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Influence of the Production Process on the Key Aroma Compounds of Rum: from Molasses to the Spirit

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1 **ABSTRACT:** The production of rum consists of fermentation, distillation, and 2 ageing. To check the influence of each step on the final rum aroma, molasses, mash, 3 distillate, and the final rum were analyzed using the sensomics concept. The 4 changes in key aroma compounds were determined by application of aroma extract 5 dilution analysis (AEDA) in combination with gas chromatography-mass spectrometry 6 for identification and by stable isotope dilution assays (SIDAs) for quantitation. Odor 7 activity values (OAVs; ratio of concentration to respective odor threshold) were 8 calculated for the compounds determined in the rum and, finally, the rum aroma was 9 successfully simulated by recombination. (E)- β -Damascenone showed by far the 10 highest OAV (3280) in rum. Although this compound was determined already in 11 molasses, its concentration increased significantly during distillation indicating a 12 thermo-labile precursor. Vanillin, 4-ethylphenol, 2-methoxyphenol, 4-ethyl-2-13 methoxyphenol, and 4-propyl-2-methoxyphenol are well-known compounds mainly 14 stemming from the wood barrels used for ageing and showed all an OAV \geq 1. 15 Another important group of aroma-active compounds in rum were ethyl esters, for 16 which a significant increase was determined during fermentation, but also to a lesser 17 extent during ageing. Altogether, the concentrations of 68% of the aroma-active 18 compounds increased during the process, demonstrating its influence on the overall 19 rum aroma.

20

21 **KEYWORDS:** *rum, production, fermentation, distillation, ageing, aroma extract* 22 *dilution analysis, stable isotope dilution analysis, odor activity value, aroma* 23 *recombination*

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24 INTRODUCTION

25 The production of rum traditionally starts with the fermentation of molasses, the 26 remainder after sugar production, using yeasts like Saccharomyces cerevisiae. 27 During fermentation, many aroma-active compounds are formed, including alcohols, 28 aldehydes, and ethyl esters. Besides for rum production, molasses is used for many 29 foods because of its sweetness and special aroma. First studies on molasses by gas 30 chromatography were done by Hashizume et al.,¹ who identified anisole, benzoic acid, vanillin, and vanillic acid. Abe et al.² analyzed for the first time the so-called 31 32 "sugary flavor" by gas chromatography-mass spectrometry (GC-MS), postulating 5-33 methyl-2-acetylfuran, cyclotene, y-nonalactone, y-decalactone, δ -decalactone, and 34 vanillin as potential key compounds. In a further study, nine new compounds were 35 identified, e.g., damascenone (no information about stereochemistry was mentioned), but, a mixture of all compounds did not show the characteristic aroma of molasses.³ 36 37 Later, 3-hydroxy-4,5-dimethylfuran-2(5H)-one was characterized as a key compound 38 and was named "sotolon", a combination of "soto", the Japanese word for cane sugar, and "olon" ("enolic lactone").⁴ In addition, vanillin, maltol, δ -nonalactone, 4-39 40 vinylphenol, and 2,6-dimethoxyphenol were characterized as key compounds for the 41 "sugary flavor" of molasses.

The next step of rum production is the distillation of the mash. Thereby, undesired compounds, but also solids, are separated from the distillate. Unfortunately, the discrimination of compounds with a lower or higher boiling point compared to ethanol can lead to a loss of aroma characteristics.

The final steps are the ageing of the distillate, which is commonly performed in oak barrels firstly used in whisky or brandy production, followed by a dilution step adjusting the desired alcohol content of 40% (by vol.). Numerous reactions take

place and influence the aroma of the final product.^{5,6} Due to the thermal treatment 49 50 during barrel production, many aroma compounds are generated and, finally, 51 extracted during ageing. Cis- and trans-whisky lactone (β -methyl- γ -octalactone) are 52 marker compounds for spirits, which were stored in barrels. These compounds were 53 firstly isolated from different oak woods by Masuda and Nishimura.⁷ Although four 54 stereoisomers are possible, up to now, only two isomers (3S,4S and 3S,4R) were characterized in oak woods⁸ and, consequently, in spirits, e.g., in rum,^{9,10} whisky,⁶ or 55 cognac.¹¹ Due to the high ethanol content, the concentrations of ethyl esters are 56 57 increasing. The reaction equilibrium of the acids and ethanol strongly depends on the 58 ageing conditions. A continuous increase of ethyl esters in a model solution 59 simulating ageing conditions was already shown.⁶

First studies on rum volatiles were done by Bober and Haddaway¹² using gas 60 chromatography, followed by Maarse and Ten Noever de Brauw,¹³ who identified 61 62 over 100 volatile compounds in a Jamaican rum by gas chromatography, mass spectrometry, and infrared spectrometry. In 2006, de Souza et al.¹⁴ analyzed rum 63 64 odorants for the first time via gas chromatography-olfactometry (GC-O). Using Charm 65 analysis, β -damascenone (no information about stereochemistry was mentioned), 66 1,1-diethoxyethane, ethyl 2-methylbutanoate, whisky lactone (no information about 67 stereochemistry was mentioned), vanillin, and 2-methoxyphenol were characterized as most potent odorants. Pino et al.⁹ guantitated 98 compounds using external 68 69 calibration methods and estimated the potency of the odorants by aroma extract 70 dilution analysis (AEDA) and odor activity values (OAVs; ratio of concentration to 71 respective odor threshold) determining ethanol, (*E*)- β -damascenone, ethyl butanoate, 72 ethyl hexanoate, vanillin, 2-methoxyphenol, *cis*-whisky lactone, ethyl 73 methylpropanoate, ethyl octanoate, ethyl decanoate, 4-propyl-2-methoxyphenol, 74 2-methylbutanaote, eugenol. 1,1-diethoxyethane, ethyl v-nonalactone. 2-

phenylethanol, 4-ethyl-2-methoxyphenol, 3-methylbutyl acetate, and 2-phenylethyl acetate as key odorants. Very recently, the key aroma compounds of two commercial rums were characterized using the sensomics concept revealing ethanol, ethyl (*S*)-2methylbutanoate, (*E*)- β -damascenone, 3-methylbutanal, 2,3-butanedione, ethyl butanoate, 1,1-diethoxyethane, ethyl 3-methylbutanoate, ethyl pentanoate, and ethyl hexanoate with OAVs \geq 1 in both rums. In addition, both rum aromas were successfully simulated using 14 and 12 reference compounds, respectively.¹⁰

As briefly outlined, the production process significantly influences the chemical composition of the final spirit. Due to the fact that an aromatization of rum and an addition of ethanol is forbidden,¹⁵ the production process is, besides the raw material, the only way to improve the aroma.

86 Thus, the aim of the present study was to investigate the influence of the production process on the aroma of rum by the sensomics concept,¹⁶ consisting of (i) 87 88 the identification of the key aroma compounds in molasses, mash, distillate, and rum 89 (representing the four main steps of rum production) by aroma extract dilution 90 analysis (AEDA) in combination with gas chromatography-mass spectrometry, (ii) the 91 quantitation of the most potent odorants by stable isotope dilution assays (SIDAs) in 92 molasses, mash, distillate, and rum, (iii) the calculation of odor activity values (OAVs) 93 for the rum odorants, and (iv) the simulation of the rum aroma. These data will 94 provide rum producers knowledge about possibilities to positively influence the 95 aroma of the final spirit.

96 MATERIALS AND METHODS

97 Samples. Molasses, mash, distillate, and rum were obtained from a Bavarian
98 distillery (Feinbrennerei Simon, Alzenau, Germany) ensuring traceability of molasses,
99 mash, distillate as well as the analyzed final spirit. Molasses was from the Dominican

100 Republic. The raw material was diluted with water (1+3; v+v) and used for 101 fermentation performed at room temperature for about 2.5 weeks. Distillation was 102 carried out in a pot still. Each batch yielded 20 L of distillate, whereat the first 2 L 103 (foreshots) were withdrawn and the heart was stopped if the alcohol content reached 104 65% (by vol.) merging into the feints. The distillate obtained was stored at room 105 temperature for 3 years in heavy toasted oak barrels (225 L; wood originating from 106 the Spessart, Germany), which were already used for red wine ageing 30 months 107 before. Before bottling, a dilution step adjusting the desired alcohol content of 40% 108 (by vol.) was performed. There was no blending leading to single cask rum of high 109 quality, a specialty of the small-sized premium distillery. Prior to analysis, molasses 110 and mash were vacuum-tight packed and stored at -18 °C in the dark; distillate and 111 rum were stored at room temperature in the dark.

112 Chemicals. The following compounds used as references for identification and 113 quantitation were obtained from commercial sources: acetic acid, 2,3-butanedione, 114 (E,E)-2,4-decadienal, decanoic acid, 1,1-diethoxyethane, ethyl butanoate, 3-ethyl-115 2,5-dimethylpyrazine, ethyl hexanoate, ethyl 2-methylbutanoate, ethyl 3-116 methylbutanoate, ethyl octanoate, ethyl pentanoate, 4-ethylphenol, ethyl 3-117 phenylpropanoate, (Z)-4-heptenal, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, 4-118 hydroxy-2,5-dimethylfuran-3(2H)-one, linalool, (R)-linalool, 2-methoxyphenol, 2-119 methoxy-4-propylphenol, 2-methylbutanal, 3-methylbutanal, 3-methylbutanoic acid, 3-methyl-1-butanol, 120 2-methyl-1-butanol, (S)-2-methyl-1-butanol, 3-methylbutyl 121 acetate. 4-methyl-2-methoxyphenol, methyl octanoate. 4-methylphenol, 122 3-(methylthio)propanal, methylpropanol, γ -nonalactone, phenylacetaldehyde, 123 phenylacetic acid, 2-phenylethanol, and cis-/trans-whisky lactone (Sigma-Aldrich 124 Chemie, Taufkirchen, Germany); 2-methylbutanoic acid and 1-octen-3-one (Alfa 125 Aeasar, Karlsruhe, Germany); ethyl cyclohexanoate and 4-ethyl-2-methoxyphenol

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126 (Lancaster, Mühlheim/Main, Germany); butanoic acid and vanillin (Merck, Darmstadt, 127 Germany); and (*R*)-2-methylbutanoic acid (Akos, Steinen, Germany). 1-(2,6,6-128 Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one ((*E*)- β -damascenone) was kindly 129 provided by Symrise (Holzminden, Germany).

130 Ethyl (S)-2-methylbutanoate was synthesized as recently described.¹⁷

Ammonium chloride, hydrochloric acid, lithium aluminum hydride, sodium carbonate, sodium chloride, sodium hydrogen carbonate, sodium sulfate, and sodium thiosulfate were from Merck. 1,1,1-Tris-(acetyloxy)-1,1-dihydro-1,2-benziodoxol-3-(1*H*)-one (Dess-Martin periodinane) was obtained from Sigma-Aldrich. Diethyl ether, dichloromethane, and pentane (Merck) were freshly distilled prior to use. Liquid nitrogen was obtained from Linde (Munich, Germany). All chemicals were at least of analytical grade.

138 **Stable Isotopically Labeled Standards.** $[^{2}H_{7}]$ -4-Methylphenol, $[^{2}H_{9}]$ -2-methyl-139 butanoic acid, and $[^{2}H_{5}]$ -2-phenylethanol were obtained from C/D/N Isotopes 140 (Quebec, Canada); $[^{13}C_{2}]$ -acetic acid, $[^{2}H_{9}]$ -methylpropanol, and $[^{13}C_{2}]$ -phenylacetic 141 acid were from Sigma-Aldrich; and $[^{13}C_{2}]$ -4-hydroxy-2,5-dimethylfuran-3(2*H*)-one was 142 from Aromalab (Planegg, Germany).

143 The following stable isotopically labeled standards were synthesized according to literature: $[{}^{13}C_4]-2,3$ -butanedione; 18 $[{}^{2}H_2]$ -butanoic acid; 19 $[{}^{2}H_{4-7}]-(E)-\beta$ -144 the damascenone;²⁰ $[{}^{2}H_{2.4}]$ -(*E*,*E*)-2,4-decadienal;²¹ $[{}^{2}H_{2}]$ -decanoic acid;²² $[{}^{13}C_{2}]$ -1,1-145 diethoxyethane;²³ [²H₃]-ethyl butanoate;¹⁸ [²H₃]-ethyl cyclohexanoate analogue to 146 $[^{2}H_{3}]$ -ethyl butanoate;¹⁸ $[^{2}H_{3}]$ -ethyl hexanoate;²³ $[^{2}H_{2-4}]$ -4-ethyl-2-methoxyphenol;²⁴ 147 $[^{2}H_{3}]$ -ethyl 2-methylbutanoate;²⁵ $[^{2}H_{3}]$ -ethyl octanoate;²⁶ $[^{2}H_{5}]$ -ethyl pentanoate;²⁷ 148 [²H₂]-3-ethylphenol:²³ $[^{2}H_{5}]$ -ethyl 3-phenylpropanoate;²⁷ $[^{13}C_{2}]$ -3-hydroxy-4,5-149 dimethylfuran-2(5*H*)-one;²⁸ [²H₂]-linalool;²⁹ [²H₃]-2-methoxyphenol;³⁰ [²H₂₋₄]-2-150 methoxy-4-propylphenol;³¹ [²H₂]-2-methylbutanal and [²H₂]-3-methylbutanal;³² [²H₂]-3-151

methylbutanoic acid;³³ [${}^{2}H_{2}$]-3-methyl-1-butanol;²⁶ [${}^{2}H_{11}$]-3-methylbutyl acetate;³⁴ 153 [${}^{2}H_{3}$]-3-(methylthio)propanal;³⁵ [${}^{2}H_{2-4}$]-1-octen-3-one;²¹ [${}^{2}H_{3}$]-vanillin;²⁴ and [${}^{2}H_{2}$]-*cis*-154 whisky lactone.³⁶

155 $[^{2}H_{9}]$ -Ethyl 3-methylbutanoate was obtained by esterification of $[^{2}H_{9}]$ -3-156 methylbutanoic acid with ethanol following the procedure previously described.²⁵

157 Concentrations of the isotopically labeled standards were determined using 158 methyl octanoate as internal standard. Solutions of each unlabeled reference 159 compound and methyl octanoate were analyzed by means of a TRACE 2000 gas 160 chromatograph equipped with a flame ionization detector (FID) (ThermoQuest, 161 Egelsbach, Germany) to determine the FID response factor. Next, the labeled 162 standard was spiked with methyl octanoate and analyzed by GC-FID. The 163 concentration was calculated by the peak areas of the labeled compound and methyl 164 octanoate, the concentration of methyl octanoate, and the determined FID response 165 factor for the unlabeled compound.

166 Synthesis. (R)-2-Methyl-1-butanol. A solution of (R)-2-methylbutanoic acid 167 (2 mmol) in anhydrous diethyl ether (10 mL) was added to a solution of LiAlH₄ 168 (5 mmol) in anhydrous diethyl ether (10 mL) and stirred for 120 min at room 169 temperature. The solution was cooled (0 °C) using an ice-bath and a saturated 170 ammonium chloride solution (15 mL) was added, followed by hydrochloric acid 171 (2 mol/L; 10 mL). The aqueous phase was extracted with diethyl ether (3 x 30 mL), 172 the organic phases were combined, washed with a saturated aqueous solution of 173 sodium hydrogen carbonate (50 mL), and dried over anhydrous sodium sulfate. 174 Yield: 91%.

175 MS-EI, *m/z* (%): 70 (57, [M - H₂O]⁺), 58 (5), 57 (94), 56 (100), 55 (19), 43 (9), 42 (12),
176 41 (75).

177 MS-CI, m/z (%): 71 (100, [(M - H₂O) + H]⁺), 70 (15), 57 (17), 56 (25), 55 (16).

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178 Retention index (RI) on DB-FFAP: 1213; RI on BGB-174E: 1105.

(*R*)-2- and (*S*)-2-Methylbutanal. Dess-Martin periodinane (1.4 mmol) was added
to a solution of (*R*)-2- or (*S*)-2-methyl-1-butanol (1.2 mmol) dissolved in dichloromethane (15 mL) and stirred for 90 min at room temperature. The mixture was
washed with an aqueous solution of sodium thiosulfate (1 mol/L), which was
saturated with sodium hydrogen carbonate (15 mL), and the organic phase was dried
over anhydrous sodium sulfate. Yield: 88% for (*R*)-2-Methylbutanal and 85% for (*S*)2-Methylbutanal, respectively.

186 (*R*)-2-Methylbutanal: MS-EI, *m/z* (%): 86 (15, M⁺), 71 (5), 58 (66), 57 (100), 56
187 (10), 55 (6), 43 (12), 41 (82).

188 MS-CI, *m/z* (%): 87 (100, [M + H]⁺), 86 (20), 84 (6), 69 (70), 58 (47), 57 (60), 55(5).

189 RI on OV-1701: 731; RI on BGB-174E: 1061.

(S)-2-Methylbutanal: MS-EI, *m/z* (%): 86 (16, M⁺), 71 (5), 58 (67), 57 (100), 56
(6), 55 (7), 43 (9), 41 (79).

192 MS-CI, m/z (%): 87 (100, [M + H]⁺), 85 (7), 69 (65), 58 (33), 57 (37), 55 (5).

193 RI on OV-1701: 731; RI on BGB-174E: 1067.

Isolation of the Volatiles. *Molasses and Mash.* To the molasses (35 g), water (25 mL) and diethyl ether (70 mL) were added and the mixture was stirred for 30 min at room temperature. To the mash (50 mL), diethyl ether (50 mL) was added and also stirred for 30 min at room temperature. After the addition of sodium sulfate to improve phase separation, the organic phase was separated and the extraction was repeated twice. The organic phases were combined and dried over anhydrous sodium sulfate.

Distillate and Rum. To the distillate (20 mL), water (20 mL) was added due to the high amount of ethanol improving phase separation, followed by an extraction with diethyl ether (3 x 40 mL) in a separating funnel. The rum (50 mL) was directly

extracted with diethyl ether ($3 \times 50 \text{ mL}$). The organic phases were washed with an aqueous solution of sodium chloride (1 mol/L; $3 \times 150 \text{ mL}$) to remove the high amount of ethanol and dried over anhydrous sodium sulfate.

All four organic extracts were subjected to high vacuum distillation using solvent assisted flavor evaporation (SAFE) technique³⁷ to separate the volatiles from the non-volatiles. The distillates obtained were concentrated to a final volume of ~200 μ L using a Vigreux-column (50 cm x 1 cm i.d.) and micro-distillation.³⁸

High-Resolution Gas Chromatography-Olfactometry (HRGC-O). HRGC-O was performed as recently described.¹⁰ RIs for each compound were determined by a series of *n*-alkanes (C6-C26 (DB-FFAP) and C6-C18 (DB-5), respectively).³⁹

Aroma Extract Dilution Analysis (AEDA). For AEDA, molasses, mash, distillate, and rum were extracted and the volatiles were isolated by SAFE distillation³⁷ as described above. The distillates obtained were concentrated to the same final volume and diluted stepwise 1+1 (v+v) with diethyl ether. The original distillate and each dilution were analyzed by HRGC-O to determine the flavor dilution (FD) factors. The original distillate was analyzed by three experienced panelists to avoid overlooking of odor-active compounds.

221 High-Resolution Gas Chromatography-Mass Spectrometry (HRGC-MS) for 222 Identification. A gas chromatograph 5890 series II (Hewlett-Packard, Waldbronn, 223 Germany) coupled to a sector field mass spectrometer type MAT 95 S (Finnigan, 224 Bremen, Germany) was used for HRGC-MS. An aliquot of the sample (0.5 µL) was 225 manually injected by the cold on-column technique at 40 °C. The initial temperature 226 was held for 2 min, then raised at 6 °C/min to 230 °C and held for 5 min. Mass 227 spectra were generated in the electron ionization (EI) mode at 70 eV and in the 228 chemical ionization (CI) mode using isobutane as reactant gas at 115 eV.

229 Quantitation by Stable Isotope Dilution Analysis (SIDA). Water (in case of 230 molasses and distillate), diethyl ether (1+1 by vol., but a minimum of 10 mL), and the 231 internal standards (dissolved in ethanol or dichloromethane; amounts depending on 232 the concentration of the analytes determined in preliminary experiments) were added 233 to the respective samples. After stirring for 30 min at room temperature for 234 equilibration, the work-up procedure was performed as described above for the 235 isolation of the volatiles prior to HRGC-MS or two-dimensional high-resolution gas 236 chromatography-mass spectrometry (HRGC/HRGC-MS). All guantitation experiments 237 (complete work-up procedure) were performed in triplicates.

For determination of the respective response factors (R_f) (**Table 1**), mixtures of known amounts of the unlabeled and labeled compounds were analyzed in five different ratios (5:1, 3:1, 1:1, 1:3, 1:5) in the same way as described below.

241 Quantitation of butanoic acid, decanoic acid as well as 2- and 3-methylbutanoic 242 acid were performed as recently described.¹⁰

243 High-Resolution Gas Chromatography-Mass Spectrometry (HRGC-MS) for 244 **Quantitation.** For quantitation experiments, a gas chromatograph type 431 (Varian, Darmstadt) equipped with a DB-FFAP column (30 m x 0.25 mm i.d., 0.25 µm film 245 246 thickness; J&W Scientific) coupled to a 220 ion trap mass spectrometer (Varian) was 247 used. An aliguot of the sample (2 µL) was injected by means of a Combi PAL 248 autosampler (CTC Analytics, Zwingen, Switzerland) using a cold on-column injector 249 at 40 °C. The initial temperature of 40 °C was held for 2 min, and then raised at 250 230 °C using a gradient of 6 °C/min. The final temperature was held for 5 min. Mass 251 spectra were generated in CI mode (70 eV) using methanol as reactant gas.

252 **Two-Dimensional High-Resolution Gas Chromatography-Mass** 253 **Spectrometry (HRGC/HRGC-MS).** In case that a trace compound was overlapped 254 by a major volatile, HRGC/HRGC-MS was performed by a TRACE 2000 series GC

255 (ThermoQuest) equipped with a DB-FFAP column (30 m x 0.32 mm i.d., 0.25 µm film 256 thickness) coupled to a CP 3800 gas chromatograph (Varian) equipped with an OV-257 1701 column (30 m x 0.25 mm i.d., 0.25 µm film thickness) (both J&W Scientific). An 258 aliquot of the sample (2 µL) was injected by means of a Combi PAL autosampler 259 using a cold on-column injector at 40 °C. Heart cuts were transferred to the second 260 column by a moving capillary stream switching system (ThermoQuest). Thereby, the 261 volatiles were cryo-focused in a cold trap cooled to -100 °C with liquid nitrogen, and 262 separated on the second column after increasing the trap temperature to 230 °C. A 263 Saturn 2000 ion trap mass spectrometer (Varian) was used to generate mass spectra 264 in CI mode (70 eV) using methanol as reactant gas.

Quantitation of Acetic acid and Ethanol. Acetic acid (in the distillate and rum) and ethanol (in the mash, distillate, and rum) were determined using a commercial enzyme-kit (R-Biopharm, Darmstadt). Analyses were done according to the instructions of the manufacturer and measuring was performed by means of a UV-2401PC photometer (Shimadzu, Duisburg, Germany).

270 Separation of Ethyl (R)-2- and Ethyl (S)-2-methylbutanoate, (R)- and (S)-271 Linalool as well as (R)-2-, (S)-2-, and 3-Methylbutanoic acid. Ethyl (R)-2- and 272 ethyl (S)-2-methylbutanoate as well as (R)-2-, (S)-2-, and 3-methylbutanoic acid were 273 separated by HRGC/HRGC-MS using a DB-FFAP column (30 m x 0.32 mm i.d., 274 0.25 µm film thickness; J&W Scientific) in the first dimension and a chiral BGB-176 275 column (30 m x 0.25 mm i.d., 0.25 µm film thickness; BGB Analytik, Böckten, Switzerland) in the second dimension as recently reported.¹⁷ The separation of (R)-276 and (S)-linalool was performed using the same column combination.²⁹ 277

Separation of (*R*)-2- and (*S*)-2-Methylbutanal as well as (*R*)-2-, (*S*)-2-, and 3-Methyl-1-butanol. (*R*)-2- and (*S*)-2-methylbutanal as well as (*R*)-2-, (*S*)-2-, and 3methyl-1-butanol were separated by HRGC/HRGC-MS using a DB-FFAP column Journal of Agricultural and Food Chemistry

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(30 m x 0.32 mm i.d., 0.25 μ m film thickness; J&W Scientific) in the first dimension and a chiral BGB-174E column (30 m x 0.25 μ m i.d., 0.25 μ m film thickness; BGB Analytik) in the second dimension as recently reported.¹⁷

284 **Determination of Orthonasal Odor Thresholds.** Orthonasal odor thresholds 285 were determined in an aqueous solution containing 40% of pure ethanol (by vol.) by 286 our in-house sensory panel (at least 20 assessors) as previously described.⁴⁰

287 Aroma Profile Analysis (APA). For APA, a sensory panel rated the intensities of 288 selected odor attributes (ethanolic, malty, butter-like, fruity, clove-like/phenolic, 289 caramel-like, vanilla-like, and baked apple-like) on a linear seven-point scale in steps 290 of 0.5 from 0 (not perceivable) to 3 (strongly perceivable). The panel consisted of 20 291 experienced assessors participating in weekly sensory sessions to train their abilities 292 to recognize and describe different aroma qualities. The samples (15 g of molasses; 293 15 mL of mash, distillate, and rum) were presented in covered glass vessels (40 mm 294 i.d., total volume = 45 mL). Sensory experiments were performed in a sensory room 295 at 21 \pm 1 °C equipped with single booths.

296 Aroma Recombination. For aroma recombination experiments, all analyzed 297 aroma-active compounds with an OAV ≥ 1 were prepared in aqueous solutions 298 containing 40% of pure ethanol (by vol.) and mixed in the natural occurring 299 concentrations determined in the rum. For an odorless (proven by the sensory panel 300 performing APA) matrix, rum (200 mL) was extracted with pentane (3 x 200 mL) and 301 diethyl ether (3 x 200 mL), and the remaining liquid was lyophilized. The mixture of 302 aroma compounds was added to the lyophilisate simulating the original ratio. The 303 original rum and the recombinate were evaluated in parallel by the sensory panel as 304 explained above for the APA.

305 **Results and Discussion**

306 To compare the overall aroma of the four different process steps (molasses, 307 mash, distillate, and rum), aroma profile analyses were performed (Figure 1). As 308 expected, the ethanolic odor quality showed no intensity in the molasses, clearly 309 increased in the mash, and showed the highest intensity in the distillate and in the 310 rum. A clear difference was observed for the fruity and malty attributes revealing the 311 highest intensities in the mash. In contrast, the butter-like odor quality was evaluated 312 with the highest intensity in the distillate and at the same level in the molasses, the 313 mash, and the rum. The molasses as starting material showed the highest rating for 314 the caramel-like attribute, whereas the rum elicited the highest intensities in the 315 clove-like/phenolic, vanilla-like, and baked apple-like attributes. To identify the aroma compounds responsible for these different overall aromas, the sensomics concept¹⁶ 316 317 was applied.

318 Identification of Aroma-Active Compounds. After work-up of all samples including solvent extraction and high vacuum distillation using SAFE technique,³⁷ 319 320 droplets of the distillates on strips of filter paper elicited the typical aromas of 321 molasses, mash, distillate, and rum, proving a successful extraction and clean-up of 322 the volatile fraction of the raw material, the intermediates, and the final rum. The 323 SAFE distillates were subjected to aroma extract dilution analysis (AEDA) by high-324 resolution gas chromatography-olfactometry (HRGC-O). For identification of the 325 compounds responsible for the single odor impressions, retention indices of the 326 aroma-active regions determined on two GC columns of different polarity were 327 compared to an in-house database containing ~1000 aroma-active reference 328 compounds. In addition, the aroma quality and intensity of the odor impressions 329 detected at the sniffing port were compared to those of the suggested compounds at

similar concentration ranges. For an unequivocal identification, mass spectra (EI andCI mode) were recorded for the odorants and compared to those of the references.

332 Aroma-Active Compounds in the Molasses. Twenty-nine aroma-active regions 333 were detected in the flavor dilution (FD) factor range between 8 and 2048 in the 334 SAFE distillate of the molasses (**Table 2**). Following the above mentioned procedure 335 for identification, 2-methoxyphenol (27; gammon-like, smoky) and vanillin (42; vanilla-336 like) were identified with the highest FD factor of 2048, followed by acetic acid (16; 337 vinegar-like), 3-(methylthio)propanal (17; cooked potato-like), butanoic acid (20; 338 sweaty), 2- and 3-methylbutanoic acid (22a,b; sweaty), 4-hydroxy-2,5-dimethylfuran-339 3(2H)-one (34; caramel-like; all FD factor of 1024), 2,3-butanedione (2; butter-like), 2-340 and 3-methyl-1-butanol (**10a**,**b**; malty), and 3-hydroxy-4,5-dimethylfuran-2(5H)-one 341 (**39**; seasoning-like, spicy; all 512). Using a chiral column in the second dimension of 342 the two-dimensional HRGC/HRGC-MS system to determine the ratio of (R)-2- and 343 (S)-2-methylbutanoic acid as well as of (R)-2- and (S)-2-methyl-1-butanol, a 344 separation of 2- and 3-methylbutanoic acid as well as of 2- and 3-methyl-1-butanol 345 was also achieved. 2-Methylbutanoic acid and 2-methyl-1-butanol showed 81% and 346 84% of the (S)-enantiomer, respectively.

347 Aroma-Active Compounds in the Mash. AEDA of the mash SAFE distillate 348 showed 38 aroma-active regions in the FD factor range between 8 and 2048 349 (Table 2). The highest FD factor of 2048 was determined for 2- and 3-methyl-1-350 butanol (**10a**,**b**; >99% of the (S)-enantiomer), followed by ethyl 2-methylbutanoate (**5**; 351 fruity; 95% of the (S)-enantiomer), 3-(methylthio)propanal (17), butanoic acid (20), 2-352 and 3-methylbutanoic acid (22a,b; 94% of the (S)-enantiomer), 2-methoxyphenol 353 (27), 2-phenylethanol (30; flowery, honey-like; all 1024), ethyl butanoate (4; fruity), 354 and acetic acid (16; both 512).

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355 Aroma-Active Compounds in the Distillate. Thirty-seven aroma-active compounds 356 were determined in the FD factor range between 8 and 2048 during AEDA of the 357 distillate (Table 2). Among them, 2- and 3-methyl-1-butanol (10a,b; >99% of the (S)-358 enantiomer) and 2-methoxyphenol (27) were determined with the highest FD factor of 359 2048, followed by 2,3-butanedione (2; butter-like), 3-methylbutyl acetate (8; banana-360 like; both 1024), ethyl 2-methylbutanoate (5; 98% of the (S)-enantiomer), ethyl 361 cyclohexanoate (14; fruity, sweet), ethyl octanoate (15; fruity, green), and 3-362 (methylthio)propanal (17; all 512).

363 Aroma-Active Compounds in the Rum. During AEDA of the rum, 38 aroma-active 364 regions were determined in the FD factor range between 8 and 2048 (Table 2). 2-365 and 3-methyl-1-butanol (**10a**,**b**; >99% of the (S)-enantiomer), 2-methoxyphenol (**27**), 366 and *cis*-whisky lactone (32; coconut-like) showed the highest FD factor of 2048, 367 followed by ethyl cyclohexanoate (14), ethyl octanoate (15), trans-whisky lactone (29; 368 coconut-like), 4-ethyl-2-methoxyphenol (35; smoky, gammon-like), vanillin (42; all 369 1024), ethyl 2-methylbutanoate (5; 97% of the (S)-enantiomer), 3-methylbutyl acetate 370 (8), ethyl 3-phenylpropanoate (28; flowery), 2-phenylethanol (30), 2-methoxy-4-371 propylphenol (37; phenolic), 4-ethylphenol (38; phenolic), and decanoic acid (40; 372 soapy, musty; all 512).

Quantitation of the Aroma-Active Compounds. AEDA is a screening method to reduce the huge amount of volatiles to a reduced set of aroma-active substances, which have to be quantitated for the characterization of the overall aroma of a certain food. Thus, 44 odorants, showing an FD factor \geq 64 in at least one of the analyzed process steps, were quantitated by means of SIDA and enzyme kits (acetic acid and ethanol).

379 *Concentrations of the Odorants in Molasses.* The highest concentration was 380 determined for acetic acid (2.6 g/kg), followed by butanoic acid (8.12 mg/kg), 3Journal of Agricultural and Food Chemistry

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381 methyl-1-butanol (6.57 mg/kg), methylpropanol (3.40 mg/kg), phenylacetic acid 382 (2.79 mg/kg), (S)-2-methyl-1-butanol (2.15 mg/kg), and vanillin (1.09 mg/kg). The 383 acids 3-methylbutanoic acid (764 μ g/kg), (S)-2-methylbutanoic acid (571 μ g/kg), 384 decanoic acid (242 μ g/kg), and (*R*)-2-methylbutanoic acid (108 μ g/kg), the aldehydes 385 (S)-2-methylbutanal (519 µg/kg), (R)-2-methylbutanal (449 µg/kg), 3-methylbutanal 386 (388 µg/kg), and 3-(methylthio)propanal (255 µg/kg) as well as the furanones 4-387 hydroxy-2,5-dimethylfuran-3(2H)-one (734 µg/kg) and 3-hydroxy-4,5-dimethylfuran-388 2(5H)-one (216 µg/kg) showed concentrations > 100 µg/kg (**Table 3**).

389 *Concentrations of the Odorants in the Mash.* In the mash, ethanol showed by far 390 the highest concentration (75.5 g/L), followed by acetic acid (3.21 g/L), 3-methyl-1-391 butanol (248 mg/L), methylpropanol (86.3 mg/L), (*S*)-2-methyl-1-butanol (54.4 mg/L), 392 and 2-phenylethanol (43.6 mg/L). Lower amounts were found for the acids butanoic 393 acid (3.48 mg/L), 3-methylbutanoic acid (1.55 mg/L), (*S*)-2-methylbutanoic acid 394 (1.37 mg/L), phenylacetic acid (1.14 mg/L), decanoic acid (721 µg/L), and (*R*)-2-395 methylbutanoic acid (93.3 µg/L) (**Table 3**).

396 *Concentrations of the Odorants in the Distillate.* By far the highest concentration 397 was again determined for ethanol (620 g/L), followed by 3-methyl-1-butanol 398 (2.86 g/L), methylpropanol (732 mg/L), (*S*)-2-methyl-1-butanol (586 mg/L), 1,1-399 diethoxyethane (79.9 mg/L), and acetic acid (43.6 mg/L). Besides, 2,3-butanedione 400 (9.65 mg/L), ethyl octanoate (7.86 mg/L), 3-methylbutyl acetate (6.12 mg/L), 2-401 phenylethanol (3.54 mg/L), 3-methylbutanal (3.49 mg/L), and (*S*)-2-methylbutanal 402 (1.68 mg/L) were determined with high concentrations (**Table 3**).

403 *Concentrations of the Odorants in the Rum.* As expected, ethanol showed by far 404 the highest concentration (317 g/L). In a concentration range between 1 mg/L and 405 1 g/L, 3-methyl-1-butanol (909 mg/L), methylpropanol (183 mg/L), (*S*)-2-methyl-1-406 butanol (148 mg/L), acetic acid (121 mg/L), 1,1-diethoxyethane (20.8 mg/L), ethyl

407 octanoate (3.61 mg/L), 2-phenylethanol (2.67 mg/L), 3-methylbutanal (2.08 mg/L), 3-408 methylbutyl acetate (1.86 mg/L), 4-ethylphenol (1.45 mg/L), decanoic acid 409 (1.24 mg/L), and vanillin (1.20 mg/L) were determined. Somewhat lower amounts 410 were found for the ethyl esters (e.g., ethyl butanoate (441 μ g/L), ethyl hexanoate 411 $(424 \mu g/L)$, and ethyl pentanoate $(36.6 \mu g/L)$ and the phenols (e.g., 2-412 methoxyphenol (399 µg/L), 4-ethyl-2-methoxyphenol (110 µg/L), and 4-methylphenol 413 (32.3 µg/L)). In addition, *cis*- and *trans*-whisky lactone were analyzed in the rum with 414 quite high concentrations (cis: 210 µg/L; trans: 191 µg/L) (Table 3).

415 Calculation of Odor Activity Values of the Odorants in Rum. To evaluate 416 which odorants contribute to the overall aroma of rum, odor activity values (OAVs; 417 ratio of concentration to respective odor threshold) were calculated (**Table 4**). By far 418 the highest OAV in rum was determined for (E)- β -damascenone (3280), followed by 419 3-methylbutanal (743), ethanol (320), 2,3-butanedione (188), ethyl (S)-2-420 methylbutanoate (97), vanillin (55), ethyl butanoate (46), (S)-2-methylbutanal (44), 421 and 2-methoxyphenol (43). Only 11 of 41 compounds showed an OAV < 1, e.g., 422 butanoic acid, trans-whisky lactone, and 4-methylphenol, and thus, these compounds 423 should not contribute to the overall aroma. The results were in good agreement with previously reported key odorants in rum.^{9,10} 424

Aroma Simulation. Recombination experiments were performed to verify that all key odorants contributing to the overall aroma of rum were successfully identified and quantitated. Therefore, all 30 aroma compounds showing an OAV \geq 1 were added to an odorless rum matrix in their determined naturally occurring concentrations in a mixture of ethanol/water (40/60 by vol.). The recombinate was evaluated in parallel with the original rum by a trained sensory panel using aroma profile analysis and showed a very good similarity (**Figure 2**). Page 19 of 53

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432 Changes of the Concentrations of the Key Odorants during the Single 433 **Processing Steps.** To enable a comparison of the concentrations of the aroma-434 active compounds during the process, data are shown on the basis of the production 435 of 1 L of rum, for which 0.72 L of molasses were needed, resulting in 2.9 L of mash 436 and 0.4 L of distillate, respectively.

437 Changes during Fermentation. 0.72 L of molasses was used for the production of 438 1 L of rum, resulting in 2.9 L of mash. A significant change in the concentrations was 439 determined for (R)-2-methyl-1-butanol, (S)-2-methyl-1-butanol, 3-methyl-1-butanol, 440 methylpropanol, and 2-phenylethanol. Except of (R)-2-methyl-1-butanol, the 441 concentrations of the alcohols clearly increased (Table 5). Following the Ehrlich pathway, amino acids can react to the corresponding odor-active alcohols.⁴¹ First, an 442 443 enzymatic deamination leads to the respective α -keto acid, followed by a 444 decarboxylation and a reduction step to the respective alcohols. Further reactions 445 can also lead to the corresponding aldehydes and acids. As the reaction is catalyzed 446 by enzymes, the degradation is stereo specific. Hence, the degradation of L-447 isoleucine during fermentation led to an increased concentration of (S)-2-methyl-1-448 butanol, whereas (R)-2-methyl-1-butanol decreased due to secondary reactions. This 449 finding was in agreement with a recent study also revealing 99% of (S)-2-methyl-1butanol in spirits.¹⁷ 450

The differences in FD factors during AEDA found for esters were confirmed by increased concentrations during fermentation. The fatty acid metabolism of yeasts leads to the formation of ethyl esters due to the reaction of acyl-CoA and ethanol.⁴² Depending on the yeast strain, different concentrations of the esters 3-methylbutyl acetate, ethyl butanoate, ethyl hexanoate, and ethyl decanoate were formed.⁴³ An increase of the branched chain esters ethyl (*R*)-2-methylbutanoate, ethyl (*S*)-2methylbutanoate, and ethyl 3-methylbutanoate was also shown during fermentation

458 in the production of Williams Christ pear brandy.⁴⁴ Ethanol can react with 2- and 3-459 methylbutanoic acid, stemming from amino acid degradation following the Ehrlich 460 pathway. Consequently, (*R*)-2- and (*S*)-2-methylbutanoic acid and the corresponding 461 ethyl esters showed almost the same ratio. An increase by a factor of 8 was shown 462 for the concentration of ethyl cyclohexanoate, which was already determined in 463 wines, brandy, and whiskys.⁴⁵

464 Interestingly, also an increase of the phenols (2-methoxyphenol, 2-methoxy-4-465 propylphenol, 4-methylphenol, 4-ethyl-2-methoxyphenol, and 4-ethylphenol) was found. Steinke and Paulson⁴⁶ postulated the formation of 4-ethylphenol and 4-ethyl-466 2-methoxyphenol from *p*-coumaric acid and ferulic acid. Chatonnet et al.⁴⁷ suggested 467 468 a formation pathway by yeasts: after decarboxylation of the acids, the phenols are 469 reduced by vinylphenol reductase. The formation of 2-methoxyphenol, 4-ethyl-2-470 methoxyphenol, and 4-methylphenol was also shown in a synthetic must containing a 471 precursor extract obtained from four different non-floral grape varieties. However, the formation and the concentrations were strongly dependent on the used yeast.⁴⁸ 472

The concentration of (*R*)-linalool increased by a factor of 48 compared to only 8 for the (*S*)-enantiomer, indicating that the enzyme-catalyzed release may occur stereo specifically from the respective precursors.

476 Changes during Distillation. 2.9 L of mash yielded 0.4 L of distillate and, thereby, 477 the concentrations of 61% of the quantitated odorants decreased (Table 6). 478 Compounds showing a higher or lower boiling point compared to ethanol might be 479 discriminated during the distillation process leading to a loss of aroma. A significant 480 decrease was shown for the acids (butanoic acid, (R)-2-methylbutanoic acid, (S)-2-481 methylbutanoic acid, acetic acid, phenylacetic acid, and decanoic acid) and the 482 phenols (2-methoxyphenol, 4-ethyl-2-methoxyphenol, 2-methoxy-4-propylphenol, 4-483 methylphenol, 4-ethylphenol, and vanillin), but also for the both furanones (3Journal of Agricultural and Food Chemistry

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hydroxy-4,5-dimethylfuran-2(5*H*)-one and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one),
and 2-phenylethanol (**Table 6**). A significant decrease of 2-phenylethanol and vanillin
during simultaneous distillation/extraction (SDE) was already shown for a model
solution.⁶

The highest increase was determined for the concentration of 1,1-diethoxyethane (factor of 20). In a model experiment, a solution of acetaldehyde (pH = 4.5; 10 Vol.-% EtOH) was distilled for 60 min. However, no significant increase of the concentration of 1,1-diethoxyethane was determined,⁶ although the conditions for the formation of the acetal of acetaldehyde and ethanol were given. Thus, 1,1diethoxyethane is probably released during distillation from precursors of the mash or catalysts from the mash promote its formation.

The concentration of 2,3-butanedione increased by a factor of 12 indicating a thermal induced formation. A possible pathway was postulated by an aldol reaction from acetaldehyde and hydroxyacetaldehyde (glycolaldehyde).⁴⁹

An increase by a factor of 4 was determined for (*E*)- β -damascenone, which confirmed data obtained for unheated and heated grapes.⁵⁰ The formation rate was higher if heat treatment was performed under acidic conditions. Therefore, the formation or the release of (*E*)- β -damascenone from precursors present in the mash during the distillation is very likely. Interestingly, the concentrations of (*R*)- and (*S*)linalool increased by ~30 µg/L each, showing that the acidic conditions and the heat treatment enabled a non-stereo specific release of linalool from precursors.

In addition, the aldehydes (*R*)-2-, (*S*)-2-, and 3-methylbutanal showed higher concentrations in the distillate compared to the mash. The heat-induced formation of these Strecker aldehydes by oxidative decarboxylation of amino acids is well-known. An increase of the concentration of the amino acids revealed an increase of the concentration of the aldehydes.⁵¹ During distillation, the concentrations of (*R*)-2- and

510 (*S*)-2-methylbutanal rose almost by the same factor indicating that the formation was 511 thermally induced and, consequently, not stereo specific. However, the concentration 512 of 3-(methylthio)propanal decreased, which was already shown in another study on 513 American whisky,⁶ concluding that the higher boiling point leads to a discrimination 514 during distillation.

515 Changes during Ageing. 0.4 L of the distillate are necessary for the production of 516 1 L of rum. Cis- and trans-whisky lactone were detected during AEDA of the rum for 517 the first time in the process and were determined with guite high concentrations (cis-518 whisky lactone: 210 µg/L; trans-whisky lactone: 191 µg/L; Table 7). These aroma compounds derived from the oak barrels, which are commonly used for ageing.⁵² The 519 520 concentrations strongly depended on the used wood, on the ageing conditions, and on the working life of the barrel.⁵³⁻⁵⁵ Lower concentrations were found for *cis*- and 521 trans-whisky lactones in wines, which were stored in used barrels.⁵³ In contrast, 522 523 higher concentrations were found in Merlot (pH = 3.7; 13.6 Vol.-% ethanol) compared 524 to Cabernet Sauvignon (pH = 3.45; 12.3 Vol-% ethanol) differing in pH value and 525 ethanol content.⁵⁴ In addition, wines aged in European wood barrels showed a ratio 526 of cis- and trans-whisky lactone between 1 and 1.5, whereas the ratio of wines aged in American wood barrels was between 5 and 8.55 In the actual study, a ratio of 1.1 527 528 was determined confirming on a molecular level the ageing in barrels produced of 529 European wood (Spessart).

A significant increase was also shown for the phenols 4-ethyl-2-methoxyphenol, 2-methoxyphenol, 4-propyl-2-methoxyphenol, 4-methylphenol, 4-ethylphenol, and vanillin. During barrel making, the formation of these phenolic compounds occurs due to the thermal treatment and are extracted during ageing.^{56,57} To a lesser extent, an increase of the phenol concentrations might also be caused by contaminations of the yeasts used. Microbial activity can lead to the degradation of ferulic acid and *p*-

coumaric acid resulting in the formation of ethylphenols, which was already shown for
 wine.⁴⁷

538 The concentration of 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one increased by a 539 factor of 49. The formation from acetaldehyde and α -ketobutyric acid was already 540 shown in a synthetic wine by aldol condensation, followed by cyclization.⁵⁸ Thereby, 541 α -ketobutyric acid is formed from threonine by enzyme-catalyzed dehydratization and 542 deamination.

543 Also increased concentrations of ethyl esters, like ethyl butanoate, ethyl (S)-2-544 methylbutanoate, ethyl pentanoate, ethyl 3-phenylpropanoate, ethyl (*R*)-2-545 methylbutanoate, ethyl 3-methylbutanoate, and ethyl cyclohexanoate were found. 546 Only the concentrations of ethyl hexanoate and ethyl octanoate were more or less 547 the same after ageing and 3-methylbutyl acetate was present even to a lesser extent, which was already reported in studies on wines.^{59,60} In a model study, 2- and 3-548 549 methylbutanoic acid, methylpropanoic acid, butanoic acid, and hexanoic acid were 550 stored for 479 days in an ethanolic solution (40 Vol.-%) spiked with white oak wood 551 chips. Due to the reaction of ethanol and the respective acids, a continuous increase 552 of the ethyl esters was determined. In contrast, in a solution without white oak wood chips the concentrations only marginally increased.⁶ 553

A small increase of (*R*)-2-, (*S*)-2-, and 3-methylbutanal was found, whereas 3-(methylthio)propanal showed an increase by a factor of 8. In an ethanolic solution (12 Vol.-%) of amino acids (valine, leucine, isoleucine, methionine, and phenylalanine) and α -dicarbonyls (pH 3.5; 25 °C; in the dark), Pripis-Nicolau et al.⁶¹ reported about the formation of the respective aldehydes following the Strecker reaction, even at these moderate temperatures. In the actual study, a non-stereo specific formation (decarboxylation of isoleucine) of 2-methylbutanal probably

561 occurred ((*R*)-enantiomer: + 243 μ g; (*S*)-enantiomer: + 213 μ g; **Table 7**) leading to a 562 changed enantiomeric ratio of 24/76 (*R*/*S*) in the distillate to 34/66 (*R*/*S*) in the rum. 563 In contrast, a significant decrease by almost 90% was determined for the highly

563 In contrast, a significant decrease by almost 90% was determined for the highly 564 volatile 2,3-butanedione, already proven in a model solution also showing a decrease 565 of 90% after five months.⁶

566 **Changes of the Key Odorants during the Whole Process.** In the following, a 567 short overview is given about the processing steps mostly influencing the formation 568 of the specific aroma compounds. The concentration trends of selected compounds 569 during the whole production process are summarized in **Table 8**. In addition, a factor 570 representing the ratio of the concentration in the rum (1 L) divided by the respective 571 concentration in the molasses (0.72 L, initial amount needed for the production of 1 L 572 of rum) was calculated.

573 (*E*)- β -Damascenone was determined as key aroma compound in the rum and 574 showed by far the highest OAV (3280). A concentration leading to a high OAV was 575 already determined in the molasses (87 µg/0.72 L). The fermentation and the ageing 576 showed almost no influence, but during distillation a significant increase of the 577 concentration was determined.

For (*R*)-2, (*S*)-2-, and 3-methylbutanal, 2,3-butanedione, and 1,1-diethoxyethane, an OAV \geq 1 was determined in rum. The aldehydes increased during distillation and ageing, whereas 2,3-butanedione and 1,1-diethoxyethane increased during fermentation and distillation.

582 Vanillin, 2-methoxyphenol, 4-ethyl-2-methoxyphenol, 2-methoxy-4-propylphenol, 583 and 4-ethylphenol were already determined in the molasses. Their concentrations 584 increased during fermentation and significantly decreased during distillation. 585 However, during ageing in the wood barrels, their concentrations increased again. Journal of Agricultural and Food Chemistry

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Most of the ethyl esters (e.g., ethyl butanoate, ethyl 2-methylbutanoate, ethyl hexanoate) and the alcohols (e.g., (*S*)-2-, 3-methyl-1-butanol, methylpropanol, and 2phenylethanol) were formed during fermentation in very high concentrations. Although they were discriminated during distillation, an OAV \geq 1 was determined in rum.

4-Hydroxy-2,5-dimethyl-3-(2*H*)-furanone and 3-hydroxy-4,5-dimethylfuran-2(5*H*)one showed a relative high concentration in the molasses, but, a quantitative transfer
from the raw material into the rum was not found.

594 In summary, the present study demonstrated for the first time the importance 595 and the high influence of the single processing steps on the key aroma compounds in 596 rum. The concentrations of 68% of the quantitated aroma-active compounds 597 increased, e.g., ethyl esters, phenols, and alcohols. In contrast, a decrease was 598 shown for the acids. With these data at hand, manufacturer can draw conclusions at 599 which step they have potential to optimize their product in matters of its overall 600 aroma, which is an important quality criteria for consumers. However, also the raw 601 material might have a significant influence on the key aroma compounds due to the 602 fact that many compounds might be released or formed from precursors present in 603 the raw material. Thus, further studies on aroma-active compounds in rums produced 604 from molasses or sugar cane juice are currently undertaken.

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788 **Figure Captions**

- 789
- 790 Figure 1. Aroma profile analyses of molasses (solid line), mash (broken line),
- 791 distillate (dotted line), and rum (dashed line).
- 792
- **Figure 2**. Aroma profile analyses of the original rum (solid line) and the respective
- aroma recombinate (dotted line).

Table 1. Stable Isotopically Labeled Standards, Selected Ions, and ResponseFactors (R_f) used in the Stable Isotope Dilution Assays

	isotope	ion		
compound	lahel	analyte	internal	R_{f}^{b}
	label	analyte	standard	
acetic acid	¹³ C ₂	61	63	0.76
2,3-butanedione	¹³ C ₄	87	91	0.99
butanoic acid	$^{2}H_{2}$	87	89	1.00
(<i>E</i>)-β-damascenone	${}^{2}H_{4-7}{}^{c}$	191	195-198 ^c	0.91
(E,E)-2,4-decadienal	² H ₂₋₄ ^c	153	155-157 ^c	0.60
decanoic acid	$^{2}H_{2}$	187	189	0.89
1,1-diethoxyethane	¹³ C ₂	73	75	0.90
ethyl butanoate	$^{2}H_{3}$	117	120	0.98
ethyl cyclohexanoate	$^{2}H_{3}$	157	160	1.00
ethyl hexanoate	$^{2}H_{3}$	145	148	0.98
4-ethyl-2-methoxyphenol	² H ₂₋₄ ^c	153	155-157 ^c	0.66
ethyl 2-methylbutanoate	$^{2}H_{3}$	131	134	0.99
ethyl 3-methylbutanoate	$^{2}H_{9}$	131	140	1.00
ethyl octanoate	$^{2}H_{3}$	173	176	0.96
ethyl pentanoate	$^{2}H_{5}$	131	136	0.96
3-ethylphenol	$^{2}H_{2}$	123	125 ^d	0.87
4-ethylphenol ^d	_d	123	125 ^d	0.87
ethyl 3-phenylpropanoate	$^{2}H_{5}$	179	184	1.00
3-hydroxy-4,5-dimethylfuran-	¹³ C ₂	129	131	0.70
2(5 <i>H</i>)-one				
4-hydroxy-2,5-dimethylfuran-	¹³ C ₂	129	131	0.99
3(2 <i>H</i>)-one				
linalool	$^{2}H_{2}$	137	139	1.00
2-methoxyphenol	$^{2}H_{3}$	125	128	0.98
2-methoxy-4-propylphenol	² H ₂₋₄ ^c	167	169-171 ^c	0.98
2-methylbutanal	$^{2}H_{2}$	87	89	0.88
3-methylbutanal	$^{2}H_{2}$	87	89	0.98
2-methylbutanoic acid	$^{2}H_{9}$	103	112	0.88

796 **Table 1.** Continued

	isotope	ion		
compound	labol	analyta	internal	R_{f}^{b}
			standard	
3-methylbutanoic acid	$^{2}H_{2}$	103	105	0.98
2-methyl-1-butanol ^e	_e	71	73 ^e	0.84
3-methyl-1-butanol	$^{2}H_{2}$	71	73	0.99
3-methylbutyl acetate	${}^{2}H_{11}$	131	142	0.90
4-methylphenol	$^{2}H_{7}$	109	116	0.79
methylpropanol	² H ₉	57	66	0.62
3-(methylthio)propanal	$^{2}H_{3}$	105	108	0.82
1-octen-3-one	² H ₂₋₄ ^c	127	129-131 [°]	0.99
phenylacetic acid	¹³ C ₂	137	139	0.94
2-phenylethanol	$^{2}H_{5}$	105	110	0.71
vanillin	$^{2}H_{3}$	153	156	1.00
<i>cis</i> -whisky lactone	$^{2}H_{2}$	157	159	0.79
<i>trans</i> -whisky lactone ^f	_f	157	159 ^f	1.00

^a lons used for quantitation. ^b Response factor determined by analyzing defined mixtures of analyte and internal standard. ^c The internal standard was used as a mixture of isotopologues. ^d Quantitation of 4-ethylphenol was performed using [²H₂]-3-ethylphenol as internal standard. ^e Quantitation of 2-methyl-1-butanol was performed using [²H₂]-3-methyl-1-butanol as internal standard. ^f Quantitation of *trans*-whisky lactone was performed using [²H₂]-*cis*-whisky lactone as internal standard.

no ^a	aampaund ^b	d ^b oder quality ^c	RI ^d		FD factors ^e			
10.	compound		DB-FFAP	DB-5	molasses	mash	distillate	rum
1a,b	2- and 3-methylbutanal	malty	930	658	64	16	128	256
2	2,3-butanedione	butter-like	980	593	512	256	1024	256
3	1,1-diethoxyethane	fruity	1013	733	32	64	256	64
4	ethyl butanoate	fruity	1033	804	32	512	256	256
5	ethyl 2-methylbutanoate	fruity	1047	854	8	1024	512	512
6	ethyl 3-methylbutanoate	blueberry-like	1067	861	< 8	256	128	256
7	methylpropanol	malty	1093	640	8	128	256	256
8	3-methylbutyl acetate	banana-like	1115	879	64	256	1024	512
9	ethyl pentanoate	fruity	1133	903	< 8	16	128	256
10a,b	2- and 3-methyl-1-butanol	malty	1213	752	512	2048	2048	2048
11	ethyl hexanoate	fruity	1230	1001	< 8	256	256	64
12	(Z)-4-heptenal	fishy, fish oil-like	1236	901	< 8	< 8	32	16
13	1-octen-3-one	mushroom-like	1296	983	32	32	256	128
14	ethyl cyclohexanoate	fruity, sweet	1409	1136	< 8	256	512	1024
15	ethyl octanoate	fruity, green	1426	1197	64	128	512	1024
16	acetic acid	vinegar-like	1443	610	1024	512	16	32
17	3-(methylthio)propanal	cooked potato-like	1457	905	1024	1024	512	256

Table 2. Comparison of FD Factors of the Most Important Aroma-Active Compounds in Molasses, Mash, Distillate, and Rum

798 **Table 2**. Continued

no ^a compound ^b	odor quality ^c	RI ^d	RI ^d		FD factors ^e			
10.	compound	odor quanty	DB-FFAP	DB-5	molasses	mash	distillate	rum
18	3-ethyl-2,5-dimethylpyrazine ^f	earthy	1437	nd	32	32	32	32
19	linalool	citrus-like, flowery	1539	1102	32	128	256	64
20	butanoic acid	sweaty	1632	821	1024	1024	16	8
21	phenylacetaldehyde	honey-like	1638	1045	32	32	32	32
22a,b	2- and 3-methylbutanoic acid	sweaty	1668	874	1024	1024	64	32
23	(E,E)-2,4-decadienal	fatty, deep-fried	1800	1323	< 8	< 8	64	128
24	(E)-β-damascenone	baked apple-like, grape	1811	1389	32	16	64	128
		juice-like						
25	unknown	spicy	1815	nd	< 8	128	< 8	< 8
26	unknown	caramel-like, spicy	1825	nd	512	64	16	< 8
27	2-methoxyphenol	gammon-like, smoky	1860	1090	2048	1024	2048	2048
28	ethyl 3-phenylpropanoate	flowery	1875	1350	< 8	256	128	512
29	trans-whisky lactone	coconut-like	1890	1300	< 8	< 8	< 8	1024
30	2-phenylethanol	flowery, honey-like	1911	1116	32	1024	256	512
31	4-methyl-2-methoxyphenol	clove-like, smoky	1190	1966	32	32	< 8	< 8
32	cis-whisky lactone	coconut-like	1950	1331	< 8	< 8	< 8	2048
33	γ-nonalactone	coconut-like	2029	1360	< 8	32	8	32

800 Table 2. Continued

no a	compound ^b	odor quality ^c		RI ^d		FD factors ^e		
no.	compound		DB-FFAP	DB-5	molasses	mash	distillate	rum
34	4-hydroxy-2,5-dimethylfuran-	caramel-like	2030	1071	1024	256	<8	< 8
	3(2 <i>H</i>)-one							
35	4-ethyl-2-methoxyphenol	smoky, gammon-like	2029	1284	32	64	256	1024
36	4-methylphenol	fecal, horse stable-like	2094	1077	128	256	32	256
37	2-methoxy-4-propylphenol	phenolic	2106	1306	< 8	32	128	512
38	4-ethylphenol	phenolic	2182	1169	32	128	16	512
39	3-hydroxy-4,5-dimethylfuran-	seasoning-like, spicy	2206	1108	512	128	128	256
	2(5 <i>H</i>)-one							
40	decanoic acid	soapy, musty	2266	1373	< 8	64	32	512
41	phenylacetic acid	beeswax-like, honey-like	2555	1261	128	256	64	64
42	vanillin	vanilla-like	2573	1406	2048	128	16	1024

^a Odorants were consecutively numbered according to their retention indices on capillary DB-FFAP. ^b Odorant identified by comparison of its odor quality and intensity, retention indices on capillaries DB-FFAP and DB-5 as well as mass spectra (EI and CI mode) with data of reference compounds. ^c Odor quality detected at sniffing port. ^d Retention indices determined using a homologous series of *n*-alkanes. ^e Flavor dilution factors determined by AEDA using a DB-FFAP column. ^f No unequivocal mass spectrum (EI mode) was obtained. Identification was performed by the remaining criteria mentioned in footnote b. nd: not determined.

Table 3. Concentrations of the Most Important Aroma-Active Compounds inMolasses, Mash, Distillate, and Rum

compound	concentrations ^a (µg/L)					
compound	molasses ^b	mash	distillate	rum		
ethanol	nd	75500000	620000000	317000000		
acetic acid	2600000	3210000	43600	121000		
butanoic acid	8120	3480	640 ^c	462 ^c		
3-methyl-1-butanol	6570	248000	2860000	909000		
methylpropanol	3400	86300	732000	183000		
phenylacetic acid	2790	1190 ^c	26.6 ^c	35.8		
(S)-2-methyl-1-butanol	2150	54400	586000	148000		
Vanillin	1090	412 ^c	27.0	1200		
3-methylbutanoic acid	764	1550	354	368		
4-hydroxy-2,5-	734	1820 ^c	14.3 ^c	3.54 ^c		
dimethylfuran-3(2H)-one						
(S)-2-methylbutanoic acid	571	1370	365	216		
(S)-2-methylbutanal	519	49.9	1680	885		
(R)-2-methylbutanal	449	12.9	529	456		
(R)-2-methyl-1-butanol	409	nd	nd	nd		
3-methylbutanal	388	103	3490	2080		
3-(methylthio)propanal	255	91.3	1.06 ^c	3.36 ^c		
decanoic acid	242	721 ^c	654	1240		
3-hydroxy-4,5-	216	53.9	0.16 ^c	2.95		
dimethylfuran-2(5H)-one						
2,3-butanedione	188	111	9650	526		
1,1-diethoxyethane	155	560	79900	20800		
2-phenylethanol	114	43600	3540	2670		
(R)-2-methylbutanoic acid	108	93.9	29.7	22.7		
(<i>E</i>)-β-damascenone	94.0	21.0	558	328		
2-methoxyphenol	64.0	178	169	399		
ethyl butanoate	34.0	110	445	441		
3-methylbutyl acetate	13.7	634	6120	1860		

Table 3. Continued

compound	concentrations ^a (µg/L)				
compound .	molasses ^b	mash	distillate	rum	
ethyl octanoate	13.6	399	7860	3610	
ethyl hexanoate	12.8	83.0	975	424	
4-ethylphenol	6.67	140 ^c	50.2	1450	
4-methylphenol	6.30	33.8	5.94	32.3	
4-ethyl-2-methoxyphenol	2.45	28.3	77.7	110	
1-octen-3-one	1.74	4.86 ^c	11.4 ^c	1.01 ^c	
2-methoxy-4-	1.53	5.20	19.0	60.5	
propylphenol					
(S)-linalool	1.52	4.53	64.3	23.3	
ethyl pentanoate	1.27	5.95	27.5	36.6	
(R)-linalool	1.04	16.4	144	25.5	
ethyl (S)-2-	0.93	3.67	28.4	19.4	
methylbutanoate					
ethyl 3-methylbutanoate	0.71	2.00	13.0	32.0	
(E,E)-2,4-decadienal	0.69	1.62 ^c	9.31 ^c	2.32	
ethyl 3-phenylpropanoate	0.44 ^c	3.93	21.3	26.7	
ethyl cyclohexanoate	0.16 ^c	0.39 ^c	0.91 ^c	1.07	
ethyl (<i>R</i>)-2-	0.05	0.23	0.58	0.60	
methylbutanoate					
<i>cis</i> -whisky lactone	nd	nd	nd	210	
trans-whisky lactone	nd	nd	nd	191	

^a Mean values of triplicates; RSD \leq 5%. ^b Concentrations were determined in µg/kg.

^c Mean values of duplicates, differing not more than 12%. nd: not detected.

Table 4. Concentrations, Orthonasal Odor Thresholds, and Odor Activity Values (OAVs) of the Key Aroma-Active Compounds (OAV ≥ 1) in Rum

	concentration ^a	odor threshold ^b	OAV ^c
compound	(µg/L)	(µg/L)	
(<i>E</i>)-β-damascenone	328	0.1 ^d	3280
3-methylbutanal	2080	2.8 ^d	743
ethanol	317000000	990000 ^e	320
2,3-butanedione	526	2.8 ^d	188
ethyl (S)-2-methylbutanoate	19.4	0.2 ^d	97
vanillin	1200	22	55
ethyl butanoate	441	9.5 ^d	46
(S)-2-methylbutanal	885	20	44
2-methoxyphenol	399	9.2 ^d	43
1,1-diethoxyethane	20800	719 ^d	29
3-hydroxy-4,5-dimethylfuran-2(5H)-one	2.95	0.11	27
ethyl octanoate	3610	147	25
(S)-2-methyl-1-butanol	148000	6100	24
ethyl 3-methylbutanoate	32.0	1.6 ^d	20
4-ethyl-2-methoxyphenol	110	6.9 ^d	16
3-methyl-1-butanol	909000	56100 ^d	16
ethyl hexanoate	424	30	14
2-methoxy-4-propylphenol	60.5	4.4	14
3-methylbutyl acetate	1860	245 ^d	8
4-ethylphenol	1450	173	8
3-methylbutanoic acid	368	78	5
ethyl cyclohexanoate	1.07	0.3	4
(R)-2-methylbutanal	456	110	4
ethyl pentanoate	36.6	11	3
<i>cis</i> -whisky lactone	210	67 ^g	3
linalool	48.8	24 ^f	2
ethyl 3-phenylpropanoate	26.7	14	2
(E,E)-2,4-decadienal	2.32	1.1 ^d	2
2-phenylethanol	2670	2600 ^d	1

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Compound	concentration ^a	odor threshold ^b	OAV ^c
Compound	(µg/L)	(µg/L)	
methylpropanol	183000	160000	1
acetic acid	121000	230000	< 1
3-(methylthio)propanal	3.36 ^h	6.8	< 1
decanoic acid	1240	2800	< 1
1-octen-3-one	1.01 ^h	2.6	< 1
4-methylphenol	32.3	89	< 1
<i>trans</i> -whisky lactone	191	790 ^g	< 1
butanoic acid	462 ^h	6900	< 1
(S)-2-methylbutanoic acid	216	3500	< 1
phenylacetic acid	35.8	1400	< 1
4-hydroxy-2,5-dimethylfuran-3(2H)-one	3.54 ^h	350	< 1
(R)-2-methylbutanoic acid	22.7	13000	< 1

^a Mean values of triplicates, RSD $\leq 5\%$. ^b Odor thresholds were determined in ethanol/water (40/60 by vol.) by an in-house sensory panel. ^c Odor activity values were calculated by dividing the concentrations by the respective odor thresholds. ^d Odor threshold previously reported in reference 36. ^e Odor threshold was determined in water. ^f Odor threshold was determined for the racemate. ^g Odor threshold previously reported in reference 51. ^h Mean values of duplicates, differing not more than 12%.

Table 5. Concentrations of the Most Important Aroma-Active Compounds inMolasses and Mash. Calculated Factor Represents the Changes in theConcentrations during Fermentation

	concentrations				
compound	molasses ^a	molasses ^b	mash	factor ^c	
	(µg/L)	(µg/0.72 L)	(µg/2.9 L)		
2-phenylethanol	148	106	125000	1180	
3-methylbutyl acetate	17.7	12.7	1810	143	
3-methyl-1-butanol	8510	6080	709000	117	
ethyl octanoate	17.6	12.6	1140	90	
(S)-2-methyl-1-butanol	2780	1990	156000	78	
methylpropanol	4400	3150	247000	78	
4-ethylphenol	8.64	6.18	400	65	
(R)-linalool	1.35	0.96	46.9	49	
4-ethyl-2-methoxyphenol	3.17	2.27	80.9	36	
ethyl 3-phenylpropanoate	0.57	0.41	11.2	27	
ethyl hexanoate	16.6	11.9	237	20	
4-methylphenol	8.16	5.83	96.7	17	
ethyl pentanoate	1.64	1.18	17.0	14	
ethyl (<i>R</i>)-2-	0.06	0.05	0.66	13	
methylbutanoate					
ethyl (S)-2-	1.20	0.86	10.5	12	
methylbutanoate					
1,1-diethoxyethane	201	144	1600	11	
2-methoxy-4-propylphenol	1.98	1.42	14.9	11	
ethyl butanoate	44.0	31.5	315	10	
(S)-linalool	1.97	1.41	13.0	9	
1-octen-3-one	2.25	1.61	13.9	9	
2-methoxyphenol	82.9	59.3	509	9	
decanoic acid	313	224	2060	9	
4-hydroxy-2,5-	951	680	5200	8	
dimethylfuran-3(2H)-one					

Table 5. Continued

		concentrations		
compound	molasses ^a	molasses ^b	mash ^c	factor ^d
	(µg/L)	(µg/0.72 L)	(µg/2.9 L)	
ethyl 3-methylbutanoate	0.92	0.66	5.72	9
ethyl cyclohexanoate	0.21	0.15	1.12	7
(E,E)-2,4-decadienal	0.89	0.64	4.60	7
(S)-2-methylbutanoic acid	739	529	3920	7
3-methylbutanoic acid	989	707	4430	6
acetic acid	3370000	2410000	9180000	4
(R)-2-methylbutanoic acid	140	100	269	3
2,3-butanedione	243	174	317	2
butanoic acid	10500	7520	9950	1.3
phenylacetic acid	3610	2580	3400	1.3
vanillin	1410	1010	1180	1.2
3-(methylthio)propanal	330	236	261	1.1
3-methylbutanal	502	359	295	0.8
3-hydroxy-4,5-	280	200	154	0.8
dimethylfuran-2(5H)-one				
(E)-β-damascenone	122	87.0	60.1	0.7
(S)-2-methylbutanal	672	481	143	0.3
(R)-2-methylbutanal	581	416	36.9	0.1
(R)-2-methyl-1-butanol	529	378	nd	-

^a Concentrations were converted from μg/kg into μg/L using the density of 1.295 g/L. ^b Concentrations in 0.72 L of molasses, which was the amount used for the production of 1 L of rum. ^c Concentrations in 2.9 L of mash, resulting from the fermentation of 0.72 L of molasses. ^d Factor was calculated by dividing the concentration of the compound in the mash (2.9 L) by the respective concentration in the molasses (0.72 L). nd: not detected.

Table 6. Concentrations of the Most Important Aroma-Active Compounds in Mash

 and Distillate. Calculated Factor Represents the Changes in the Concentrations

 during Distillation

	conce		
compound	mash ^a	distillate ^b	factor ^c
	(µg/2.9 L)	(µg/0.4 L)	
1,1-diethoxyethane	1600 32000		20
2,3-butanedione	317	3860	12
(R)-2-methylbutanal	36.9	212	6
(S)-2-methylbutanal	143	672	5
3-methylbutanal	295	1400	5
(<i>E</i>)-β-damascenone	60.1	223	4
ethyl octanoate	1140	3140	3
(S)-2-methyl-1-butanol	156000	234000	2
3-methyl-1-butanol	709000	1140000	2
ethyl hexanoate	237	390	2
(S)-linalool	13.0	25.7	2
3-methylbutyl acetate	1810	2450	1.4
methylpropanol	247000	293000	1.2
(R)-linalool	46.9	57.6	1.2
ethyl (S)-2-methylbutanoate	10.5	11.4	1.1
ethanol	216000000	248000000	1.1
ethyl 3-methylbutanoate	5.72	5.20	0.9
(E,E)-2,4-decadienal	4.60	4.60 3.70	
ethyl 3-phenylpropanoate	11.2 8.52		0.8
ethyl butanoate	315	182	0.6
ethyl pentanoate	17.0	11.0	0.6
2-methoxy-4-propylphenol	14.9	7.60	0.5
4-ethyl-2-methoxyphenol	80.9	31.1	0.4
ethyl (R)-2-methylbutanoate	0.66	0.23	0.4
1-octen-3-one	13.9 4.56		0.3
ethyl cyclohexanoate	1.12	0.16	0.1
2-methoxyphenol	509	67.6	0.1

	concen		
compound	mash ^a	distillate ^b	factor ^c
	(µg/2.9 L)	(µg/0.4 L)	
decanoic acid	2060	262	0.1
4-ethylphenol	400	20.1	0.05
(R)-2-methylbutanoic acid	269	11.9	0.04
(S)-2-methylbutanoic acid	3920	146	0.04
3-methylbutanoic acid	4430	142	0.03
butanoic acid	9950	256	0.03
4-methylphenol	96.7	2.38	0.02
2-phenylethanol	125000	1420	0.01
vanillin	1180	10.8	0.01
phenylacetic acid	3400	10.6	0.003
acetic acid	9180000	17440	0.002
3-(methylthio)propanal	261	0.42	0.002
4-hydroxy-2,5-dimethylfuran-3(2H)-	5200	5.72	0.001
one			
3-hydroxy-4,5-dimethylfuran-2(5H)-	154	0.06	0.0004
one			

^a Concentrations in 2.9 L of mash, which was the amount used for the production of 1 L of rum. ^b Concentrations in 0.4 L of distillate, resulting from the distillation of 2.9 L of mash. ^c Factor was calculated by dividing the concentration of the compound in the distillate (0.4 L) by the respective concentration in the mash (2.9 L).

Table 7. Concentrations of the Most Important Aroma-Active Compounds in Distillate

 and Rum. Calculated Factor Represents the Changes in the Concentrations during

 Ageing

compound	concentrat	factor ^C	
compound	distillate ^a (µg/0.4 L)	rum ^b (µg/L)	Taclor
<i>cis</i> -whisky lactone	nd	210	-
<i>trans</i> -whisky lactone	nd	191	-
vanillin	6.80	1200	176
4-ethylphenol	20.1	1450	72
3-hydroxy-4,5-dimethylfuran-	0.06	2.95	49
2(5 <i>H</i>)-one			
4-methylphenol	2.38	32.3	14
3-(methylthio)propanal	0.42	3.36	8
2-methoxy-4-propylphenol	7.60	60.5	8
acetic acid	17400	121000	7
ethyl cyclohexanoate	0.16	1.07	7
2-methoxyphenol	67.6	399	6
ethyl 3-methylbutanoate	5.20	32.0	6
decanoic acid	262	1240	5
4-ethyl-2-methoxyphenol	31.1	110	4
ethyl butanoate	182	441	3
ethyl (R)-2-methylbutanoate	0.23	0.60	3
ethyl 3-phenylpropanoate	8.52	26.7	3
ethyl pentanoate	11.0	36.6	3
phenylacetic acid	10.6	35.8	3
3-methylbutanoic acid	142	368	3
(R)-2-methylbutanal	212	456	2
2-phenylethanol	1420	2670	2
3-methylbutanal	1400	2080	2
butanoic acid	256	462	2
ethyl (S)-2-methylbutanoate	11.4	19.4	2
(R)-2-methylbutanoic acid	11.9	22.7	2
(S)-2-methylbutanoic acid	146	216	1.5

Table 7. Continued				
compound	concentra	factor ^c		
compound	distillate ^a (µg/0.4 L)	rum ^b (µg/L)	lactor	
(E)-β-damascenone	223	328	1.5	
(S)-2-methylbutanal	672	885	1.3	
ethanol	248000000	317000000	1.3	
ethyl hexanoate	390	424	1.1	
ethyl octanoate	3140	3610	1.1	
(S)-linalool	25.7	23.3	0.9	
3-methyl-1-butanol	1140000	909000	0.8	
3-methylbutyl acetate	2450	1860	0.8	
1,1-diethoxyethane	32000	20800	0.7	
(E,E)-2,4-decadienal	3.72	2.32	0.6	
(S)-2-methyl-1-butanol	234000	148000	0.6	
methylpropanol	293000	183000	0.6	
4-hydroxy-2,5-dimethylfuran-3(2H)-	5.72	3.54	0.6	
one				
(R)-linalool	57.6	25.5	0.4	
1-octen-3-one	4.40	1.01	0.2	
2,3-butanedione	3860	526	0.1	

^a Concentrations in 0.4 L of distillate, which was the amount used for the production of 1 L of rum. ^b Concentrations in 1 L of rum, based on 0.4 L of distillate. ^c Factor was calculated by dividing the concentration of the compound in the rum (1 L) by the respective concentration in the distillate (0.4 L). nd: not detected.

	concentrations				
compound	molasses ^a	mash ^b	distillate ^c	rum ^d	factor ^e
	(µg/0.72 L)	(µg/2.9 L)	(µg/0.4 L)	(µg/L)	
ethyl octanoate	12.6	1140	3140	3610	287
ethyl hexanoate	11.9	237	390	424	36
2-phenylethanol	106	125000	1420	2670	25
ethyl (S)-2-methylbutanoate	0.86	10.5	11.4	19.4	23
2-methoxyphenol	59.3	509	67.6	399	7
(<i>E</i>)-β-damascenone	87.0	60.1	223	328	4
2,3-butanedione	174	317	3860	526	3
(S)-2-methylbutanal	481	143	672	885	2
vanillin	1010	1180	10.8	1200	1
butanoic acid	7520	9950	256	462	0.06
3-hydroxy-4,5-dimethylfuran-2(5H)-one	200	154	0.06	2.95	0.02
phenylacetic acid	2580	3400	10.6	35.8	0.01
4-hydroxy-2,5-dimethylfuran-3(2H)-one	680	5200	5.72	3.54	0.005

Table 8. Changes in the Concentrations of 13 Aroma-Active Compounds during the Whole Rum Production Process

^a Concentrations in 0.72 L of molasses, which was used for the production of 1 L of rum. ^b Concentration in 2.9 L of mash, resulting from the fermentation of 0.72 L of molasses. ^c Concentrations in 0.4 L of distillate, resulting from the distillation of 2.9 L of mash. ^d Concentrations in 1 L of rum, based on 0.4 L of distillate. ^e Factor was calculated by dividing the concentration of the compound in 1 L of rum by the respective concentration in 0.72 L of molasses.

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Figure 1.



Figure 2.

TOC graphic

