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Changes in the Key Odorants and Aroma Profiles of Hamlin and Valencia Orange Juices not from Concentrate (NFC) During Chilled Storage

Ines Sellami[#], Veronika Mall[#] and Peter Schieberle[§*]

[#] Deutsche Forschungsanstalt für Lebensmittelchemie[a],

Lise-Meitner-Strasse 34, D-85354 Freising, Germany

[§] Technische Universität München, Department für Chemie,

Lise-Meitner-Strasse 34, D-85354 Freising, Germany

*Corresponding Author

[a]The institute was renamed as Leibniz Institute for Systems Biology at the Technical University Munich

Phone: +49 871 97698160

E-mail: peter.schieberle@ch.tum.de

ABSTRACT. Application of the aroma extract dilution analysis (AEDA) on the volatiles isolated by extraction/SAFE distillation from NFC (not from concentrate) juice from Hamlin oranges revealed 51 odor-active constituents in the flavor dilution (FD) factor range of 8 to 8192 among which vanillin, wine lactone and (R)-linalool appeared with the highest FD factors. The AEDA applied on the volatile fraction of the same batch of juice stored at 0 °C for ten months under aseptic conditions showed clear changes in the aroma profile as well as in the FD factors of key odorants. The reduction in the intensity of the citrus-like, pungent, green odor attributes in the aroma profile correlated with the loss of 1-penten-3-one, acetaldehyde and (*Z*)-3-hexenal and a clear decrease in hexanal, octanal, nonanal, decanal and (*E*,*E*)-2,4-decadienal. Quantitation done by stabile isotope dilution assays followed by a calculation of odor activity values (ratio of concentration to odor thresholds in citrate buffer) confirmed that the quick loss of 1-penten-3-one and acetaldehyde already within a few weeks, and a significant reduction in nearly all aldehydes over the storage time of 10 months was responsible for the changes in the overall aroma profile of the juice. The same approach applied on Hamlin juice from the next harvest year as well as on chilled stored NFC juice from Valencia oranges confirmed the results for another harvest year and another orange variety.

KEY WORDS: NFC orange juice, storage, aroma extract dilution analysis, stable isotope dilution assay, odor activity value; 1-penten-3-one; acetic acid

INTRODUCTION

Orange trees (*Citrus sinensis, L. Osbeck*) are one of the oldest cultivated plants and the fruit and juice production constantly increased due to the characteristic flavor and beneficial nutrient content. Today, orange juice ranks among the world`s most produced fruit juices, and the per-capita-consumption in Germany was about 8 Liter in 2014.[1] Two types of orange juices are prepared commercially; juice from concentrate (CJ) which undergoes a thermal treatment to evaporate the major part of the water as well as the aroma fraction, and NFC (not from concentrate) juice, which is only pasteurized and then chilled stored before consumption. Although freshly squeezed orange juice is said to have a superior aroma compared to both, CJ and NFC juice, in particular the overall aroma of NFC juices comes close to the aroma of freshly squeezed juices.[2]

Investigations on the volatile fraction of orange juices were already started about 100 years ago,[3] and until now more than 400 volatiles have been identified.[4] Next to (R)-limonene, (R)-α-pinene and myrcene, the esters (S)-ethyl 2-methylbutanoate, ethyl butanoate and ethyl 2-methylpropanoate as well as the aldehydes (*Z*)-3-hexenal, hexanal and acetaldehyde were previously characterized [5,6] among the key aroma compounds in freshly squeezed orange juice using the Sensomics concept. But, the aroma of freshly squeezed orange juice is not stable, and a significant change in the overall aroma profile rapidly occurs during processing and storage.[7] For example, enzymatic reactions, thermal reactions during pasteurization and bottling[8-12] are well-known to induce changes in the aroma composition.

In addition, several aroma compounds are newly formed or generated either during storage in glass bottles in the trade or during chilled storage before bottling. Several investigations have already been carried out to understand the formation of off-flavors.[7,9,13,14] Kirchner and Miller[9] were among the first to study the changes in the

volatiles between fresh and stored juices and found an increase in, e.g. carvone, acetic acid and α-terpineol, while esters, aldehydes and terpene hydrocarbons decreased. Tatum et al.[13] later on suggested 2-methoxy-4-vinylphenol and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone to be responsible for the aged off-flavor formed after storage at 35 °C. Additionally, it is long known that the concentration of α-terpineol increases significantly depending on the storage temperature.[9,13-16]

However, most studies did not use approaches directly combining sensory evaluation and analytical measurements, such as gas chromatography-olfactometry (GC-O) or odor activity value calculations. In a first study trying to confirm the above mentioned compounds as marker substances for juice storage using the Sensomics concept[16] the authors did not confirm the influence of these compounds on the formation of off-flavors during storage of juice from concentrate in glass bottles at 20 °C. Instead, a drastic decrease in acetaldehyde, octanal and decanal was found and it was suggested that the increase in concentration of certain juice compounds together with the decrease of key juice odorants is responsible for the overall changes in the aroma of the orange juice during storage.[16]

But, to date, no comprehensive approach using the Sensomics concept including systematic sensory experiments has been used to clarify the molecular reason for changes in the overall aroma of chilled stored NFC orange juice. Therefore, it was the aim of this study to clarify the molecular background of aroma changes occurring during chilled storage of NFC orange juices from the two varieties, Hamlin and Valencia, by means of the Sensomics concept. The particular focus was on compounds degrading during storage in order to establish a basis for further studies on the pathways leading to their instability even under the mild conditions of a chilled storage.

MATERIALS AND METHODS

Materials. Orange juices were produced and stored by a commercial juice supplier (Tropicana Products Inc). Fruits from the same batch of either Hamlin (Ham) or Valencia (Val) oranges were extracted and stored in aseptic tanks at 0 °C. Samples were taken every month for a total period of 10 months, filled in aseptic bottles, sent frozen to Germany and stored at – 60 °C prior to analysis.

Reference Odorants. Reference compounds were obtained from the commercial sources given in parentheses: acetaldehyde, (*E,E*)-2,4-decadienal, decanal, ethyl 2-methylbutanoate, ethyl 3-hydroxyhexanoate, ethyl hexanoate, ethyl propanoate, hexanal, (*Z*)-3-hexenal, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 2-hydroxy-3-butanone, 3-isopropyl-2-methoxy-pyrazine, (*R*)-limonene, 3-(methylthio)propanal, methyl 3-hydroxyhexanoate, 2- and 3-methylbutanoic acid, myrcene, (*E,Z*)-2,6-nonadienal, octanal, 1-penten-3-one, phenylacetic acid, α-pinene, α-terpineol, vanillin and 2-ethyl-3,5-methylpyrazine, 1,8-cineol, ethyl butanoate and linalool (Sigma-Aldrich Chemie, Taufkirchen, Germany). Carvone, nootkatone, 1-octen-3-one and 2-methoxy-4-vinylphenol (Lancaster, Mühlheim, Germany). Acetic acid and 2,3-butanedione (Merck, Darmstadt, Germany). Nonanal and β-ionone (Roth, Karlsruhe, Germany).

The following reference odorants were synthesized as reported in the literature: *trans*-4,5-epoxy-*(E)*-2-decenal[17] and 3α,4,5,7α-tetrahydro-3,6-dimethyl-2(3*H*)-benzofuranone (wine lactone).[18]

Chemicals Ascorbic acid, citric acid (water free), ethanol, fructose, glucose, lithium aluminium hydride, *trans*-p-menth-2-ene, potassium peroxomonosulfate, potassium acetate, potassium ferrocyanide, sucrose, sodium thiosulfate, p-toluene sulfonic acid and zinc sulfate were purchased from Sigma-Aldrich Chemie (Taufkirchen, Germany). Aceton (suprasolv), acetic anhydride, methyl octanoate, silica gel, sodium chloride, sodium hydrogen carbonate, anhydrous sodium sulfate, and sulfuric acid

were from Merck (Darmstadt, Germany). Diethyl ether and dichloromethane (Merck) were freshly distilled before use.

Isotopically Labeled Internal Standards. These were synthesized as previously reported: [$^2H_4$]-carvone and [$^2H_4$]- decanal,[15] [$^2H_4$]-octanal,[20] [$^2H_6$]-α-terpineol,[6] [$^2H_2$]-linalool,[21] [$^2H_3$]-(*E,E*)-2,4-decadienal,[22] [$^2H_3$]-ethyl 2-methylbutanoate,[23] [$^2H_3$]-vanillin,[24] [$^2H_3$]-ethyl hexanoate,[25] [$^2H_4$]-nonanal,[26] [$^2H_2$]-butanoic acid,[27] [$^2H_3$]-β-ionone,[28] [$^{13}C_4$]-2,3-butanedione,[29] [$^2H_2$]-ethyl butanoate,[30] [$^2H_3$]-2-methoxy-4-vinyl-phenol,[31] [$^2H_4$]-(*Z*)-3-hexenal[32] and [$^2H_4$]-hexanal.[33]

Most of the syntheses were performed on a micro scale basis. Thus, concentrations were determined by GC/FID as follows: An FID-response factor was calculated by analyzing defined amounts of the respective unlabeled compound and methyl octanoate as internal standard. Using the same internal standard and the FID response factor determined with the unlabeled compound, the concentration of the labeled compound was calculated on the basis of a defined volume of the respective stock solution.

[$^{13}C_2$]-Acetaldehyde, [$^2H_3$]-acetic acid, [$^{13}C_8$]-octanoic acid and [$^2H_2$]-1-penten-3-ol were obtained from Sigma-Aldrich (Taufkirchen, Germany).

Syntheses.

Synthesis of [$^2H_2$]-1-penten-3-one. To obtain the target compound [$^2H_2$]-1-penten-3-ol was oxidized with freshly prepared Dess Martin periodinane [1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3-(1*H*) one].

Preparation of Dess Martin periodinane.[34] 2-Iodobenzoic acid (40 mmol) and potassium peroxomonosulfate (60 mmol) were suspended at 70 °C in distilled water (300 mL) and stirred for 3 h. After cooling to 5 °C, the reaction mixture was stirred for another 90 min. The white crystals formed were filtered off, washed with distilled water (240 mL) followed by acetone (80 mL) and finally dried in a stream of nitrogen. A

mixture of the reaction product obtained (35 mmol), was refluxed with acetic acid anhydride (50 mL) and p-toluene sulfonic acid (0.6 mmol) for 2 h at 80 °C. After cooling to 0 °C, the crystals formed were filtered off and washed with diethyl ether (50 mL). Dess Martin periodinane was stored under argon prior to use.

Oxidation of [$^{2}H_{2}$]-1-penten-3-ol. The alcohol (2.85 mmol in 10 mL anhydrous dichloromethane) was added within 10 min to a solution of Dess Martin periodinane (3.42 mmol in 10 mL anhydrous dichloromethane), and the solution was stirred for 1 h at room temperature. Diethyl ether (50 mL) and an aqueous sodium thiosulfate-solution (100 mL; 1 mol/L, saturated with sodium hydrogen carbonate) was added and the reaction mixture was stirred for 10 min. The aqueous layer was separated and extracted with diethyl ether (total volume: 90 mL). The combined organic layers were subsequently washed with sodium thiosulfate (50 mL; 1 mol/L, saturated with sodium hydrogen carbonate), an aqueous saturated sodium hydrogen carbonate solution (100 mL) and finally with distilled water (100 mL). The solution was then dried over sodium sulfate and the target compound was characterized by mass spectrometry.

MS (EI): *m/z* (%): 57 (100), 56 (92), 86 (83), 58 (50), 85 (46), 82 (21), 48 (15).

MS (CI): 87 (M+1, 100).

Synthesis of [$^{13}C_{8}$]-octanal. The compound was prepared by reduction of $^{13}C_{8}$-octanoic acid to [$^{13}C_{8}$]-Octanol followed by an oxidation into the target compound using Dess Martin periodinane.

Synthesis of [$^{13}C_{8}$]-octanol. [$^{13}C_{8}$]-octanoic acid (0.7 mmol in 20 mL diethyl ether) was slowly added to a solution of lithium aluminium hydride (0.6 mmol in 50 mL diethyl ether) and the mixture was refluxed for 1 h. Ice water was slowly added until the hydrogen formation stopped, and sulfuric acid (10%) was added dropwise to dissolve the aluminium hydroxide formed. The aqueous layer was extracted with diethyl ether

(150 mL), the combined organic layers were washed with an aqueous saturated sodium chloride solution (50 mL) and finally dried over sodium sulfate.

Synthesis of [$^{13}C_8$]-octanal. The alcohol was oxidized with Dess Martin periodinane as described above for 1-penten-3-ol. To remove impurities, the aldehyde was purified by chromatography on silica gel using an n-pentane/diethyl ether gradient. The [$^{13}C_8$]-octanal was eluted with n-pentane/diethyl ether (9:1; 100 mL) and was characterized by mass spectrometry.

MS (EI): *m/z* (%): 49 (100), 44 (68), 60 (66), 42 (33), 59 (30), 90 (16), 74 (15), 106 (4), 118 (2).

MS (CI): 136 (M+1, 100).

Isolation of the Volatiles. Orange juice (100 mL) was extracted with diethyl ether (total volume: 200 mL) by vigorous stirring for 2 h, and the volatiles were isolated by solvent assisted flavor evaporation (SAFE).[35] The distillate was separated into an acidic fraction (AF) and a fraction containing the neutral/basic volatiles (NBF).[36] Each fraction was concentrated at 40 °C to obtain a final volume of about 250 µL using a Vigreux column (50 cm x 1.5 cm i.d.), and a micro distillation device.[37]

High Resolution Gas Chromatography/Olfactometry (HRGC/O), Aroma Extract Dilution Analysis (AEDA) and High Resolution Gas Chromatography/Mass Spectrometry (HRGC/MS). The SAFE distillates were injected by the cold on column injector onto a GC column installed in a Carlo Erba Instruments gas chromatograph HRGC 8000 (Hofheim, Germany). The volatiles were separated with a helium flow rate of 1.9 mL per min on a J&W Scientific DB-FFAP column (30 m x 0.32 mm i.d., 0.25 µm film thickness) (Folsom, USA) using the following parameters: 2 min held at 40 °C, then increased at 20 °C/min to 60 °C; held 2 min at 60 °C, then increased at 6 °C/min to 180 °C. Finally, the temperature was increased to 230 °C and held for 5 min. HRGC-O was performed by dividing the effluent 1:1 at the end of the capillary

177 column using a Y-shaped glass splitter (Chromatographie Handel Müller, Germany)
178 and two deactivated, non-coated fused-silica capillaries of 50 cm each. One was
179 connected with the flame ionization detector (250 °C) and the other with the sniffing
180 port (190 °C). The extract was diluted 1:1 until no odor-active compound could be
181 smelled. Thus, the flavour dilution factor indicates the last dilution in which an odorant
182 could be smelled.

183 The parameters used for the second column, a J&W Scientific DB 5 (30 m x 0.32
184 mm i.d., 0.25 µm film thickness) (Folsom, USA) were as follows: 2 min held at 40 °C,
185 then increased at 9 °C/min to 50 °C; held for 2 min and increased at 6 °C/min to 180
186 °C, Finally the temperature was increased at 9 °C/min to 240 °C and held for 5 min.

187 Linear retention indices (RI) of the compounds were calculated using a series of n-
188 alkanes (C6−C26 for DB-FFAP and C6−C18 for DB-5) as previously described.[36]

189 HRGC/MS was performed by means of a Hewlett-Packard gas chromatograph
190 5890 series II (Waldbronn, Germany) connected to a Finnigan sector field mass
191 spectrometer type MAT 95 S (Bremen, Germany) using the capillaries described
192 above. Mass spectra were generated in the electron impact mode (MS-EI) at 70 eV
193 and in the chemical ionization mode (MS-CI) at 115 V using isobutane as the reac-
194 tant gas.

195 **Quantitation by Stable Isotope Dilution Assays.**

196 **Gas Chromatography/Ion Trap Mass Spectrometry (GC/ITMS).** Orange juice (5
197 to 1000 mL, depending on the concentration of the respective odorant determined in
198 preliminary experiments) was spiked with the respective isotopically labeled internal
199 standards either dissolved n-pentane or diethyl ether. The amount of the standard
200 was chosen in a similar concentration as the analyte, and the samples were equili-
201 brated for 30 min with stirring. After extraction with dichloromethane (10 to 1000 mL,
202 depending on the initial volume of the juice used), the volatiles and the labeled inter-

nal standards were isolated by SAFE distillation.[35] After drying over sodium sulfate and concentration to about 200 uL, the resulting solutions were analyzed by GC/MS. Quantitation of major compounds was performed using a Varian 431 gas chromatograph (Darmstadt, Germany) equipped with the DB-FFAP column coupled to a Varian mass spectrometer 220 (Darmstadt, Germany). Mass spectra were generated by MS-CI using methanol as the reactant gas.

Two dimensional High Resolution Gas Chromatography-Gas Chromatography-Ion Trap-Mass Spectrometry (GCxGC/ITMS). For the quantitation of trace aroma compounds, two dimensional GCxGC/mass spectrometry was applied, using a Thermo Finnigan Trace 2000 series gas chromatograph (Braunschweig, Germany) equipped with a Fison Instruments moving capillary stream switching system (MCSS) (Mainz-Kastel, Germany) and linked to a Varian CP 3800 gas chromatograph and a Varian Saturn 2000 ion trap mass spectrometer (Darmstadt, Germany).

The samples were injected by the cold-on-column technique and after chromatography on the first column (DB-FFAP), the odorant and the respective internal standard were cut out by means of the MCSS and transferred to the second column via a heated transfer line. The effluent was condensed in a cold trap before the separation was continued on the second column (DB-1701). The effluent was monitored by means of the ion trap mass spectrometer in the CI-mode with methanol as reactant gas. The heart cut time in the first dimension was determined using the respective reference compound.[38] Response factors (R_f) were determined by analyzing mixtures containing known amounts of the unlabeled target compound and the respective isotopically labeled internal standard in five different ratios (5:1, 3:1, 1:1, 1:3, and 1:5) by either GC-MS or GC×GC/MS.

Quantitation of Acetaldehyde. Acetaldehyde was quantitated using an enzymatic assay (Boehringer Mannheim/ R-Biopharm AG, Darmstadt, Germany) with photomet-

ric monitoring. The workup procedure and photometric measurements were carried out according to the instruction manual using a 0.5 mL solution. To get rid of color and proteins, orange juice (16g) was clarified with Carrez-I-solution (1 mL; potassium ferrocyanide, 85 mM = 3.6 g $K_4[Fe(CN)_6]$ x 3H2O / 100 mL) and Carrez-II-solution (1 mL; zinc sulfate, 250 mM = 7.20 g $ZnSO_4$ x 7 H2O / 100 mL) and the samples were filled up in a 20 mL volumetric flask. The assay was calibrated with freshly distilled acetaldehyde.

Quantitation of Terpene Hydrocarbons. The quantitation of the major terpene hydrocarbons limonene, myrcene and α-pinene was carried out by HRGC-FID using *trans*-p-menth-2-ene as the internal standard. For the quantitation of myrcene and α-pinene 50 g of juice were used. The concentration of *trans*-p-menth-2-ene was chosen between the expected concentrations of myrcene and of α-pinene. For limonene, 10 g juice were used. Extraction and SAFE distillation was done as described above. The terpene hydrocarbons were enriched by silica column chromatography as follows: The concentrated distillate (1 mL) was placed on top of the glass column filled with silica gel column in n-pentane, and the terpene hydrocarbons were eluted using n-pentane. The solution was concentrated to 200 µL and subjected to HRGC-FID. Response factors were calculated from mixtures of known concentrations of *trans*-p-menth-2-ene, limonene, myrcene and α-pinene.

Sensory Evaluation. *Aroma Profile Analysis.* Sensory analyses were carried out in a sensory room designed for this purpose with individual sections for each panelist. The room temperature was adjusted to 20 – 25°C, and to avoid any influence of colored solutions, the evaluations were carried out in yellow tainted light. 20 panelists were recruited from the German Research Center for Food Chemistry (Freising, Germany), who were regularly trained in orthonasal odor perception.[39] The panelists were asked to evaluate the intensity of the following odor attributes: pungent (1-

penten-3-one), green/grassy ((*Z*)-3-hexenal), orange/green (octanal), vanilla-like (vanillin), citrus-like/flowery (linalool), clove-like (2-methoxy-4-vinylphenol), fruity (ethyl butanoate), caramel-like (4-hydroxy-2,5-dimethyl-3(2*H*)-furanone), sour (acetic acid), cabbage-like (dimethyl sulfide) and fresh (acetaldehyde) on a linear scale from 0 (not perceivable) to 3 (strongly perceivable) in steps of 0.5 units. The attributes were defined in a first session with the panelists as most relevant to describe the overall aroma of orange juice. For the subsequent aroma profile analysis reference solutions of the aroma compounds given in parentheses in 20 – 40-fold threshold concentration (in water) were presented for each attribute. Samples (15 mL) were presented in covered glass vessels (i.d. = 40 mm, total volume = 45 mL) at room temperature, and the results obtained in two sessions were averaged and plotted in a spider web diagram.

Triangle Test. In order to provide information on the differentiation of sensory properties of samples, triangle tests were designed and interpreted according to the method ISO 4120:2004.

Determination of Odor Thresholds. Odor thresholds were determined in tap water as recently described.[39] Because of its pH dependent molecular properties, the orthonasal odor threshold of acetic acid was determined in an orange juice model matrix, consisting of an aqueous solution of 4.5 % sucrose, 2% glucose, 2 % fructose, 1.1 % citric acid, 0.5 % ascorbic acid and 0.1 % sunflower oil (the pH was adjusted to 3.6).

Odor Activity Values. These were calculated as previously described.[38]

Results and Discussion

Aroma Profile of unstored NFC juice from Hamlin oranges. First, the overall aroma of the unstored juice was evaluated by an aroma profile analysis using 11 odor attributes agreed upon by the sensory panel in preliminary sessions. The evaluation of the intensity of each attribute was trained using solutions of single odorants representing the respective odors in a concentration by factors of approximately 50 above their odor threshold. The major odor attributes described in the unstored Hamlin orange juice were fresh with an intensity of 2.3 (Figure 1), followed by orange/green (1.8), pungent (1.7), fruity (1.6) and green/grassy (1.5). The other six attributes citrus/flowery, sour, caramel-like, clove-like, vanilla-like and cabbage-like were ranked with a lower intensity.

Identification of Aroma-Active Compounds in Hamlin orange juice. To elucidate the compounds contributing to the overall aroma of the unstored Hamlin juice, an aroma extract dilution analysis (AEDA) was carried out on the entire set of volatiles isolated by extraction and SAFE distillation. A total of 51 aroma-active regions could be located in the FD factor range of 8 to 8192 (Figure 2) among which two compounds with a vanilla-like (**51**) and a coconut-like odor attribute (**45**) showed the highest FD factors of 8192, followed by two compounds with a flowery (**26**) and a smoky odor (**47**). Further compounds with a high FD factor were **3** (fir needle -like), **7** (green), **10** (citrus-like), **21** (sour), **36** (deep-fried), **41** (metallic) and **42** (caramel-like). A comparison of the retention indices, mass spectra and perceived odors (quality and intensity) with the institute's database containing analytical and sensory data of approximately 1000 aroma compounds resulted in proposals for the chemical structures. These were confirmed by the analysis of reference compounds.

The results of the identification experiments in combination with the FD factors showed that vanillin (**51;** Figure 3), 3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3*H*)-

benzofuranone (winelactone) (**45**) followed by (R)-linalool (**26**) caused the respective odors of the odor-active areas detected by AEDA (Figure 2). The enantiomeric purity of linalool was higher than 95% (data not shown). Further odorants with high FD factors were α-pinene (**3**), hexanal (**7**), (R)-limonene (**10**), acetic acid (**21**), (*E,E*)-2,4-decadienal (**35**), *trans*-epoxy-(*E*)-2-decenal (**40**) and 4-hydroxy-2,5-dimethyl-3(2*H*)furanone (**41**). The results of the further identification experiments (Table 1) showed that 45 out of the 51 odorants detected by GC/Olfactometry could be identified. In general, the results confirmed data of previous studies[5,9,12,13,15,40-50] aimed at identifying either volatile or odor-active compounds in orange juices of different origins and varieties. However, 2-ethyl-3- and 2-ethyl-5-methylpyrazine (**16**) and 3-hydroxy-4,5-dimethyl-2(5*H*) furanone (**46**) are reported here as additional odorants in orange juice, although both showed only low FD factors.

Influence of a chilled storage To elucidate changes occurring during chilled storage of the Hamlin juice, its aroma profile was determined after 1, 2 and 10 months of storage (Figure 4). A comparison with the profile of the unstored juice (black line in Figure 4) indicated that in particular the freshness of the juice was decreased during storage and also the pungent and green, grassy odor attributes were clearly lower in the stored juices. This loss in odor intensity was already detectable after 1 month of storage (dark green line in Figure 4).

To clarify the odorants responsible for these differences, the AEDA was applied on a SAFE distillate obtained from the 10 months stored juice (10m; Table 2). A comparison with the results obtained for the unstored juice (Ust; Table 2) revealed that the majority of the 51 odorants was qualitatively identical in both samples. But, 4 compounds were no longer detectable by GC/O in the stored juice, i.e., 1-penten-3-one (**4**), (*Z*)-3-hexenal (**8**), (*E*,*Z*)-2,6-nonadienal (**28**) and 3-hydroxy-4,5-dimethyl-2(5*H*) furanone (**46**). Because differences of two or one dilution step lie within the error

range of the AEDA, only the differences in FD factors determined for the following six compounds may give additional hints, which odorant loss may contribute to the aroma differences detected in the aroma profiles: hexanal (**7**), octanal (**14**), nonanal (**18**), (*E,E*)-2,4-decadienal (**35**), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**41**) and vanillin (**51**). On the other hand, the FD factor of only one compound, namely 2-methoxy-4-vinylphenol (**44**) was higher in the stored juice compared to the unstored juice (Table 2).

Quantitative Analysis. To get nearer to the reason for the aroma differences in the juice, quantitative data were correlated with the odor thresholds of the odorants by calculating odor activity values (ratio of concentration to odor threshold). Thus, first a quantitative analysis of aroma compounds showing clear differences in their FD factors as well as of compounds proposed in the previous literature as markers for off-flavor in orange juices was performed mainly by means of stable isotope dilution assays.

In the unstored juice, (R)-limonene showed by far the highest concentration of about 121 mg/kg; followed by acetaldehyde with 16.1 mg/kg, acetic acid with 5.5 mg/kg and myrcene with 1.5 mg/kg (Table 3). Moreover, α-pinene, linalool, hexanal, ethyl butanoate, decanal, octanal and nonanal were found in concentrations between 99.6 and 757 µg/kg. 1-Penten-3-one, vanillin, (*Z*)-3-hexenal and 2-methoxy-4-vinylphenol were measured in a lower concentration range between 0.74 and 35 µg/kg.

Compared to the unstored juice, already after one month the concentrations of acetaldehyde and of (*Z*)-3-hexenal decreased significantly (Table 3), and 1-penten-3-one was decomposed to about one tenth of its initial concentration. It should, however, be mentioned that (*Z*)-3-hexenal is not stable during a freezing/thawing process.[2,51] So, its initial concentration in the unstored juice was probably much higher.

Additionally, hexanal, nonanal and decanal decreased in their concentrations. On the other hand, acetic acid and 2-methoxy-4-vinylphenol showed an increase already after 1 month (Table 3). Monitoring the changes in concentrations of the 15 aroma compounds after ten months indicated a continuous loss of (R)-limonene, myrcene, a-pinene, (*Z*)-3-hexenal as well as of hexanal, octanal, nonanal and decanal. In particular, 1-penten-3-one and acetaldehyde fell below their detection threshold already after two months of chilled storage. While e.g., (R)-linalool and ethyl butanoate were quite stable, 3 compounds increased after ten months, namely acetic acid, 2-methoxy-4-vinylphenol and vanillin (Table 3).

To confirm the trend in the different stabilities of the juice odorants, the storage trial was repeated using NFC juice from Hamlin oranges of the following harvest year. In this trial, further 6 compounds were added to the set of 15 aroma compounds analyzed in the first year: ethyl hexanoate, (*E*,*E*)-2,4-decadienal, β-ionone, α-terpineol and carvone The latter two odorants have previously been suggested as off-flavor compounds in orange juice.[7,9,14] The same trends in the stability of the odorants were observed (Table 4). Besides ethyl butanoate, also further two esters, (S)-ethyl 2-methylbutanoate and ethyl hexanoate, were quite stable during storage for six months, and the same was true for (R)-linalool. However, 1-penten-3-one and acetaldehyde rapidly disappeared during storage as already observed for the juice from the year before. In addition, (*Z*)-3-hexenal and (*E,E*) 2,4-decadienal as well as all saturated aldehydes were clearly reduced during storage. But, the previously suggested off-flavor compounds in orange juice, carvone and vanillin, did not show an increase compared to the unstored juice. But, the concentration of α-terpineol increased from 183 µg/kg to nearly the ten-fold amount of 1100 µg/kg.

As a next step, a calculation of odor activity values was done on the unstored Hamlin juice as well as on the five stored samples (Table 4 and Table 5). In the un-

stored juice, (R)-limonene, (R)-linalool, myrcene and acetaldehyde followed by ethyl butanoate showed the highest odor activity values. Neither α-terpineol, carvone and vanillin nor 2-methoxy-4-vinylphenol were present above their odor threshold, while acetic acid was already present with an OAV of 8.9 already in the unstored juice. A comparison of the OAVs in the unstored juice and the 6 months stored juice clearly indicated that in particular 1-penten-3-one and acetaldehyde fell below their odor threshold after 6 months and, are, thus, lost as contributors to the overall aroma profile. A reduced effect in the aroma contribution indicated by lower OAVs also occurred for hexanal, octanal, nonanal, decanal, (*Z*)-3-hexanal and (*E*,*E*)-2,4-decadienal.

Aroma-Active Compounds in NFC Valencia Orange Juice.

To indicate whether similar aroma losses also occur in juices from other varieties, the same series of experiments was done on NFC juice from Valencia oranges. The study started with the aroma profile analysis of the unstored juice and a juice stored for 10 months (Figure 5). The most distinct aroma impression in the unstored juice was orange/green" (2.0) and "fresh" (1.7), followed by the attributes "pungent" (1.4), "fruity" (1.5) and "citrus/flowery" (1.4). The qualities "green/grassy" (1.3), "sour" (1.1) and "caramel-like" (1.0) were rated less intense, while the odor impressions "clove-like" (0.7), "vanilla-like" (0.5) and "cabbage-like" (0.2) showed a low impact on the overall aroma of this juice. In the 10 months stored juice, the most obvious loss of intensity was detected for the odor quality orange/green. The changes were similar compared to those observed for the Hamlin juice (Figure 4).

An aroma extract dilution analysis applied on the unstored NFC juice from Valencia oranges followed by identification experiments revealed the same odor-active compounds in the Valencia NFC juice as in the Hamlin juice, although the FD factors were different (see supplementary information) pointing to differences in the amounts

of several odorants between both unstored juices. Furthermore, an AEDA applied on the stored Valencia juice also resulted in changes for the same aroma compounds (data not shown). Therefore, the same set of aroma compounds as analyzed in the Hamlin juices was also quantitated in the unstored and the stored Valencia juice (Table 6). In the unstored juice, (R)-limonene, acetic acid and acetaldehyde followed by linalool and myrcene showed the highest concentrations. Compared to the unstored juice from the Hamlin oranges, in particular the concentrations of (R)-limonene, linalool as well as octanal, nonanal and decanal showed higher concentrations in the Valencia juice (compare data in Tables 4 and 6). These differences might be the reason for a more intense orange-like/green note in the overall aroma profile of the Valencia juice (Figure 1 and Figure 5).

When the stability of the compounds during chilled storage was monitored, the same trends as for Hamlin orange juice were observed (Table 6). For example, 1-penten-3-one and acetaldehyde showed a very rapid decrease and all unsaturated and saturated aldehydes were significantly degraded during storage. On the other hand, (R)-linalool and all esters were stable, and besides vanillin, in particular acetic acid, α-terpineol as well as 2-methoxy-4-vinylphenol increased in concentration. The calculation of odor activity values revealed (R)-linalool followed by (R)-limonene and myrcene with the highest odor activities followed by acetaldehyde and ethyl butanoate in the unstored NFC juice (Table 7). Except acetaldehyde, which was below its odor threshold in the 10 months stored juice, the other 4 compounds still showed the highest odor activity after a 10 months storage. However, significant losses in odor activity were measured for (*E*,*E*)-2,4-decadienal, hexanal, octanal, nonanal and decanal. But, also in the NFC Valencia juice neither 2-methoxy-4-vinylphenol, vanillin and α-terpineol nor carvone were found as contributors to the changes in the overall aroma, i.e. as off-flavor compounds. By application of the same concept on a Valen-

cia NFC juice from a second harvest year nearly identical results were obtained (data not shown).

Sensory experiments on odorant contribution

During storage of the Hamlin juice, exclusively a degradation of odorants was detected, but no significant increase in any odor active compound was observed. Thus, spiking a stored juice with the lost amounts of the odorants should bring back the aroma profile of the unstored juice. Thus, the following sensory experiments were carried out to study this assumption (Figure 6). In a six months stored juice the concentrations of 12 aroma compounds were made up to their initial concentrations determined in the unstored juice (Table 8). While the six months stored juice and the unstored juice were clearly distinguishable by their overall aroma profiles (data not shown), the spiked juice could not be distinguished from the unstored juice (Table 8) (α= 0.2). In a second set of experiments, aroma compounds were grouped according to three aroma impressions, namely “green”, “fresh/ pungent”, and “citrus/ orange-like” and subgroups correlating with this odor attribute were separately administered to the six months stored juice. The stored juice spiked with the two “green” compounds (*Z*)-3-hexenal and hexanal was clearly differentiated (α= 0.01) (Table 8) from the unstored juice. However, after administering only the “fresh, pungent” compounds 1-penten-3-one and acetaldehyde, the panel could not differentiate between the spiked and the unstored sample (α= 0.2). In addition, also spiking 4 aldehydes, namely octanal, nonanal, decanal and (*E*,*E*)-2,4-decadienal showed a positive effect on the aroma of the stored juice. Also this partial recombinate could no longer be distinguished from the unstored juice (α= 0.2). These results corroborate the importance of 1-penten-3-one as well as acetaldehyde, octanal, nonanal, decanal and (*E*,*E*)-2,4-decadienal for the overall aroma of orange juice.

With respect to fresh orange juice, these results confirm data of previous investigations from our working group on fresh orange juices[2,5,6,15] suggesting that besides esters, such as ethyl butanoate, and terpenoids like (R)-limonene, several compounds known as degradation products of lipid hydroperoxides, such as 1-octen-3-one, (*Z*)-3-hexenal and (*E*,*E*)-2,4-decadienal and saturated aldehydes are important contributors to the aroma of unstored (fresh) orange juices. In addition, 1-penten-3-one and acetaldehyde were established as an important key odorant in NFC orange juices. The formation of 1-penten-3-one can be assumed from the 15-hydroperoxide of linolenic acid by a radical mechanism (Figure 7) which is similar to a mechanism proposed for the formation of 1-octen-3-one from linoleic acid in mushrooms.[52] However, in particular the oxidation of the intermediate alcohol to the ketone has not yet been proven in both cases. Thus, it might be assumed that 1-penten-3-one is enzymatically formed in the orange fruit.

Furthermore, while previous investigations[15,16] focused on the storage of juice made from concentrate at room temperature or even under forced conditions, these investigations focused for the first time on changes of the aroma compounds of NFC orange juice during chilled storage, which is common in the juice industry. Our results clearly show that despite these gentle storage conditions, changes in the overall aroma profiles of juices from two orange varieties occurred leading to a decrease in the intensity of several aroma attributes compared to the unstored juice. However, an important role of previously suggested off-flavor compounds in orange juice, such as carvone, 2-methoxy-4-vinylphenol or vanillin for changes in the overall juice aroma could be ruled out for such chilled stored juices.

But, as confirmed by sensory experiments, the rapid decomposition of 1-penten-3-one and acetaldehyde are particularly involved in the loss of the typical aroma of the unstored juice. In addition, the continuous degradation of (*Z*)-3-hexenal, hexanal, oc-

tanal, nonanal, decanal, and (*E,E*)-2,4-decadienal for sure contribute to the overall changes in juice aroma. However, neither the pathways for the decomposition of the 1-penten-3-one nor for the losses of the aldehydes are yet known. This challenge will be addressed in further investigations.

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SUPPORTING INFORMATION

Table S1. Odorants, isotopically labelled standards, response factors and mass traces used for the quantitation of aroma compounds in orange juices

Table S2. Most odor-active volatiles (FD ≥ 16) in the unstored NFC juice from Valencia oranges

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FIGURE CAPTIONS

Figure 1. Aroma profile of the unstored NFC juice from Hamlin oranges.

Figure 2 Flavor dilution (FD) chromatogram obtained by application of the aroma extract dilution analysis on the volatiles isolated from unstored NFC juice from Hamlin oranges.

Figure 3. Structures of the aroma active compounds identified with the highest flavor dilution factors in NFC juice from Hamlin oranges. Numbering refers to Table 1.

Figure 4. Comparative aroma profile of the unstored NFC juice (black), the 1 month stored juice (dark green), the 2 months stored juice (light green) and the 10 months stored juice (orange) prepared from Hamlin oranges.

Figure 5. Aroma profile of the unstored NFC juice from Valencia oranges (green) and the 10 months stored juice (blue)

Figure 6. Triangle test designed for the spiking experiments of the 6 months stored Hamlin orange juice (6 m) in comparison to the unstored juice (Ust).

Figure 7. Hypothetical pathway leading to the formation of 1-penten-3-one by a degradation of the 15-hydroperoxide of linolenic acid.

Table 1. Most odor-active volatiles (FD ≥ 8) in the unstored NFC juice from Hamlin oranges

No.[a]	Odorant[b]	Odor quality[c]	Fraction[g]	RI on[d] FFAP	RI on[d] DB-5	FD factor[e]	Literature[h]
1	ethyl propanoate	fruity, glue-like	NBF 3	950	713	8	40
2	2,3-butanedione	butter-like	NBF 3	972	607	32	41
3	α-pinene	resin-like, fir needle-like	NBF 1	1011	933	512	42
4	1-penten-3-one	pungent, train oil-like	NBF 3	1024	682	32	40
5	ethyl butanoate	fruity	NBF 2	1029	801	256	43
6	(*S*)-ethyl 2-methylbutanoate	fruity	NBF 2	1040	845	8	44
7	hexanal	green, grassy	NBF 3	1074	799	512	9
8	(*Z*)-3-hexenal	green, grassy	NBF 3	1100	810	16	45
9	myrcene	geranium-like, carrot-like	NBF 1	1160	991	128	9
10	(*R*)-limonene	citrus-like, carrot-like	NBF 1	1198	1028	512	9
11	1,8-cineol	eucalyptus-like	NBF 3	1209	1035	64	46
12	ethyl hexanoate	fruity, pineapple-like	NBF 2	1229	999	64	47
13	2-hydroxy-3-butanone	butter-like, carrot-like	NBF 3	1273	800	64	12
14	octanal	citrus-like, green	NBF 3	1282	1003	256	9
15	1-octen-3-one [f]	mushroom-like	NBF 3	1291	976	64	5
16	2-ethyl-3- and 2-ethyl-5-methylpyrazine [f]	earthy	NBF 3	1361	1000	64	-

Table 1. Continued

No.[a]	Odorant[b]	Odor quality[c]	Fraction [g]	RI on[d] FFAP	RI on[d] DB-5	FD[e]	Literature[h]
17	(*Z*)-1,5-octadien-3-one	geranium-like, metallic	NBF 3	1365	983	16	[5]
18	nonanal	citrus-like, soapy	NBF 3	1373	1103	128	[43]
19	3-isopropyl-2-methoxypyrazine [f]	earthy, pea-like	NBF 3	1420	1094	128	[5]
20	unknown	flowery, citrus	NBF 3	1429	-	16	-
21	acetic acid	vinegar-like	AF	1437	652	512	[9]
22	methional[f]	cooked potato-like	NBF 3	1449	903	64	[5]
23	decanal	citrus-like, soapy	NBF 3	1488	1205	32	[9]
24	2-isobutyl-3-methoxypyrazine	bell pepper-like, earthy	NBF 3	1510	1094	64	[48]
25	unknown	pungent	NBF 3	1514	-	128	-
26	(*R*)-linalool	citrus-like, flowery	NBF 4	1531	1102	2048	[9]
27	unknown	coconut-like	NBF 3	1537	-	16	-
28	(*E,Z*)-2,6-nonadienal [f]	cucumber-like	NBF 3	1567	1153	32	[5]
29	butanoic acid	sweaty	AF	1620	814	16	[49]
30	2- and 3-methylbutanoic acid	sweaty	AF	1667	850	64	[5]
31	ethyl 3-hydroxyhexanoate	fruity	NBF 5	1670	1120	128	[47]
32	α-terpineol	flowery, citrus-like	NBF 3	1691	1192	64	[9]

Table 1. Continued

No.[a]	Odorant[b]	Odor quality[c]	Fraction[g]	RI on[d] FFAP	RI on[d] DB-5	FD[e]	Literature[h]
33	valencene	fatty, woody	NBF 3	1700	-	8	12
34	carvone	caraway-like	NBF 3	1720	1243	64	9
35	(*E,E*)-2,4-decadienal	fatty, deep-fried	NBF 3	1805	1318	512	19
36	(*E*)-β-damascenone [f]	baked apple-like	NBF 2	1811	1385	32	15
37	hexanoic acid	sweaty	AF	1847	994	32	49
38	β-ionone	flowery, violet-like	NBF 3	1931	1468	64	12
39	unknown	fatty, spicy, smoky	NBF 3	1979	-	16	-
40	*trans*-4,5-epoxy-(*E*)-2-decenal	metallic	NBF 3	1992	1382	512	5
41	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like	AF	2026	1071	512	13
42	unknown	metallic	NBF 3	2032	-	64	-
43	4-methylphenol	fecal, horse stable-like	NBF 5	2074	1077	16	15
44	2-methoxy-4-vinylphenol	smoky, clove-like	NBF 5	2197	1314	8	50
45	wine lactone [f]	coconut-like, dill-like	NBF 3	2233	1455	8192	5
46	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone [f]	seasoning-like, spicy	NBF 5	2274	-	32	-
47	unknown	sweet, smoky	NBF 5	2360	-	4096	-
48	unknown	soapy, olibanum-like	NBF 5	2430	-	256	-

Table 1. Continued

No.[a)]	Odorant[b)]	Odor quality[c)]	Fraction[g)]	RI on[d)] FFAP	RI on[d)] DB-5	FD[e)]	Literature[h)]
49	nootkatone	grapefruit-like, soapy	NBF 3	2526	1814	64	12
50	phenyl acetic acid	honey-like, beeswax-like	AF	2557	1275	128	5
51	vanillin	vanilla-like, sweet	AF	2578	1399	8192	5

[a)] The odorants detected during aroma extract dilution analysis (AEDA) were numbered according to their elution order from the FFAP stationary phase. [b)] The odorant was identified by comparing it with the reference substance on the basis of the following criteria: retention index (RI) on a FFAP and on a DB-5 capillary column, mass spectra obtained by MS (EI) and odor quality perceived at the sniffing port. [c)] Odor quality perceived at the sniffing port. [d)] RI: Retention index [e)] FD: Flavor dilution factor. [f)] The MS signals were too weak for an unequivocal interpretation. The compound was identified on the basis of the remaining criteria given in footnote [b)]. [g)] Fraction in with an odor-active compound was eluted from the gc column. [h)] Literature on the first report on the presence of the volatile in an orange juice.

Table 2. Comparison of the most odor-active volatiles (FD ≥ 16) in the unstored (USt) and the 10 months (10 m) stored Hamlin orange juice

			FD factor in a distillate from[d]	
No.[a)]	Odorant[b)]	Odor quality[c)]	USt	10 m
1	ethyl propanoate	fruity, glue-like	8	16
2	2,3-butanedione	butter-like	32	64
3	α-pinene	resin-like, fir needle-like	512	256
4	1-penten-3-one	pungent, train oil-like	32	<1
5	ethyl butanoate	fruity	256	256
6	(*S*)-ethyl 2-methylbutanoate	fruity	8	16
7	hexanal	green, grassy	512	64
8	(*Z*)-3-hexenal	green, grassy	16	<1
9	myrcene	geranium-like, carrot-like	128	64
10	(*R*)-limonene	citrus-like, carrot-like	512	256
11	1,8-cineol	eucalyptus-like	64	32
12	ethyl hexanoate	fruity, pineapple-like	64	128
13	2-hydroxy-3-butanone	butter-like, carrot-like	64	64
14	octanal	citrus-like, green	256	64
15	1-octen-3-one	mushroom-like	64	32
16	2-ethyl-3- and 2-ethyl-5-methylpyrazine[e)]	earthy	64	32
17	(*Z*)-1,5-octadien-3-one	geranium-like, metallic	16	32
18	nonanal	citrus-like, soapy	128	32
19	3-isopropyl-2-methox-ypyrazine[e)]	earthy, pea-like	128	256
20	unknown	flowery, citrus	16	32
21	acetic acid	vinegar-like	512	1024
22	methional[e)]	cooked potato-like	64	64
23	decanal	citrus-like, soapy	32	16
24	2-isobutyl-3-methoxypyrazine	bell pepper-like, earthy	64	16
25	unknown	pungent	128	64
26	(*R*)-linalool	citrus-like, flowery	2048	1024

Table 2. Continued

No.[a)]	Odorant[b)]	Odor quality[c)]	FD factor in a distillate from[d]	
			USt	10 m
27	unknown	coconut-like	16	16
28	(*E,Z*)-2,6-nonadienal[e)]	cucumber-like	32	<1
29	butanoic acid	sweaty	16	32
30	2- and 3-methylbutanoic acid	sweaty	64	128
31	ethyl 3-hydroxyhexanoate	fruity	128	32
32	α-terpineol	flowery, citrus-like	64	64
33	valencene	fatty, woody	8	16
34	carvone	caraway-like	64	64
35	(*E,E*)-2,4-decadienal	fatty, deep-fried	512	32
36	(*E*)-β-damascenone[e)]	baked apple-like	32	4
37	hexanoic acid	sweaty	32	4
38	β-ionone	flowery, violet-like	64	128
39	unknown	fatty, spicy, smoky	16	32
40	*trans*-4,5-epoxy-(*E*)-2-decenal	metallic	512	64
41	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like	512	128
42	unknown	metallic	64	16
43	4-methylphenol	fecal, horse-stable-like	16	16
44	2-methoxy-4-vinylphenol	smoky, clove-like	8	256
45	winelactone[e)]	coconut-like, dill-like	8192	4096
46	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone[e)]	seasoning-like, spicy	32	<1
47	unknown	sweet, smoky	4096	256
48	unknown	soapy, olibanum-like	256	128
49	nootkatone	grapefruit-like, soapy	64	128
50	phenylacetic acid	honey-like, beeswax-like	128	256
51	vanillin	vanilla-like, sweet	8192	2048

[a)] The odorants detected during aroma extract dilution analysis (AEDA) were numbered according to their elution order from the FFAP stationary phase. [b)] The odorant was identified

by comparing it with the reference substance on the basis of the following criteria: retention index (RI) on a FFAP and on a DB-5 capillary column, mass spectra obtained by MS (EI) and odor quality perceived at the sniffing port. [c)] Odor quality perceived at the sniffing port. [d)] FD: Flavor dilution factor. [e)] The MS signals were too weak for an unequivocal interpretation. The compound was identified on the basis of the remaining criteria given in footnote b.

Table 3. Changes in the concentrations of 15 odor-active compounds in NFC juice from Hamlin oranges (harvest year 1) during storage

	Concn. [μg/kg] after[a)]					
Odorant	0 (Ust)	1	2	4	6	10 months
(*R*)-limonene	121,000	n.m.	n.m.	n.m.	n.m.	65,500
acetaldehyde	16,100	9.84	n.d.	n.d.	n.m.	n.m.
acetic acid	5,570	6,690	9,580	11,900	14,500	21,000
myrcene	1,540	n.m.	n.m.	n.m.	n.m.	916
α-pinene	757	n.m.	n.m.	n.m.	n.m.	599
linalool	561	520	518	n.m.	n.m.	561
hexanal	545	482	402	324	226	222
ethyl butanoate	278	264	277	n.m.	n.m.	245
decanal	275	175	138	112	38.1	40.7
octanal	179	135	54.2	51.2	52.1	49.9
nonanal	99.6	83.8	61.1	55.5	47.8	58.9
1-penten-3-one	34.8	2.15	n. d.	n.d.	n.m.	n.m.
vanillin	20.6	17.5	16.9	19.6	21.5	25.0
(*Z*)-3-hexenal	2.9	1.00	0.47	0.36	0.43	n.m.
2-methoxy-4-vinylphenol	0.74	1.04	1.34	n.m.	2.6	3.8

[a)] Data are mean values of triplicates; standard deviation ≥ 10%; n.m.: not measured; n.d.: not detected.

Table 4. Changes in the concentrations of 20 odor-active compounds in NFC juice from Hamlin oranges (harvest year 2) during storage

	Concn.[a)] [μg/kg] after					
Odorant	0	1	2	3	4	6 months
(*R*)-limonene	101,000	93,200	n.m.	n.m.	n.m.	82,400
acetic acid	12,800	13,100	n.m.	n.m.	21,500	24,200
acetaldehyde	13,900	5,180	760	n.d.	n.m.	n.m.
myrcene	1,710	1,620	n.m.	n.m.	n.m.	1,250
α-pinene	520	449	n.m.	n.m.	n.m.	374
linalool	357	418	387	375	374	380
ethyl butanoate	332	325	336	323	336	340
hexanal	321	257	203	190	189	179
decanal	254	154	122	118	109	96.1
octanal	237	218	175	162	151	157
α-terpineol	185	n.m.	n.m.	n.m.	n.m.	1100
nonanal	90.8	72.5	57.2	58.6	54.6	52.8
ethyl hexanoate	72.5	65.9	61.4	59.0	56.3	54.0
carvone	72.9	n.m.	n.m.	n.m.	n.m.	72.9
1-penten-3-one	34.6	1.40	n.d.	n.d.	n.m.	n.m.

Table 4. Continued

	Concn.[a)] [μg/kg] after					
Odorant	0	1	2	3	4	6 months
vanillin	15.7	17.8	17.3	n.m.	n.m.	17.3
(*E,E*)-2,4-decadienal	3.00	1.46	1.00	0.60	0.42	0.25
(S)-ethyl 2-methylbutanoate	2.54	3.17	2.40	2.74	2.68	2.86
β-ionone	1.92	2.1	1.97	1.94	1.94	1.98
2-methoxy-4-vinylphenol	0.48	0.6	0.95	1.23	1.50	1.81

[a)] Data are mean values of triplicates; standard deviation ≥ 10%; n.m.: not measured; n.d.: not detected.

Table 5. Changes in odor activity values (OAV) of 21 aroma compounds in Hamlin orange juice (harvest year 2) during storage

	Threshold[a)]	OAV [b)] in juice after					
Odorant	[µg/kg]	0	1	2	3	4	6 months
(*R*)-limonene	13	7700	7200	n.c.	n.c.	n.c.	6300
(*R*)-linalool	0.087	4100	4800	4400	4300	4300	4400
myrcene	1.2	1400	1300	n.c.	n.c.	n.c.	1000
acetaldehyde	16	870	320	48	< 1	n.c.	n.c.
ethyl butanoate	0.75	440	430	450	430	450	450
(S)-ethyl 2-methylbutanoate	0.0080	320	400	300	340	340	360
hexanal	2.4	130	110	84	79	79	75
(*E,E*)-2,4-decadienal	0.027	110	54	37	22	15	9
β-ionone	0.021	91	100	94	92	92	94
octanal	3.4	70	64	51	48	44	46
(*Z*)-3-hexenal	0.12	69	10	4	3	2	2
ethyl hexanoate	1.2	60	55	51	49	47	45
1-penten-3-one	0.94	37	1	< 1	< 1	n.c.	n.c.
nonanal	2.8	32	26	20	21	19	19
decanal	9.3	27	17	13	13	12	10
acetic acid	1500[c)]	8.6	8.7	n.c.	n.c.	14	16
α-pinene	58	9.0	7.7	n.c.	n.c.	n.c.	6.4

Table 5. Continued

	Threshold[a)]	OAV [b)] in juice after					
Odorant	[µg/kg]	0	1	2	3	4	6 months
α-terpineol	1200[d)]	< 1	n.c.	n.c.	n.c.	n.c.	1
carvone	95[e)]	< 1	n.c.	n.c.	n.c.	n.c.	< 1
vanillin	53	< 1	< 1	< 1	n.c.	n.c.	< 1
2-methoxy-4-vinylphenol	21	< 1	< 1	< 1	< 1	< 1	< 1

[a)] Odor threshold concentrations in water if not stated otherwise. [b)] Odor acitivity values were calculated by dividing the concentrations in table 4 by the odor threshold concentrations; n.c.: not calculated. [c)] The threshold concentration of acetic acid was determined in an orange juice matrix at a pH value of 3.6. [d)] Threshold concentration in water according to [23]. [e)] Threshold concentration of the (S)-isomer in water.

Table 6. Changes in the concentrations of 21 odor-active compounds in Valencia NFC orange juice (harvest year 2) during storage.

	Concn.[a)] [μg/kg] after			
Odorant	0	1	6	10 months
(*R*)-limonene	188000	175000	153000	124000
acetic acid	12700	20900	21700	23700
acetaldehyde	16100	11900	n.d.	n.m.
(*R*)-linalool	1710	1560	1630	1560
myrcene	1620	1550	1380	1150
α-pinene	800	630[)]	458	337
octanal	790	650	384	377
decanal	651	520	253	225
α-terpineol	412	808	1870	2740
ethyl butanoate	374	373	371	372
hexanal	277	192	140	93.3
nonanal	160	141	96.6	84.3
carvone	105	105	107	106
ethyl hexanoate	80.5	67.2	56.6	49.5
vanillin	44.0	51.8	58.9	63.5
1-penten-3-one	30.2	1.5	n.d.	n.m.
2,3-butanedione	21.1	20.1	21.5	21.1
β-ionone	10.3	10.4	9.8	10.0
(*E,E*)-2,4-decadienal	8.3	3.1	0.5	0.3
(*S*)-ethyl 2-methylbutanoate	3.2	3.3	3.6	3.7
(*Z*)-3-hexenal	1.6	0.4	0.1	0.1
2-methoxy-4-vinylphenol	1.5	2.3	3.2	6.0

[a)] Data are mean values of triplicates; standard deviation ≥ 10%; n.m.: not measured; n.d.: not detected.

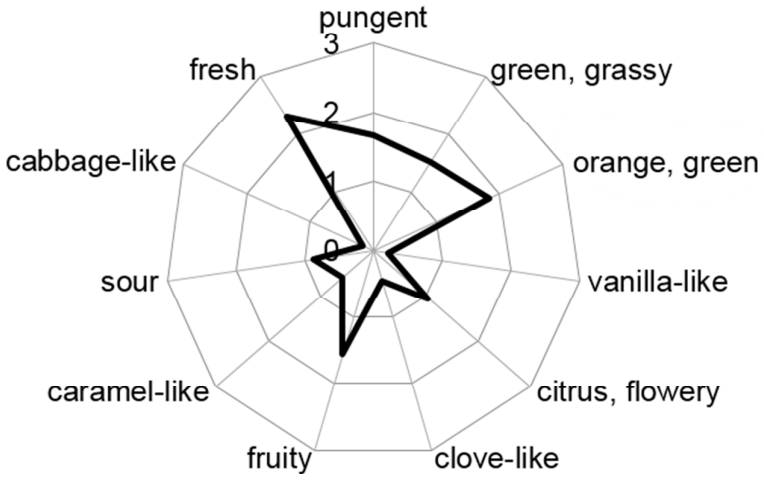
Table 7. Changes in odor activity values (OAV) of 21 aroma compounds in NFC juice from Valencia oranges (harvest year 2) during storage

		OAV [b] after storage for			
Odorant	Threshold[a] [µg/kg]	0	1	6	10 months
(*R*)-linalool	0.087	20000	18000	19000	18000
(*R*)-limonene	13	14000	13000	12000	10000
myrcene	1.2	1300	1300	1100	960
acetaldehyde	16	1000	740	n.c.	n.c.
ethyl butanoate	0.75	500	500	490	500
β-ionone	0.021	490	500	470	480
(S)-ethyl 2-methylbutanoate	0.0080	400	410	450	460
(*E,E*)-2,4-decadienal	0.027	310	120	17	10
octanal	3.4	230	190	110	110
hexanal	2.4	120	80	58	39
decanal	9.3	70	56	27	24
ethyl hexanoate	1.2	67	56	47	41
nonanal	2.8	57	50	35	30
1-penten-3-one	0.94	32	2	< 1	< 1
2,3-butanedione	1.0	21	20	21	21
α-pinene	58	14	11	7.9	5.8
(*Z*)-3-hexenal	0.12	13	3	< 1	< 1
acetic acid	1500[c]	8.5	14	14	16
carvone	95[d]	1.1	1.1	1.1	1.1
vanillin	53	< 1	1	1	1
2-methoxy-4-vinylphenol	21	< 1	< 1	< 1	< 1
α-terpineol	1200[e]	< 1	< 1	2	2

[a] Odor threshold concentrations in water if not stated differently. [b] Odor acitivity values were calculated by dividing the concentrations in table 6 by the odor threshold concentrations; n.c.: not calculated. [c] The threshold concentration of acetic acid was determined in an orange juice matrix at a pH value of 3.6. [d] Threshold concentration of the (S)-isomer in water. [e] Threshold concentration in water according to [23].

Table 8. Design and outcome of triangle tests for spiking experiments for unstored (Ust) Hamlin juice (second harvest year) compared to the 6 months stored juice (6 m) spiked by adding reference aroma compounds to their initial concentrations in Ust

Reference sample	Spiked sample	Spiked aroma compounds	Raised to conc. [µg/kg]	Statistical significance triangle test
Ust	6 m + all compounds with reduced concn.	acetaldehyde	139000	α=0.2
		1-penten-3-one	34.6	
		(*Z*)-3-hexenal	8.23	
		hexanal	321	
		octanal	237	
		nonanal	90.8	
		decanal	254	
		(*E*,*E*)-2,4-decadienal	3.02	
		limonene	101000	
		α-pinene	520	
		myrcene	1710	
		ethyl hexanoate	72.5	
Ust	6 m + “green”	(*Z*)-3-hexenal	8.23	α= 0.01
		hexanal	321	
Ust	6 m + “fresh/ pungent”	acetaldehyde	139000	α= 0.2
		1-penten-3-one	34.6	
Ust	6 m + “citrus/ orange-like”	octanal	237	α= 0.2
		nonanal	90.8	
		decanal	254	
		(*E*,*E*)-2,4-decadienal	3.02	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2
1
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orange, green
vanilla-like
citrus, flowery
clove-like
fruity
caramel-like
sour
cabbage-like
fresh

Figure 1

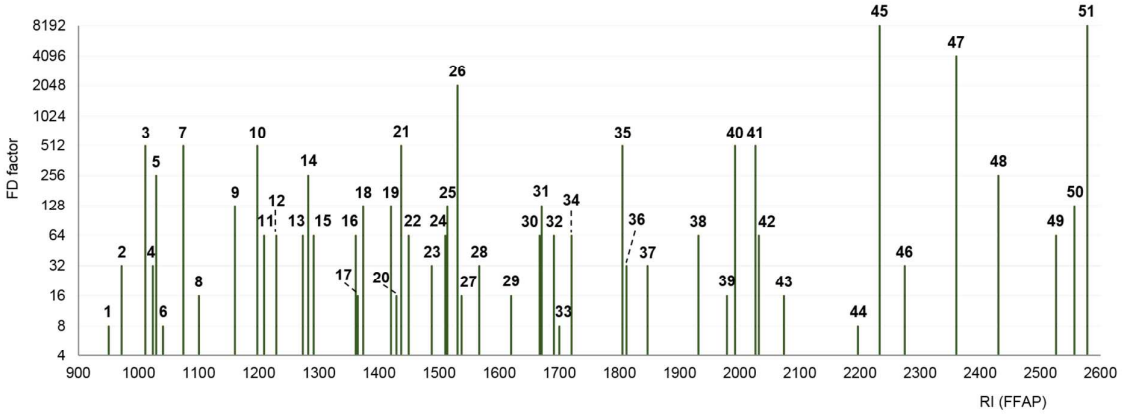
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Figure 2

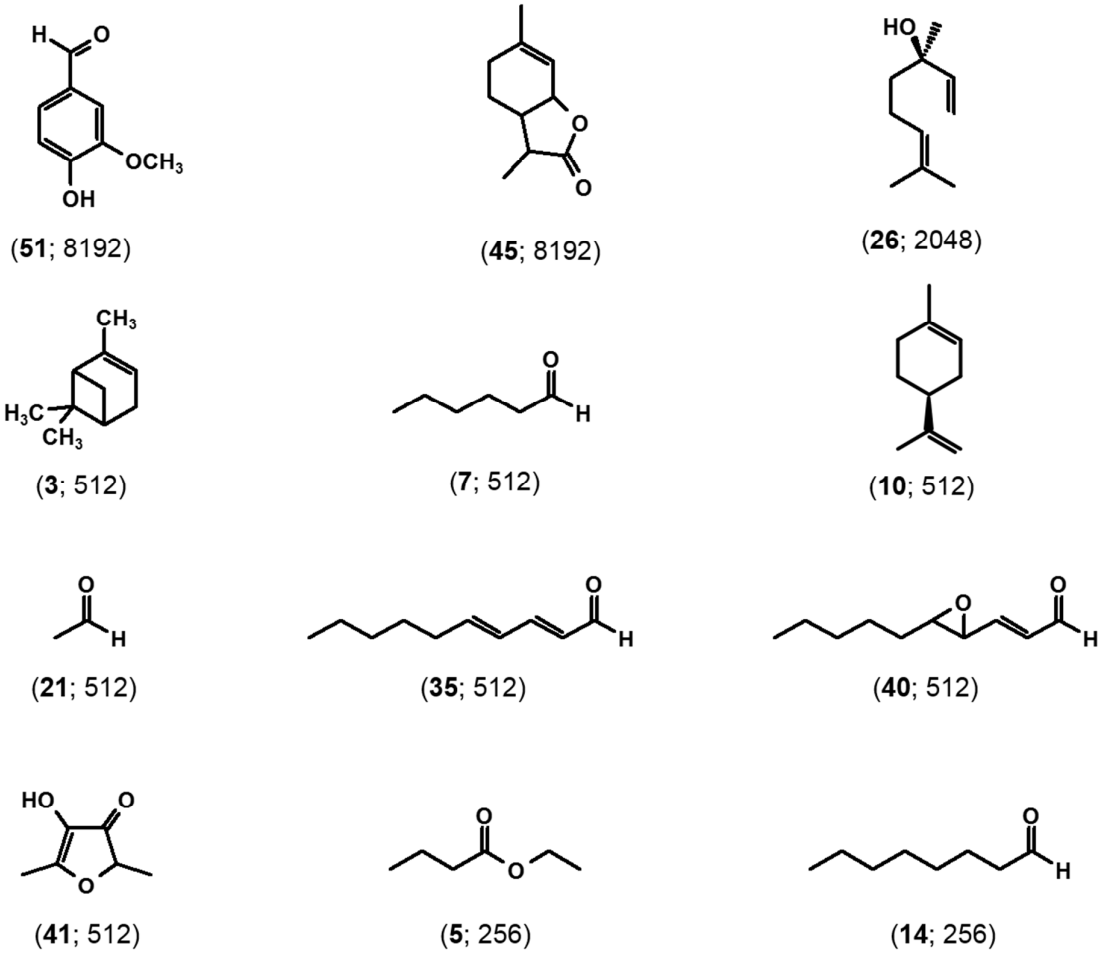
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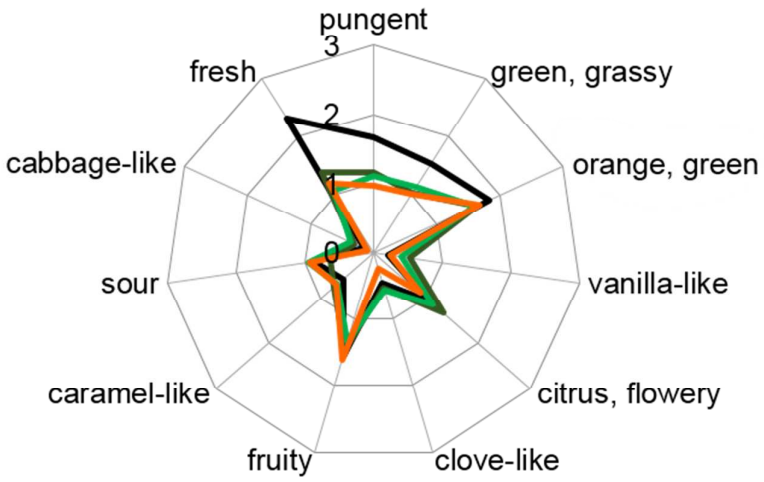
Figure 3


pungent
3
2
1
0
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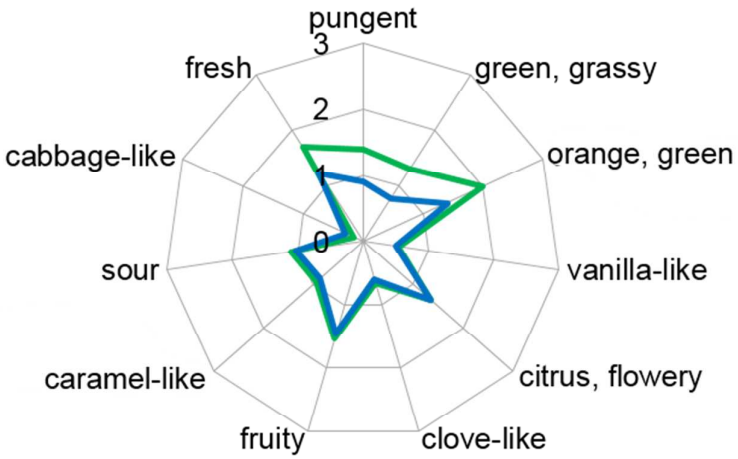
orange, green
vanilla-like
citrus, flowery
clove-like
fruity
caramel-like
sour
cabbage-like
fresh

Figure 4


pungent
3
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2
orange, green
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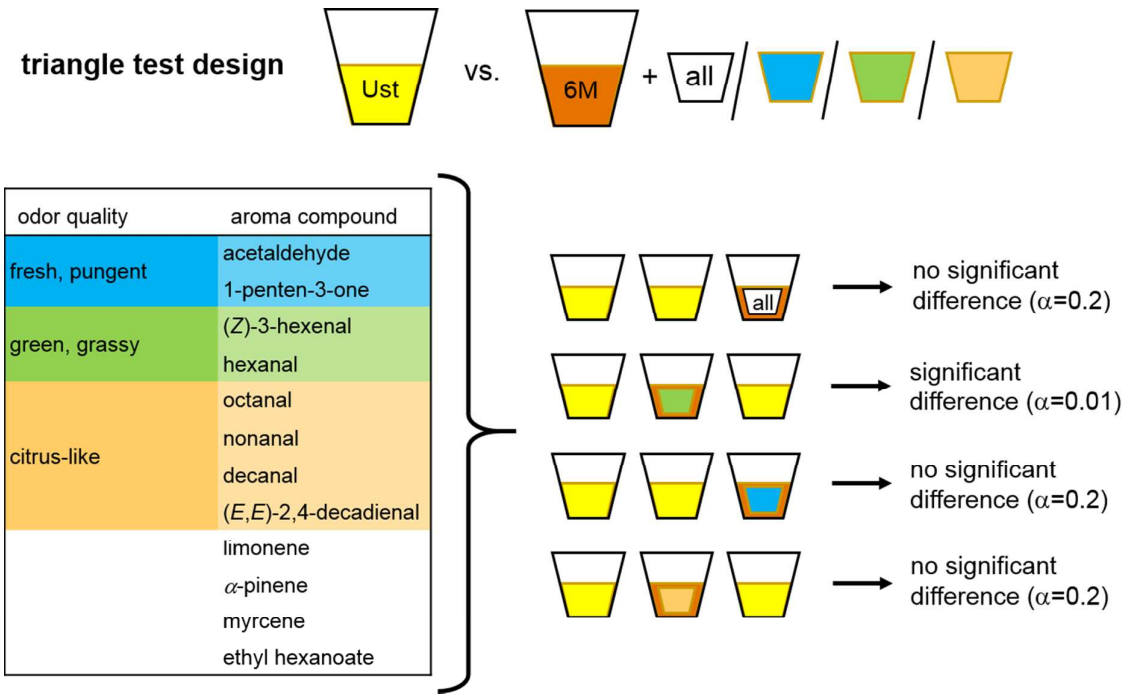
sour
cabbage-like
fresh

Figure 5


triangle test design
Ust
vs.
6M
+
all
odor quality
aroma compound
fresh, pungent
acetaldehyde
1-penten-3-one
green, grassy
(Z)-3-hexenal
hexanal
citrus-like
octanal
nonanal
decanal
(E,E)-2,4-decadienal
limonene
α-pinene
myrcene
ethyl hexanoate
all
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significant difference (α=0.01)
no significant difference (α=0.2)
no significant difference (α=0.2)

Figure 6

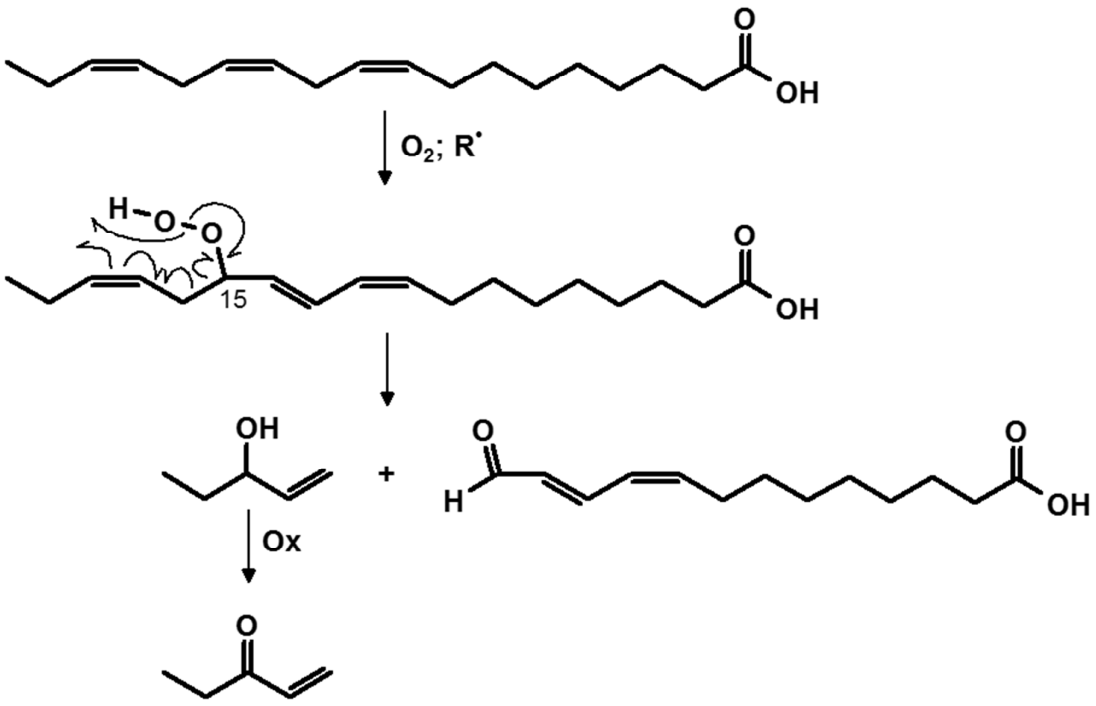
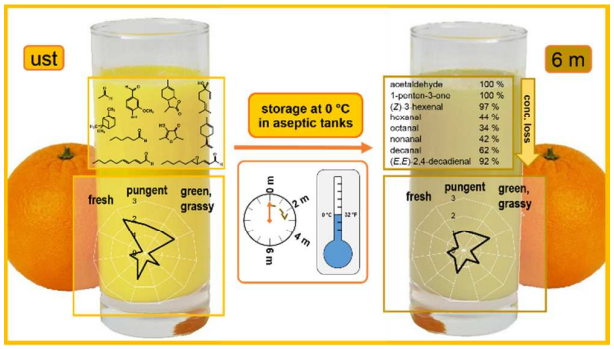


Figure 7

TOC-Graphic


ust
6 m
storage at 0 °C
in aseptic tanks
acetaldehyde 100 %
1-penten-3-one 100 %
(Z) 3 hexenal 97 %
hexanal 44 %
octanal 34 %
nonanal 42 %
decanal 62 %
(E,E) 2,4-decadienal 92 %
conc. loss
fresh
pungent
green, grassy
fresh
pungent
green, grassy