‘Renaissance’ of flavor: portrait of a new European strawberry cultivar

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Abstract

In the history of strawberry breeding, selection for aroma was mainly conducted to avoid untypical flavor notes. The depleted aroma patterns of modern cultivars resulted from fixation on breeding goals like high yield, large, firm and optically attractive fruit. Genetic erosion was clearly demonstrated in comparative studies using old and modern cultivars. Wild species introgression can serve to improve flavor in pedigrees. In this context, chemical and sensory analysis can assist the selection process in strawberry breeding and is essential for a successful selection of highly aromatic genotypes. In the present study, we compared the aroma profiles of three European strawberry cultivars released in 1933, 1975 and 2009 to demonstrate the steady loss of character impact compounds using HS-SPME-GC-FID (and MS identification). Furthermore, we present one clone selection, which is characterized by an aroma profile that was reached by cultivar crossbreeding, and two selections with profiles obtained from wild species introgression with *Fragaria chiloensis* ssp. *lucida* in F_2 and F_3 backcrosses. Obviously, the use of wild species accessions results in quantitatively and qualitatively improved aroma potentials. Such a broadened aroma spectrum in combination with a high brix-acid-ratio has never been found in cultivars and clones originating from cultivar crossbreeding. Backcrossing with modern cultivars resulted in genotypes with a combination of diverse esters as well as high values of linalool and lactones. One of the selected clones was successfully tested for horticultural high-performance traits and for stability over five years. This selection won the comparison with other cultivars and breeding clones in a consumer study and several sensory tests and has a high potential for the processing industry. As a result, it is under application for European Plant Breeders Rights and will be introduced to the market with the designation ‘Renaissance’.

Keywords: wild species introgression, aroma profile, VOC, acceptance, ‘Renaissance’

INTRODUCTION

Flavor has been merely a secondary breeding goal in recent strawberry breeding history. Instead, modern cultivars with high performance in yield, fruit size, firmness and shelf life emerged. This process caused negative domestication effects characterised by poor flavor and reduced resistances (Ulrich et al., 2005; Aharoni et al., 2004; Hancock et al., 2010; Ulrich and Olbricht, 2013). About 20 out of approximately 360 volatiles have been identified as important for sensory impression (so-called character impact compounds) (Pyysalo et al., 1979; Hirvi and Honkanen, 1982; Ulrich et al., 1997; Fischer and Hammerschmidt, 1997; Schieberle and Hofmann, 1997; Olbricht et al., 2008). In particular, range and amount of low-boiling esters and of methyl anthranilate (MA) are limited or missing in modern cultivars. Vice versa, terpenoids and lactones are consistently found in these cultivars (Olbricht and Ulrich, 2017).

The combination of analytical methods such as gas chromatography (GC), mass spectrometry (MS), GC-olfactometry and the evaluation by human sensory panels can

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support the selection of high-flavor aroma types in strawberry breeding.

Crossbreeding with wild species accessions is a method to increase the diversity of strawberry breeding material. In particular, the two octoploid species *F. virginiana* Miller and *F. chiloensis* (L.) Miller are promising breeding partners (Hancock et al., 2010).

Inheritance analysis of several important aroma compounds are scarce but help breeders to select suitable breeding partners with a high flavor potential and to assist in the selection process (Carrasco et al., 2007; Olbricht et al., 2008, 2011; Wang et al., 2011).

MATERIAL AND METHODS

Plant material and cultivation

All investigated plants were planted as fresh plants on the open field in Dresden-Weixdorf, (elevation 188 m a.m.s.l., latitude: 51.142703°, longitude: 13.772751°), Germany, on sandy loam on gravel ground. In the subsequent year, all available and typical healthy fruit from ten plants per genotype were harvested and stored at -20°C until sample preparation and analysis.

Three cultivars of *Fragaria ×ananassa* Duch. were chosen as typical representatives of 80 years of European breeding history: 'Frau Mieze Schindler' (1933), 'Elsanta' (1975), and 'Elegance'(2009). Further, three breeding clones were chosen: F_1 of 'Daroyal' × 'Elsanta' and two clones with two backcrossing levels after introgression of *Fragaria chiloensis* ssp. *lucida* (E. Vilmorin) Staudt (F_2 and F_3) with high-performance cultivars. Additionally, four other cultivars ('Evie 2', 'Senga Sengana', 'Clery' and 'Honeoye') and an off-flavor breeding clone (P-713) served for comparison regarding soluble solids (SSC) and titratable acidity (TA) values as well as for the consumer test.

Horticultural testing was performed at three German trial stations in Dresden-Weixdorf (Hansabred), Dresden-Pillnitz (Sächsisches Landesamt für Umwelt, Landwirtschaft und Geologie) and Köln-Auweiler (Versuchszentrum Gartenbau Straelen, Köln-Auweiler).

Fruit preparation and fruit analysis

For fruit analysis, all fully-ripe and typical fruits from ten plants per genotype were used for sample preparation to average the differences of fruit quality caused by different plants, different harvest dates and fruit ranks. SSC was measured in °Brix using a digital refractometer (Quick-Brix®, Mettler Toledo, Schwerzenbach, Germany). TA was determined with a titrator (716DMS-Titrino-Serie-06®, Metrohm, Filderstadt, Germany).

The sample preparation for VOC analysis followed the procedure described in Olbricht et al. (2011). For analysis, the volatiles were sampled by head-space solid phase micro-extraction (HS-SPME) with a 100 µm PDMS fiber (Supelco, Bellefonte, USA) using an MPS2 autosampler from Gerstel (Mühlheim an der Ruhr, Germany) (method described in Olbricht et al., 2011). Data processing was performed with the commercial software CHROMstat™ version 2.6 by Analyt Mühlheim (Germany). A number of 18 character impact compounds were detected and used for a comparison of aroma patterns for six genotypes (Table 1).

Table 1. 18 detected character impact compounds grouped into four compound groups.

Esters	Terpenoids	Lactones	Miscellaneous and carotinoid-derived
Methyl butanoate	Linalool	γ-decalactone	Benzaldehyd
Ethyl butonoate	Myrtenyl acetate	γ-dodecalactone	2-undecanone
Methyl hexanoate	Nerolidol		Hexanoic acid
(*E*)-2-hexenal			β-damascenone
Ethyl hexanoate			
Hexyl acetate			
(E)-2-hexenyl acetate			
2-methylbutanoic acid			
Methyl anthanilate			

RESULTS AND DISCUSSION

Cultivar description and pedigree of 'Renaissance'

The cultivar 'Renaissance' was tested as horticulturally stable over five years at several trial stations. The cultivar is characterized by the following criteria:

1. Plant growth.

Stable plantation without plant losses in the field, vigorous, fully winterhardy.

2. Plant diseases.

Highly tolerant to powdery mildew, *Botrytis*, *Phytophthora cactorum*, anthracnose, spider mites, tolerant to *Verticillium* wilt (tested also in inoculated trials), *Xanthomonas*, slightly susceptible to strawberry weevil.

3. Harvest.

Two days after 'Elsanta'.

4. Yield.

In comparison to other cultivars at different locations and under different cultivation systems:

1. Köln-Auweiler (raised bed with mulch film and dropping irrigation, single row, open field):
   a. planted as fresh plants in August 2012, harvested in 2013: 'Elsanta' 1293 g plant^{-1} in total with first class 957 g plant^{-1}; 'Elegance' 1111 g plant^{-1} in total with 994 g plant^{-1} first class fruit; 'Renaissance' 1122 g in total with first class fruit 993 g plant^{-1}.
   b. planted as fresh plants in August 2013, harvested in 2014: 'Elsanta' 1680 g plant^{-1} in total with first class 1087 g plant^{-1}; 'Darselect' 1297 g plant^{-1} in total with 880 g plant^{-1} first class fruit; 'Renaissance' 1489 g plant^{-1} in total with first class fruit 1004 g plant^{-1}.
2. Dresden-Pillnitz (open field, planted as frigo plants in May 2012, harvested in 2013): 'Elsanta' 1120 g plant^{-1} in total with first class 645 g plant^{-1}; 'Renaissance' 969 g plant^{-1} in total with first class fruit 665 g plant^{-1}.
3. Dresden-Weixdorf (open field):
   a. planted as fresh plants in August 2013, harvested in 2014: 'Elsanta' 670 g plant^{-1} in total; 'Renaissance' 821 g plant^{-1} in total.
   b. planted as fresh plants in August 2014, harvested in 2015: 'Elsanta' 868 g plant^{-1} in total; 'Malling Centenary' 1135 g plant^{-1} in total; 'Elegance' 989 g plant^{-1} in total; 'Renaissance' 1159 g plant^{-1} in total.

5. Average fruit size.

1. Köln-Auweiler:
   a. 2013: 'Elsanta' 15.9 g; 'Elegance' 22 g; 'Renaissance' 19.1 g.
   b. 2014: 'Elsanta' 14.6 g; 'Renaissance' 17.7 g.
2. Dresden-Pillnitz in 2013: 'Elsanta' 15.9 g; 'Renaissance' 16.5 g.
3. Dresden-Weixdorf in 2015: 'Elsanta' 14.6 g; 'Malling Centenary' 19.4 g; 'Elegance' 20.2 g; 'Renaissance' 15.1 g.

6. Fruit shape.

Very similar heart-shaped, no misshapen fruit.

7. Firmness, appearance and shelf life.

Firm and good shelf life, attractive glossy fruit.

8. Flavor.

Extraordinarily high flavor, stable over six years, without any comparison in the current assortment of strawberry, gourmet type, flavorful also under glass production.

9. Potential.

Open field production and under tunnel for high-aroma fruit fresh market production, direct marketing, self-picking, processing (jam, freeze-drying, aroma extraction, fruit puree and smoothies), and, last but not least, private gardening.

Brix values and acidity

SSC and TA are known as environment-dependent traits of strawberry fruit. Table 2 lists the values of the new cultivar 'Renaissance' over six years in comparison to the fresh market cultivar 'Elsanta' and the standard processing cultivar 'Senga Sengana'. Evidently, 'Renaissance' exhibits the highest values with only one exception.

Table 2. SSC and TA values (in °Brix and citric acid equivalent (in %)) over six years for 'Renaissance', 'Elsanta' and 'Senga Sengana'.

Cultivar	2010		2011		2012		2013		2014		2015	
	SSC	TA	SSC	TA	SSC	TA	SSC	TA	SSC	TA	SSC	TA
Elsanta	9.7	0.91	10.2	0.89	n.d.	n.d.	9.0	0.87	9.2	0.79	8.6	0.81
Senga Sengana	n.d.	n.d.	11.3	0.91	8.5	0.95	8.9	0.99	7.6	0.92	6.9	0.81
Renaissance	10.9	1.10	13.2	0.95	9.8	1.01	9.4	0.98	10.0	0.94	9.7	0.97

Aroma analysis

For comparison of the patterns of six genotypes, diagrams for each genotype were generated (Figure 1). In general, the aroma patterns are very diverse. The old cultivar 'Frau Mieze Schindler' (released in 1933), which is known as a standard for excellent flavor in Germany, is rich in esters like ethyl butanoate, methyl hexanoate, and ethyl hexanoate. It is the only genotype with detectable amounts of methyl anthranilate (MA). MA can be defined as important discriminative aroma compound (Ulrich et al., 1997). Due to its importance, MA is used as artificial flavoring substance in the food industry (Schlosser and Lane, 2001). Further, MA is lacking in the economically important cultivars and is only found in some old cultivars and in some wild species (Ulrich et al., 1997; Ulrich and Olbricht, 2013). The inheritance of MA is very low which explains the easy loss in the domestication of strawberry (Olbricht et al., 2008). The second cultivar 'Elsanta' (released in 1975) shows a poor ester expression. For ethyl hexanoate it shows the highest peak (relative concentration) of all analysed genotypes. On the other hand, there are higher contents in terpenoids (linalool and nerolidol) and in the lactones, γ-decalactone and γ-dodecalactone. This is in contrast to 'Frau Mieze Schindler' where no lactones were measured. This obvious trend from high to low ester contents and to high lactone and terpenoid contents is confirmed by broader studies with more cultivars in comparison (Olbricht and Ulrich, 2017). In this context, 'Elegance' (released in 2009) serves as an extreme example of this development in the breeding history of strawberry. Linalool, nerolidol, γ-decalactone and hexanoic acid show the highest peaks in the aroma pattern of 'Elegance'. By selecting for flavor after cultivar crossbreeding (F_1 cultivar cross 'Daroyal' × 'Elsanta'), higher ester contents with different qualitative composition can be achieved. Using an accession of *Fragaria chiloensis* ssp. *lucida*, completely new patterns appear in the F_2 (first backcross after introgression of *F. chiloensis* ssp. *lucida*) and in 'Renaissance' (released in 2016/17) as an F_3-clone (second backcross after introgression of *F. chiloensis* ssp. *lucida*). Here, the combination of a diverse ester pattern with high quantities is combined with high relative concentrations of linalool, nerolidol and γ-decalactone.

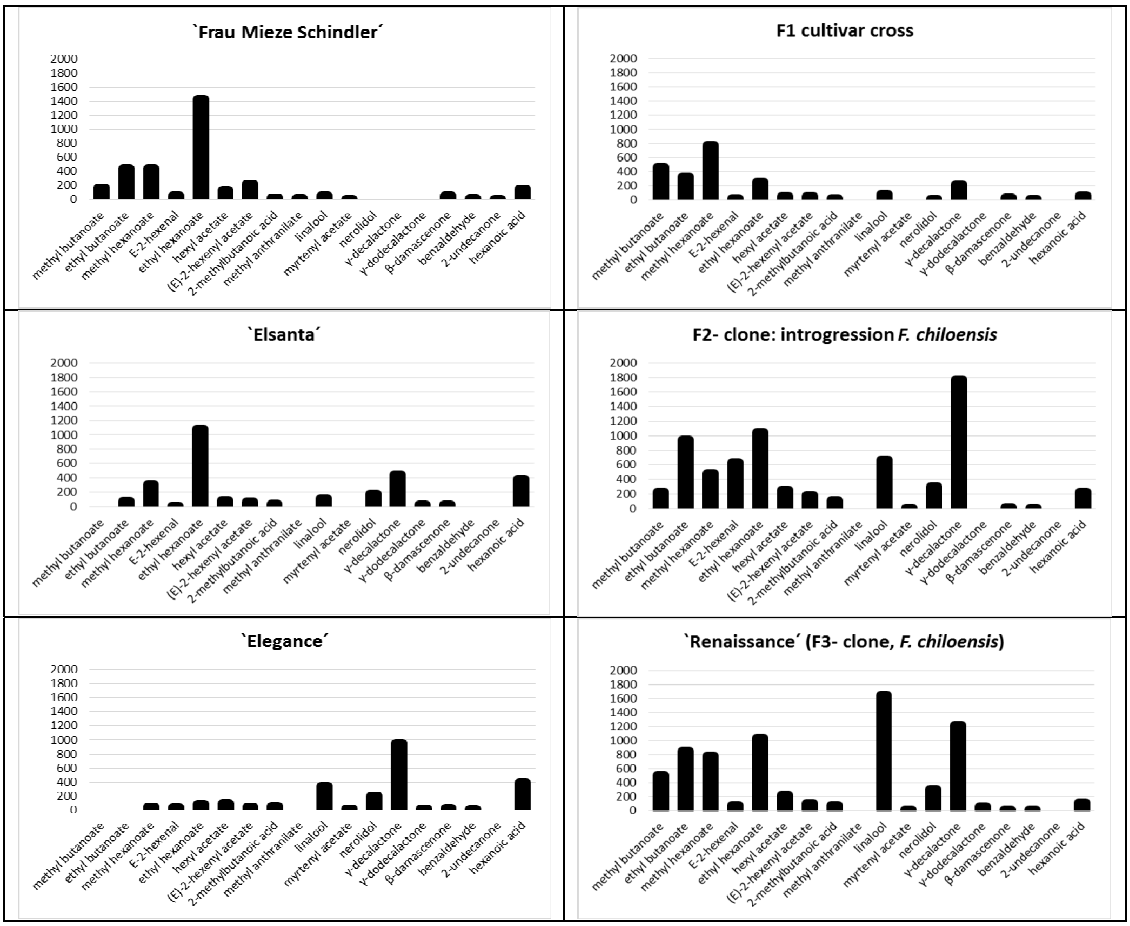

Figure 1. Character impact compounds (relative concentration) for three cultivars ('Frau Mieze Schindler', 'Elsanta', 'Elegance', F_1 cultivar cross ('Daroyal' × 'Elsanta'), F_2-clone: introgression *F. chiloensis* (first backcross after introgression of *F. chiloensis* ssp. *lucida*), 'Renaissance' (F_3-clone, second backcross after introgression of *F. chiloensis* ssp. *lucida*).

The 18 character impact compounds belong to different compound groups (cf. Table 1). The evaluation of compound groups shows clear differences with regard to the breeding history and breeding background (Figure 2). The ester content is decreasing for the three cultivars in parallel with the ascending chronological order of their release. It reached the highest values for the clone after wild species introgression (F_2 and F_3). Terpenoids and lactones increase from old to modern cultivars but are the highest for the F_2 and F_3 of wild species introgression. The relevance of terpenoids and lactones is not only important for the aroma. Importantly, terpenes and lactones are described as sweet enhancers (Schwieterman et al., 2014; Ulrich and Olbricht, 2016). Taking miscellaneous and carotenoid-derived compounds together, their concentration is increasing from old to modern cultivars. It is comparatively high in the F_2-backcross and lower for 'Renaissance' in comparison with the other cultivars.

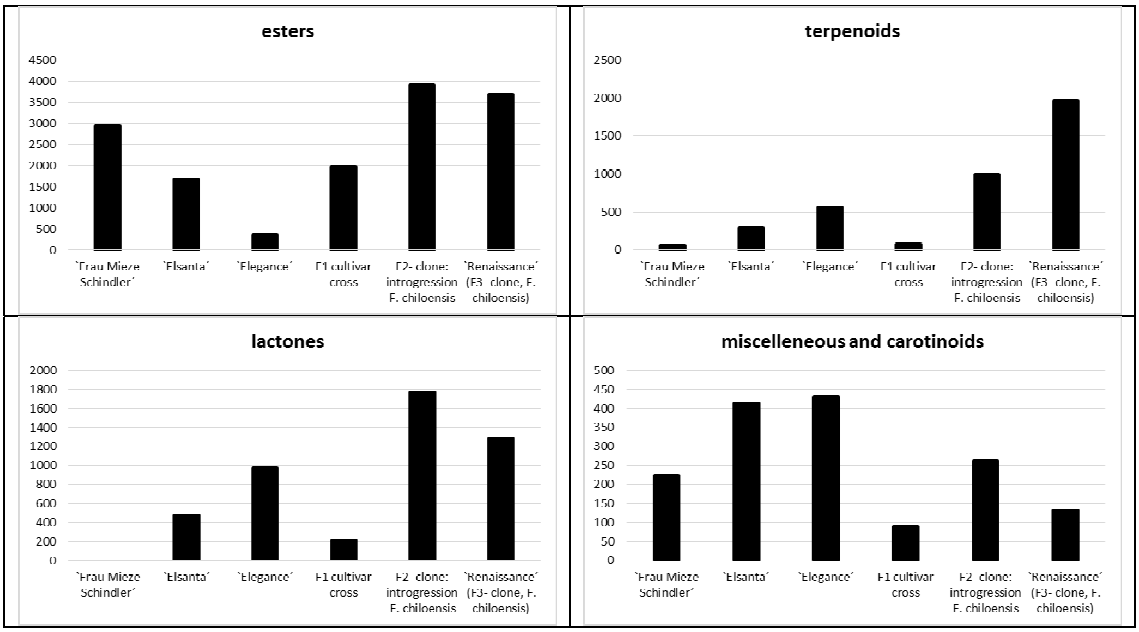

Figure 2. Peak sum of four compound groups (relative concentration) for three cultivars ('Frau Mieze Schindler', 'Elsanta', 'Elegance', F_1 cultivar cross ('Daroyal' × 'Elsanta'), F_2-clone: introgression *F. chiloensis* (first backcross after introgression of *F. chiloensis* ssp. *lucida*), 'Renaissance' (F_3-clone, second backcross after introgression of *F. chiloensis* ssp. *lucida*).

The sum of character impact compounds is dramatically increased for the F_2 and F_3 after wild species introgression reaching more than 200% of the investigated three cultivars (Figure 3). This analysis supports the suitability for processing purposes that crucially depends on the quantity of relevant aroma compounds.

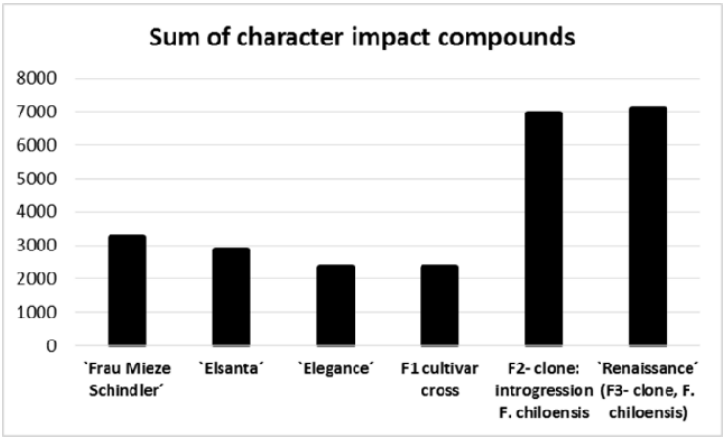

Figure 3. Sum of character impact compounds (relative concentration) for three cultivars ('Frau Mieze Schindler', 'Elsanta', 'Elegance', F_1 cultivar cross ('Daroyal' × 'Elsanta'), F_2-clone: introgression *F. chiloensis* (first backcross after introgression of *F. chiloensis* ssp. *lucida*), 'Renaissance' (F_3-clone, second backcross after introgression of *F. chiloensis* ssp. *lucida*).

Consumer study

In a consumer study, the new cultivar 'Renaissance' was tested in comparison to six cultivars and a parallel clone (F_3-clone, second backcross after introgression of *F. chiloensis*

ssp. *lucida*) that was pre-selected as off-flavor type (Figure 4). The highest score was reached for 'Renaissance'. It can be noted that clone P-713 (originating from the same breeding background) was evaluated with the lowest score for acceptance. Although this genotype has a very diverse aroma profile with extraordinary high quantities of some esters (data not shown), the whole aroma pattern resulted in off-flavor impressions (Ulrich and Olbricht, 2016).

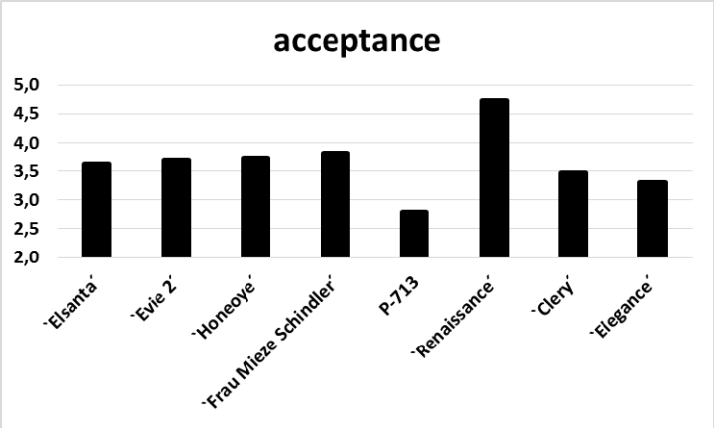

Figure 4. Acceptance in a consumer study (each genotype was evaluated for overall liking by ca. 80 testers), cultivars and one breeding clone 'Daroyal', 'Elsanta', 'Evie 2', 'Honeoye', 'Frau Mieze Schindler', 'Elegance', P-713 (F_3-clone: introgression *F. chiloensis*: second backcross after introgression of *F. chiloensis* ssp. *lucida*, off-flavor-type), 'Renaissance' (F_3-clone, second backcross after introgression of *F. chiloensis* ssp. *lucida*).

Additionally, 'Renaissance' was selected from hundreds of parallel clones especially based on flavor stability. In a former study (Olbricht et al., 2011), it was shown that in a model population the highest stability in aroma compounds is reached for genotypes with low or even zero quantities of VOCs. This appears contradictive to the breeding of highly aromatic strawberry cultivars and explains why modern high-performance cultivars are not characterized by high quantities of aroma compounds.

CONCLUSION

Modern cultivars can be distinguished from old cultivars in terms of aroma patterns because they are characterized by low sums of esters and increased values for terpenoids and lactones. *Fragaria* × *ananassa* 'Renaissance' was developed using a subspecies of *F. chiloensis*. After two backcrosses it was selected out of hundreds of parallel clones because of its high performance for horticultural characteristics and a highly appreciated stable expressed flavor. This flavor type is characterized by a manifold spectrum of esters that occur in high concentrations. In distinction to appreciated old cultivars, 'Renaissance' combines the group of esters with high contents of terpenoids and lactones, which are typical for modern cultivars.

In addition to sugar-acid balance and mouth feeling, the assessment of VOCs with positive and negative impacts on the sensory quality is very important for the breeding of preferred strawberries. High sums of character impact compounds does not guarantee high scores of acceptance, however, they are essential for processed products. In any event, the pattern of character impact compounds (in quality and quantity) is crucial for the sensorial impression.

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