1.64	0.48	0.81	0.34	0.24	0.08	0.69	0.31	0.21	0.08	0.59	0.15	0.77	0.55	0.53	0.12	1.68	0.70	0.32	0.13
25	0.28	0.23	0.49	0.82	0.25	0.13	0.45	0.25	0.12	0.04	0.19	0.18	0.21	0.16	0.65	0.32	0.27	0.26	0.49	0.42
26	0.09	0.11	0.04	0.02	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.06	0.02

The data are the mean of 3 harvest years with 3 repetitions each and expressed as relative concentration in relation to the internal standard. The point in time of maximum aroma is listed in table 1. Compound numbers: propyl acetate (11), 2-methylpropyl acetate (12), butyl acetate (13), 2-methylbutyl acetate (14), butanol (15), pentanol (16), 2-methylbutanol (17), (E)-2-hexenal (18), pentanol (19), hexyl acetate (20), hexanol (21), (E)-2-hexenol (22), 2-methyl butanoic acid (23), 3-methyl thiopropanol (24), farnesene (25), β-damascenone (26).

Fig. 3 the ester content is drawn versus the alcohol content. Typical alcohol types like 'Pilot', 'Golden Delicious' and 'Renora' are located in the lower right side of the diagramm. Some of the preferred cultivars on the markets like 'Cox' and 'Pinova' are typical ester types located on the upper left side of the plot. No cultivar is placed above the diagonal of the diagram because alcohols and esters are strongly connected as precursor and final product in the biosynthetic pathways (VILLATORO et al., 2008; ZHU et al. 2008).

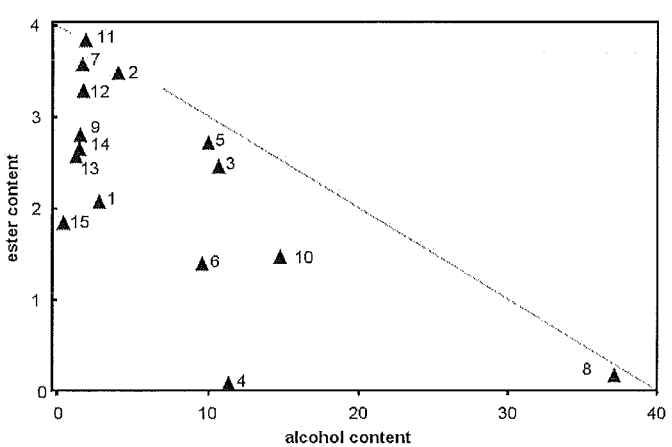

Fig. 3: Ester versus alcohol content of 15 apple cultivars. Volatiles were semiquantitated by gas chromatography using an internal standard procedure for data processing. Quantities are given as relative concentrations in relation to the internal standard (0.1 ppm v/v). Every data point represents the average of a three year trial. Alcohol content: sum of butanol, 2-methylbutanol, 1-pentanol and 1-hexanol. Ester content: sum of propyl acetate, 2-butyl acetate, 1-butyl acetate, 2-methylbutyl acetate, 1-pentyl acetate and hexyl acetate.

The correlation and visualization of all 52 estimated quality parameters was performed by a PCA (Fig. 4a and 4b). The plot of cultivars in Fig. 4a show clear clustering. The distances within a cultivar is caused by analytical repetition and harvest year. In plot 4b the relations of the 52 quality parameters and the AE factor are visualized. One of the most important parameters, the preference P, is located in the first quadrant. This location is in correspondence with the position of the ester-type apples in plot 4a. The preference P is far apert from the AE factor. Both relations show that ester type apple are preferred by the testers. The closest relation between preference and parameters were found for the retronasal sensation 'aromatic' (41), 'juiciness' (52), 'crispiness' (51), (*E*)-hexenol (22), and 2-methylbutyl acetate (14), and, surprisingly, the a* value (66) which is a degree for red color in the LAB system. A correlation between metabolites and colour of the apple skin was reported earlier in literature (DAYTON, 1964; FELLMAN et al., 2000). The authors discussed a correlation between so called color classes and aroma profiles where genotypes with higher pigmentation have higher aroma levels. The findings of this experiment are in accordance with those of Dayton and Fellmann.

A close correlation exists between the sensory taste impression 'sweet' (35) and the analytical value of the fructose concentration (60). Both parameters are located adjoining in quadrant 2 in contrast to the concentration parameters of glucose (61) and saccharose (62). The close neighboring of 'sweet' and fructose concentration is due to its high concentration in apples and the higher relative sweetness of this sugar type in relation to the others (BELITZ et al. 2008). The findings are also a strong argument for the accuracy of the used sensory profile method and the results delivered by the panel.

Conclusions

a) The used complex of sensory and instrumental analyses is a convenient methodology for comprehensive measurement of apple qua-

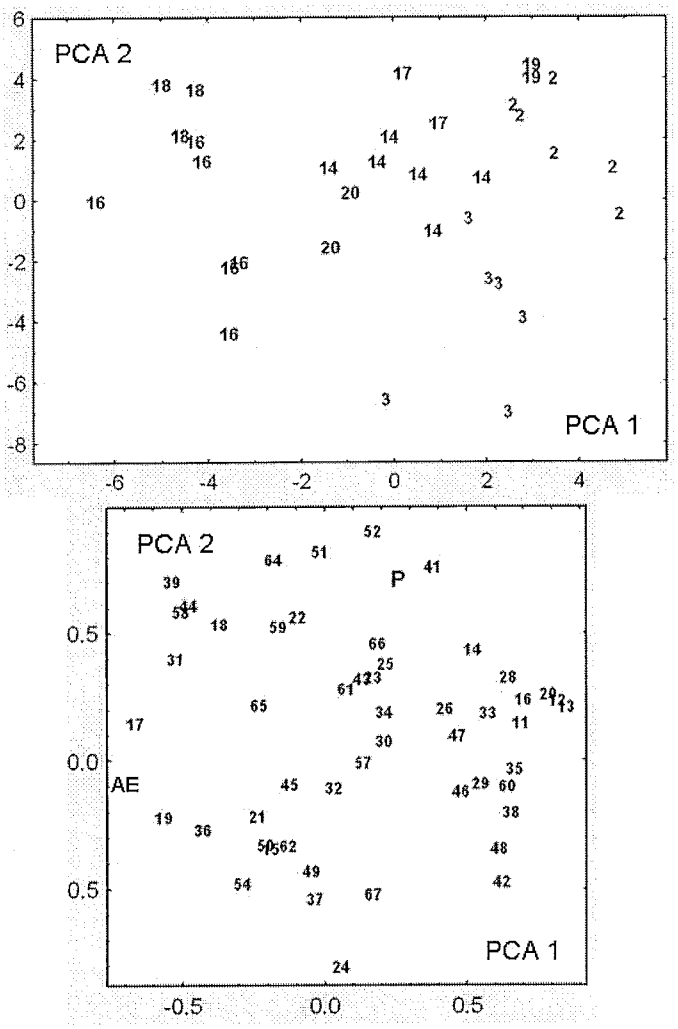

Fig. 4: Cultivar and parameter plot of a PCA. The PCA includes 7 cultivars and 52 quality parameters. The nomenclature of cultivars refer to Tab. 1. The coding of sensory and instrumental parameters is given in the materials and methods section. Further parameters: preference (P), alcohol/ester-factor (AE), dry matter (57), malic acid (58), citric acid (59), fructose (60), glucose (61), saccharose (62), firmness (64), L*-value (65), a*-value (66), b*-value (67).

lity especially for quality assessment in apple breeding programmes.
b) Particularly the aroma profile changes dramatically between harvest and time of optimal eating quality/maximum aroma content. The degree of alteration depends on genotype. This behavior is of essential importance for instance in apple breeding. A flavour selection on the basis of aroma profiles at harvest will not match necesseraly the qualitative and quantitative ratios at eating quality for different genotypes.
c) Regarding the aroma profile a more powerful influence of the cultivar than those of the harvest year was measured comparing 10 cultivars.
d) Apple cultivars develop different aroma types dominated by esters or alcohols.
e) A multivariate statistical analysis of 52 quality parameters reveals their influence on the preference. Obviously the ester types were preferred by the panel in opposite to alcohol types. Parameters which influence the preference positively were: 'aromatic' (41), 'juiciness' (52), 'crispiness' (51), (*E*)-hexenol (22), 2-methylbutyl acetate (14), the a* value (66) of the LAB colour measurement.
f) The displayed results are of evidence for defining breeding aims regarding internal and external quality of new apple lines.

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