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Characterization of the Key Aroma Compounds in Two Commercial Rums by Means of the Sensomics Approach

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ABSTRACT: Two rums differing in their overall aroma profile and price level (rum A, high price; rum B, low price) were analyzed by means of the Sensomics approach. Application of aroma extract dilution analysis (AEDA) on a distillate of volatiles prepared from rum A revealed 40 aroma-active compounds in the flavor dilution (FD) factor range from 8 to 2048. The identification experiments indicated *cis*-whiskey lactone, vanillin, decanoic acid, and 2- and 3-methylbutanol with the highest FD factors. The AEDA of a distillate prepared from rum B showed only 26 aroma-active compounds in the same FD factor range. Among them, in particular, ethyl butanoate, 1,1-diethoxyethane, ethyl (*S*)-2-methylbutanoate, and decanoic acid appeared with the highest FD factors. Thirty-seven compounds having at least an FD factor ≥32 in one of the two rums were quantitated using stable isotope dilution assays or enzyme kits (2 compounds). The calculation of odor activity values (OAVs; ratio of concentration to respective odor threshold) indicated ethanol, vanillin, ethyl (*S*)-2-methylbutanoate, and (*E*)-β-damascenone with the highest OAVs in rum A, whereas ethanol, 2,3-butanedione, 3-methylbutanal, and ethyl butanoate revealed the highest OAVs in rum B. Most compounds were present in similar concentrations in both rums, but significant differences were determined for vanillin, *cis*-whiskey lactone, and 4-allyl-2-methoxyphenol (all higher in rum A) and 3-methylbutanal, 2,3-butanedione, and ethyl butanoate (all higher in rum B). Finally, the aromas of both rums were successfully simulated by a recombinate using reference odorants in the same concentrations as they naturally occurred in the spirits.

KEYWORDS: *rum, aroma extract dilution analysis, stable isotope dilution assay, odor activity value, aroma recombination*

INTRODUCTION

Rum is an alcoholic beverage with an alcohol content of 40% (by vol), traditionally produced in the Caribbean and in Central American countries. The spirit is available either as dark or white rum; the latter is either filtered to remove any color originating from aging in wooden barrels or is stored in steel cylinders. The manufacturing process starts with the fermentation of either sugar cane juice or molasses, the remainder of sugar production, followed by distillation and storage in oak barrels. Finally, a dilution step is performed to adjust the desired alcohol content. During the manufacturing process, a rich and complex aroma is generated.[1,2] For example, the concentrations of aromatic congeners were found to increase due to reactions between ethanol and oak wood components.[3]

First studies on volatiles of rum were done by Maarse et al.,[4] who identified over 100 volatile compounds, and Nykänen et al.,[5] who analyzed in particular volatile fatty acids. In recent years, many groups identified rum volatiles,[6−9] but the contribution of single volatile compounds to the overall aroma was not proven. De Souza et al.[7] compared the aroma of cachaça, a Brazilian spirit made from sugar cane, with the aroma of rum by means of gas chromatography−olfactometry (GC-O) and postulated several compounds to be the most potent odorants, for example, eugenol, ethyl phenylacetate, ethyl butanoate, and vanillin. However, no quantitation experiments were performed. Pino et al.[9] suggested the following compounds as key aroma compounds of aged rum on the basis of an aroma extract dilution analysis (AEDA), quantitation experiments, and the calculation of odor activity values (OAVs): ethanol, 1,1-diethoxyethane, ethyl methylpropanoate, ethyl butanoate, ethyl 2-methylbutanoate, 3-methylbutyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl phenylacetate, (*E*)-β-damascenone, 2-phenylethanol, 2-methoxyphenol, 4-ethyl-2-methoxyphenol, 4-propyl-2-methoxyphenol, *trans*-whiskey lactone, *cis*-whiskey lactone, γ-nonalactone, eugenol, and vanillin. However, no isotopically labeled standards were used for quantitation, and no recombination experiments were done. Thus, up to now, a comprehensive investigation on a molecular basis of rum aroma is still missing.

The aims of the present study were, thus, to elucidate the differences in the key aroma compounds of two rums varying in their overall aroma using the Sensomics concept[10] consisting of (i) the identification of the most important odorants on the basis of AEDA in combination with gas chromatography−mass spectrometry (GC-MS), (ii) quantitation experiments by means of stable isotope dilution assays and, finally, (iii) a simulation of the aroma by recombination experiments.

MATERIALS AND METHODS

Rum Samples. A high-priced rum produced from sugar cane molasses and stored for 15 years in a solera system (rum A) was bought from an internet supplier. Solera, a process for aging spirits, is mainly used for sherry production in Spain. Manufactured by fractional blending in a pyramid of barrels, the finished product is a mixture of differently aged products. A cheaper rum (rum B) was obtained from a

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local store. There was no further information for this rum except that it was made "overseas". Both contained 40% of alcohol (by vol). Samples were stored at room temperature in the dark prior to analysis.

Chemicals. The following compounds, used as references for identification and quantitation experiments, were obtained from commercial sources: acetic acid, 4-allyl-2-methoxyphenol, 2,3-butanedione, (*E*,*E*)-2,4-decadienal, decanoic acid, 1,1-diethoxyethane, 2,3-diethyl-5-methylpyrazine, ethyl butanoate, ethyl hexanoate, ethyl (*S*)-2-methylbutanoate, ethyl 3-methylbutanoate, ethyl 4-methylpentanoate, ethyl octanoate, ethyl pentanoate, 4-ethylphenol, ethyl 3-phenylpropanoate, hexanal, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon), 2-isobutyl-3-methoxypyrazine, 2-methoxyphenol, 3-methylbutanal, (*S*)-2-methylbutanoic acid, 3-methylbutanoic acid, (*S*)-2-methylbutanol, 3-methylbutanol, 3-methylbutyl acetate, *cis*- and *trans*-β-methyl-γ-octalactone (whiskey lactone), 4-methylphenol, 3-(methylthio)propionaldehyde (methional), (*E*,*Z*)-2,6-nonadienal, (*E*)-2-nonenal, phenylacetaldehyde, 2-phenylethanol, 4-propyl-2-methoxyphenol, and 3-propylphenol (Sigma-Aldrich Chemie, Taufkirchen, Germany); 2-isopropyl-3-methoxypyrazine, 2-methylbutanal, and 1-octene-3-one (Alfa Aesar, Karlsruhe, Germany); methylpropanol and nonanoic acid (Fluka, Neu-Ulm, Germany); butanoic acid, ethanol, and 4-hydroxy-3-methoxybenzaldehyde (vanillin) (Merck, Darmstadt, Germany); ethyl cyclohexanoate, 4-ethyl-2-methoxyphenol, and 4-vinylphenol (Lancaster, Mühlheim/Main, Germany). 1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one ((*E*)-β-damascenone) was kindly provided by Symrise (Holzminden, Germany).

Liquid nitrogen was obtained from Linde (Munich, Germany). Diethyl ether and pentane (Merck) were freshly distilled prior to use. Hydrochloric acid, sodium chloride, sodium sulfate, and sodium carbonate were from Merck. All chemicals were at least of analytical grade.

Stable Isotopically Labeled Standards. [$^2H_5$]-2-Phenylethanol was obtained from C/D/N Isotopes (Quebec, Canada) and [$^2H_{12}$]-hexanal from Sigma-Aldrich. The following stable isotopically labeled standards were prepared as described previously: [$^{13}C_4$]-2,3-butanedione, [$^2H_3$]-ethyl butanoate, and [$^2H_3$]-ethyl cyclohexanoate;[11] [$^2H_2$]-butanoic acid;[12] [$^2H_{4-7}$]-(*E*)-β-damascenone;[13] [$^2H_4$]-(*E*,*E*)-2,4-decadienal;[14] [$^2H_2$]-decanoic acid;[15] [$^{13}C_2$]-1,1-diethoxyethane, [$^2H_2$]-3-ethylphenol, and [$^2H_3$]-ethyl hexanoate;[16] [$^2H_{2-4}$]-4-ethyl-2-methoxyphenol and [$^2H_3$]-4-hydroxy-3-methoxybenzaldehyde;[17] [$^2H_3$]-ethyl 2-methylbutanoate;[18] [$^2H_5$]-ethyl pentanoate and [$^2H_5$]-ethyl 3-phenylpropanoate;[19] [$^{13}C_2$]-3-hydroxy-4,5-dimethyl-2(5*H*)-furanone;[20] [$^2H_3$]-2-methoxyphenol;[21] [$^2H_3$]-2-methoxy-4-(1-propenyl)-phenol;[22] [$^2H_{2-4}$]-2-methoxy-4-propylphenol;[23] [$^2H_2$]-2-methylbutanal and [$^2H_2$]-3-methylbutanal;[24] [$^2H_2$]-3-methylbutanoic acid;[25] [$^2H_2$]-3-methyl-1-butanol;[26] [$^2H_{11}$]-3-methylbutyl acetate;[27] and [$^2H_2$]-*cis*-whiskey lactone.[28] [$^2H_9$]-Ethyl 3-methylbutanoate was obtained by esterification of [$^2H_9$]-3-methylbutanoic acid with ethanol following the procedure previously described for [$^2H_3$]-ethyl 2-methylbutanoate.[18]

Concentrations of the isotopically labeled standards were determined by means of a ThermoQuest Trace 2000 gas chromatograph equipped with a flame ionization detector (FID) (Egelsbach, Germany) using methyl octanoate as the internal standard. First, the FID response factor was determined for each unlabeled reference compound and methyl octanoate. Then, the concentration of the labeled standard was calculated via the peak areas of the labeled compound and methyl octanoate using the FID response factor determined for the unlabeled compound.

Isolation of the Volatiles. Rum (100 mL) was extracted with diethyl ether (3 × 100 mL) at room temperature. The combined organic phases were washed with aqueous NaCl solution (1 mol/L; 3 × 300 mL) to remove the major part of ethanol, then dried over anhydrous sodium sulfate, and filtered. To separate the volatile fraction from the nonvolatiles, solvent-assisted flavor evaporation (SAFE)[29] was applied. The distillate obtained was concentrated using a Vigreux column (50 cm × 1 cm) and microdistillation[30] to a final volume of ~200 μL.

High-Resolution Gas Chromatography−Olfactometry (HRGC-O). HRGC-O was performed by means of a Carlo Erba Instruments type 5160 gas chromatograph (Hofheim, Germany) and two fused silica capillaries: DB-FFAP (30 m × 0.32 mm i.d., 0.25 μm film thickness) at a flow rate of the carrier gas helium of 1.9 mL/min; and DB-5 (30 m × 0.25 mm i.d., 0.25 μm film thickness) at a flow rate of 1.2 mL/min. Both columns were from J&W Scientific (Agilent, Waldbronn, Germany). The sample was injected by using the cold-on-column technique at 40 °C. The initial temperature was held for 2 min, then raised at 6 °C/min to 230 °C, and held for 5 min. The effluent was split into two equal parts at the end of the column by means of a Chrompack Y-type quick-seal glass splitter (Frankfurt, Germany) and two deactivated fused-silica capillaries of the same length (25 cm × 0.32 mm i.d.). One part was directed to an FID held at 250 °C, the other to a sniffing port (200 °C). A series of *n*-alkanes (C6−C26 (DB-FFAP) and C6−C18 (DB-5)) was used to determine linear retention indices (RI) for each compound as previously described.[26]

Comparative Aroma Extract Dilution Analysis (cAEDA). For cAEDA, the same amount of both rums was extracted and distilled according to the SAFE method and concentrated to the same final volume, and the same volume was used in GC-O. This way, the flavor dilution (FD) factors were determined on the basis of the same amount of food, thus allowing a direct comparison of the FD factors in both samples on a semiquantitative basis. To determine the FD factors, the distillate was diluted stepwise 1:1 (by vol) with diethyl ether. The original distillate and each dilution were analyzed via HRGC-O by at least three experienced panelists to avoid possible individual differences of the panelists with regard to personal sensitivity and threshold profiles.

High-Resolution Gas Chromatography−Mass Spectrometry (HRGC-MS). HRGC-MS was performed using a Hewlett-Packard gas chromatograph 5890 series II (Waldbronn, Germany) coupled to a Finnigan sector field mass spectrometer type MAT 95 S (Bremen, Germany). The same capillary columns were used as mentioned above, and mass spectra were generated in the electron impact mode (MS-EI) at 70 eV and in the chemical ionization mode (MS-CI) at 115 eV using isobutane as reactant gas.

Quantitation by Stable Isotope Dilution Analysis (SIDA). After the addition of diethyl ether (1:1 by vol, but a minimum of 10 mL) to the rum (0.1−400 mL, depending on the concentration of each analyte), the internal standards (dissolved in ethanol or diethyl ether; amounts needed were determined in preliminary experiments) were added. After stirring for 30 min at room temperature for equilibration, the workup procedure was performed as described above for the isolation of the volatiles.

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

For quantitation of butanoic acid, 2- and 3-methylbutanoic acid, and decanoic acid, the distillate was separated into an acidic (AF) and a neutral/basic fraction (NBF) by liquid−liquid extraction with an aqueous Na_2CO_3 solution (0.5 mol/L; pH 10.0). The combined aqueous phases were adjusted to pH 2 with hydrochloric acid, and the acidic volatiles were extracted with diethyl ether (1:1 by vol, three times). The combined organic phases were dried over anhydrous Na_2SO_4 and concentrated to ~100 μL.

Quantitation was performed using a Varian 431 gas chromatograph (Darmstadt, Germany) equipped with a DB-FFAP column (30 m × 0.25 mm i.d., 0.25 μm film thickness) (J&W Scientific) coupled to a Varian 220 ion trap mass spectrometer.

In the case that a compound (e.g., 3-methylbutanal) was overlapped by a major nonsmelling volatile, two-dimensional gas chromatography−mass spectrometry was performed by means of a ThermoQuest Trace 2000 series gas chromatograph equipped with a DB-FFAP column (30 m × 0.32 mm i.d.; 0.25 μm film thickness) coupled to a Varian CP 3800 GC equipped with an OV-1701 column (30 m × 0.25 mm i.d., 0.25 μm film thickness) (J&W Scientific). Samples were injected by means of a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland). Heart cuts were transferred to the second column by means of a moving capillary stream switching system, and

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Table 1. Stable Isotopically Labeled Standards, Selected Ions, and Response Factors (R_f) Used in the Stable Isotope Dilution Assays

compound	isotope label	ion (m/z)[a] analyte	ion (m/z)[a] internal standard	R_f[b]
4-allyl-2-methoxyphenol[c]	–[c]	165	168[c]	0.86
2,3-butanedione	$^{13}C_4$	87	91	0.99
(*E*)-β-damascenone	$^{2}H_{4-7}$	191	195–198[d]	0.91
(*E*,*E*)-2,4-decadienal	$^{2}H_{3-5}$	153	156–158[d]	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	$^{2}H_2$	153	155	0.66
ethyl 2-methylbutanoate	$^{2}H_3$	131	134	0.99
ethyl 3-methylbutanoate	$^{2}H_9$	131	140	1.00
ethyl pentanoate	$^{2}H_5$	131	136	0.96
4-ethylphenol[e]	–[e]	123	125[e]	0.87
3-ethylphenol	$^{2}H_2$	123	125	0.87
ethyl 3-phenylpropanoate	$^{2}H_5$	179	184	1.00
hexanal	$^{2}H_{12}$	101	113	0.94
2-methoxyphenol	$^{2}H_3$	125	128	0.98
2-methoxy-4-(1-propenyl) phenol	$^{2}H_3$	165	168	0.86
2-methoxy-4-propylphenol	$^{2}H_{2-4}$	167	169–171[d]	0.98
2-methylbutanal	$^{2}H_2$	87	89	0.88
3-methylbutanal	$^{2}H_2$	87	89	0.98
2-methylbutanoic acid[f]	$^{2}H_9$	103	112	0.88
3-methylbutanoic acid[f]	$^{2}H_2$	103	105	0.98
2-methylbutanol[g,h]	–[g]	71	73[g]	0.84
3-methylbutanol[h]	$^{2}H_2$	71	73	0.99
3-methylbutyl acetate	$^{2}H_{11}$	131	142	0.90
methylpropanol	$^{2}H_9$	57	66	0.62
2-phenylethanol	$^{2}H_5$	105	110	0.71
sotolon	$^{13}C_2$	129	131	0.70
cis-whiskey lactone	$^{2}H_2$	157	159	0.77
trans-whiskey lactone[i]	–[i]	157	159[i]	0.79
vanillin	$^{2}H_3$	153	156	1.00

[a]Ions used for quantitation. [b]Response factor determined by analyzing defined mixtures of analyte and internal standard. [c]4-Allyl-2-methoxyphenol was quantitated using [$^{2}H_2$]-2-methoxy-4-(1-propenyl)phenol as internal standard. [d]Internal standard was used as a mixture of isotopologues. [e]4-Ethylphenol was quantitated using [$^{2}H_2$]-3-ethylphenol as internal standard. [f]Differentiation of 2- and 3-methylbutanoic acid was performed as described under Materials and Methods. [g]2-Methylbutanol was quantitated using [$^{2}H_2$]-3-methylbutanol as internal standard. [h]Differentiation of 2- and 3-methylbutanol was performed as described under Materials and Methods. [i]*trans*-Whiskey lactone was quantitated using [$^{2}H_2$]-*cis*-whiskey lactone as internal standard.

the volatiles were cryo-focused with liquid nitrogen. Mass spectra were recorded using a Varian Saturn 2000 ion trap running in CI mode using methanol as reactant gas at 70 eV.

Quantitation of Ethanol and Acetic Acid. Ethanol and acetic acid were determined using a commercial enzyme kit (R-Biopharm, Darmstadt). Analyses were done by means of a Shimadzu UV-2401PC photometer (Duisburg, Germany) according to the instructions of the manufacturer.

Separation of Ethyl (*R*)-2- and Ethyl (*S*)-2-Methylbutanoate as well as (*R*)-2-, (*S*)-2-, and 3-Methylbutanoic Acid. Ethyl (*R*)-2- and ethyl (*S*)-2-methylbutanote as well as (*R*)-2-, (*S*)-2-, and 3-methylbutanoic acid were separated by two-dimensional GC-MS using a DB-FFAP column (30 m × 0.32 mm i.d.; 0.25 μm film thickness) in the first dimension and a chiral BGB-176 column (30 mm × 0.25 mm i.d., 0.25 μm film thickness) (BGB Analytik, Böckten, Switzerland) in the second dimension as recently reported.[31]

Separation of (*R*)-2, (*S*)-2-, and 3-Methylbutanol as well as (*R*)-2- and (*S*)-2-Methylbutanal. (*R*)-2, (*S*)-2-, and 3-methylbutanol as well as (*R*)-2- and (*S*)-2-methylbutanal were separated by two-dimensional GC-MS using a DB-FFAP column (30 m × 0.32 mm i.d.; 0.25 μm film thickness) in the first dimension and a chiral BGB-174E column (30 m × 0.25 mm i.d., 0.25 μm film thickness) (BGB Analytik AG) in the second dimension.[31]

Determination of Orthonosal Odor Thresholds. For the calculation of odor activity values, orthonasal odor thresholds were determined in an aqueous solution containing 40% of pure ethanol (by vol) as previously described.[32]

Aroma Profile Analysis. For aroma profile analysis, the intensities of six selected odor attributes (butter-like, clove-like, ethanolic, fruity, malty, and vanilla-like) were rated on a seven-point linear scale from 0 (not perceivable) to 3 (strongly perceivable) by a sensory panel. The panel consisted of 20 experienced assessors participating in weekly sensory training sessions intended to train their abilities to recognize and describe different aroma qualities. Sensory analyses were performed in a sensory room at 21 ± 1 °C equipped with single booths. The samples (15 mL) were presented in covered glass vessels (40 mm i.d., total volume = 45 mL).

Aroma Recombination. For aroma recombination, each rum A (200 mL) was extracted with pentane (3 × 200 mL) and diethyl ether (3 × 200 mL) until the remaining liquid was odorless. The liquid was freeze-dried, and the lyophilisate was used as matrix. All analyzed aroma compounds with an OAV ≥ 1 were prepared in aqueous solutions containing 40% of alcohol (by vol) and were added to the matrix in the concentrations determined in the rum. The recombinate and the original rum were evaluated by the sensory panel as explained above for the aroma profile analysis. The similarity was evaluated using a seven-point scale from 0 (not identical) to 3 (identical).

RESULTS AND DISCUSSION

To get a first idea of the differences in the overall aroma of two rums differing in price level, aroma profile analyses were performed. Both rums showed the odor attribute ethanolic at the same intensity. Rum A elicited the more intense clove-like, fruity, and vanilla-like aromas, whereas rum B was higher evaluated in the attributes malty and butter-like (Figure 1). To identify the odorants responsible for the overall aroma of both rum samples, the Sensomics concept was applied.[10]

Identification of Key Aroma Compounds in Rum A. The spirit was extracted with diethyl ether, followed by separation of the volatile fraction from the nonvolatiles using

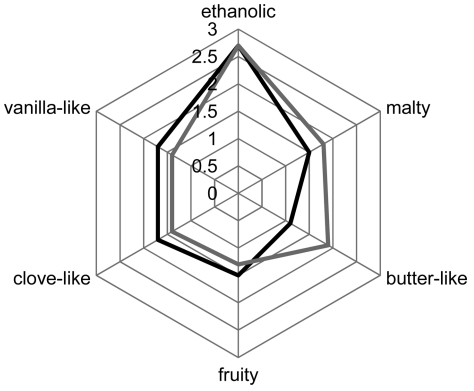

Figure 1. Aroma profiles of rum A (black) and rum B (gray).

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SAFE distillation. The distillate obtained revealed the typical rum aroma when it was evaluated on a strip of filter paper. Subsequently, the distillate was analyzed by means of HRGC-O and AEDA.

Altogether, 40 odor-active regions were detected in the FD factor range between 8 and 2048. The highest FD factor of 2048 was obtained for compounds **33** (coconut-like odor impression) and **42** (vanilla-like, sweet), followed by **12** (malty) and **40** (soapy, fusty), both showing an FD factor of 1024. Lower FD factors were determined for **37** (clove-like; FD factor 512), **39** (seasoning-like, spicy; 512), **5** (fruity; 256), **29** (smoky, sweet; 256), and **32** (flowery, honey-like; 256) (Figure 2).

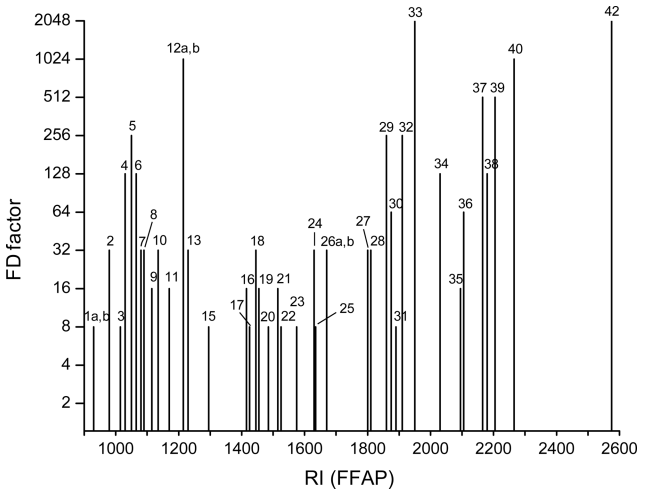

Figure 2. Flavor dilution chromatogram obtained by application of the aroma extract dilution analysis (AEDA) on a distillate of rum A. Odorants with an FD factor ≥8 are displayed. Numbering is identical with that in Table 2.

For identification of the compounds responsible for the single-odor impressions, retention indices of the aroma-active regions were determined on two GC columns of different polarities and compared to data available in an in-house database containing ~1000 volatiles known to be odor-active constituents of foods or food raw materials, respectively. Then, the aroma quality and intensity of the odor impressions detected in the rum distillate were compared by GC-O to reference compounds at similar concentration ranges. Finally, for an unequivocal identification, mass spectra (MS-EI, MS-CI) were recorded for both the odorants in the distillate and the respective reference compounds.

Following this procedure, *cis*-whiskey lactone (**33**; coconut-like), vanillin (**42**; vanilla-like, sweet), and 2- and 3-methylbutanol (**12a** and **12b**; both malty) as well as decanoic acid (**40**; soapy, fusty) were identified with the highest FD factors (Figure 3). Further important odorants were 4-allyl-2-methoxyphenol (**37**; clove-like), sotolon (**39**; seasoning-like, spicy), ethyl 2-methylbutanoate (**5**; fruity), 2-methoxyphenol (**29**; smoky, sweet), and 2-phenylethanol (**32**; flowery, honey-like) (Table 2).

Next, the enantiomeric ratio in 2-methylbutanol was determined using a chiral stationary GC phase, showing >99% of the (*S*)-enantiomer. Using this column, separation of 2- and 3-methylbutanol was achieved simultaneously, revealing 21% of 2-methylbutanol and 79% of 3-methylbutanol. Additionally, the ratio of (*R*)-2- and (*S*)-2-methylbutanal was

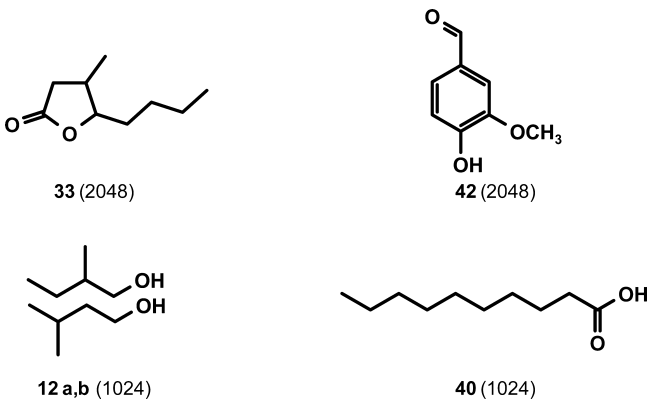

Figure 3. Structures of the most odor-active volatiles identified in rum A (numbering refers to Table 2 and Figure 2; FD factors are given in parentheses).

determined to be 44% of (*R*)-2-methylbutanal and 56% of (*S*)-2-methylbutanal.

Using another chiral column, the enantiomeric ratio in ethyl 2-methyl butanoate was determined, revealing >99% of the (*S*)-enantiomer. The same column was used for separation of (*R*)- 2- and (*S*)-2-methylbutanoic acid, showing 24% of (*R*)-2-methylbutanoic acid and 76% of (*S*)-2-methylbutanoic acid. Simultaneously, the ratio of 2- and 3-methylbutanoic acid was determined to be 49% of 2-methylbutanoic acid and 51% of 3-methylbutanoic acid.

Identification of Key Odorants in Rum B. Twenty-six aroma compounds were detected in the volatile fraction in the FD factor range between 8 and 1024. Ethyl cyclohexanoate (**14**; fruity, sweet) and ethyl butanoate (**4**; fruity) showed the highest FD factors of 1024 and 512, respectively, followed by 1,1-diethoxyethane (**3**; fruity), ethyl 2-methylbutanoate (**5**; fruity), decanoic acid (**40**; soapy, fusty), and ethyl pentanoate (**10**; fruity) (Table 2).

The enantiomeric ratio in ethyl 2-methylbutanoate showed >99% of the (*S*)-enantiomer. Separation of 2- and 3-methylbutanoic acid revealed 43% of 2-methylbutanoic acid (45% (*R*) and 55% (*S*), respectively) and 57% of 3-methylbutanoic acid. The ratio of 2- and 3-methylbutanol was determined to be 19% of 2-methylbutanol (>99% of the (*S*)-enantiomer) and 81% of 3-methylbutanol. The enantiomeric ratio in 2-methylbutanal was determined to be 31% of (*R*)-2- to 69% of (*S*)-2-methylbutanal.

Quantitation of the Key Aroma Compounds and Calculation of Odor Activity Values (OAVs). AEDA is a screening method indicating which odorants in a given set of volatiles are able to interact with the human odorant receptors. However, the amount present in the air above a food, for example, during consumption, is significantly influenced by the binding properties of the food matrix. Thus, the concentration of each aroma-active compound must be determined and correlated with the respective odor threshold. A total of 37 aroma compounds (each showing an FD factor of at least ≥32 in one of both rums) was quantitated using SIDAs.

Concentrations of Key Odorants. As expected, ethanol showed by far the highest concentration in rum A, followed by acetic acid, 3-methylbutanol, methylpropanol, 1,1-diethoxyethane, and (*S*)-2-methylbutanol (Table 3). Somewhat lower amounts were found for vanillin, *cis*-whiskey lactone, and 2-phenylethanol. Lower concentrations were obtained for esters (e.g., 3-methylbutyl acetate, ethyl butanoate, and ethyl

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Table 2. Comparison of the FD Factors of the Most Aroma-Active Compounds in Rums A and B

no.[c]	compound[d]	odor quality[e]	RI[a]		FD factor[b] in rum	
			DB-FFAP	DB-5	A	B
1a,b	2- and 3-methylbutanal[f]	malty	930	658	8	32
2	2,3-butanedione	butter-like	980	593	32	32
3	1,1-diethoxyethane	fruity	1013	733	128	128
4	ethyl butanoate	fruity	1033	804	128	256
5	ethyl 2-methylbutanoate	fruity	1047	854	256	128
6	ethyl 3-methylbutanoate	blueberry-like	1067	861	128	32
7	hexanal	green, grassy	1080	800	32	<8
8	methylpropanol	malty	1093	640	32	<8
9	3-methylbutyl acetate	banana-like	1115	879	16	32
10	ethyl pentanoate	fruity	1133	903	32	64
11	ethyl 4-methylpentanoate	fruity	1170	963	16	<1
12a,b	2- and 3-methylbutanol[f]	malty	1213	752	1024	32
13	ethyl hexanoate	fruity	1230	1001	32	32
14	ethyl cyclohexanoate	fruity, sweet	1409	1136	<8	1024
15	1-octen-3-one	mushroom-like	1296	983	8	<8
16	2-isopropyl-3-methoxypyrazine	earthy, pea-like	1417	1094	16	16
17	ethyl octanoate	fruity	1426	1197	8	<8
18	acetic acid	vinegar-like	1443	610	32	<1
19	methional	cooked potato-like	1457	905	16	<8
20	2,3-diethyl-5-methylpyrazine	earthy	1487	1158	8	8
21	2-isobutyl-3-methoxypyrazine	bell pepper-like	1517	1184	16	8
22	(*E*)-2-nonenal	fatty, green	1527	1160	8	<8
23	(*E*,*Z*)-2,6-nonadienal	cucumber-like	1573	1153	8	<1
24	butanoic acid	sweaty	1632	821	32	<8
25	phenylacetaldehyde	honey-like	1638	1045	8	<1
26a,b	2- and 3-methylbutanoic acid[f]	sweaty, fruity	1668	874	32	<8
27	(*E*,*E*)-2,4-decadienal	fatty, deep-fried	1800	1323	32	<8
28	(*E*)-*β*-damascenone	baked apple-like, grape juice-like	1811	1389	32	32
29	2-methoxyphenol	smoky, sweet	1860	1090	256	32
30	ethyl 3-phenylpropanoate	flowery	1875	1350	64	32
31	*trans*-whiskey lactone	coconut-like	1890	1300	8	32
32	2-phenylethanol	flowery, honey-like	1911	1116	256	<8
33	*cis*-whiskey lactone	coconut-like	1950	1331	2048	<8
34	4-ethyl-2-methoxyphenol	smoky, gammon-like	2029	1284	128	32
35	4-methylphenol	fecal, horse stable-like	2094	1077	16	16
36	2-methoxy-4-propylphenol	phenolic	2106	1360	64	32
37	4-allyl-2-methoxyphenol	clove-like	2165	1359	512	<8
38	4-ethylphenol	phenolic	2182	1169	128	8
39	sotolon	seasoning-like, spicy	2206	1108	512	32
40	decanoic acid	soapy, fusty	2265	1373	1024	128
41	4-vinylphenol	smoky, leather-like	2393	1211	<8	16
42	vanillin	vanilla-like, sweet	2573	1406	2048	32

[a]Retention index determined using a homologous series of *n*-alkanes. [b]Flavor dilution factor determined by AEDA on capillary DB-FFAP. [c]Numbering refers to Figure 2. [d]Odorant was identified by comparing the retention indices on two columns with different polarities, the odor quality as well as the odor intensity perceived at the sniffing port, and the mass spectra (EI, CI) with the data of reference compounds. [e]Odor quality detected at sniffing port. [f]FD factors were determined in sum.

pentanoate) and phenols (e.g., 4-allyl-2-methoxyphenol, 2-methoxyphenol, and 4-ethyl-2-methoxyphenol). Ethyl 3-phenylpropanoate, 2-methoxy-4-propylphenol, (*E*,*E*)-2,4-decadienal, and ethyl cyclohexanoate were present in concentrations below 1 μg/L (Table 3).

Also in rum B, ethanol was determined with the highest concentration, followed by 1,1-diethoxyethane, 3-methylbutanol, decanoic acid, butanoic acid, 2,3-butanedione, ethyl butanoate, methylpropanol, 3-methylbutanal, and (*S*)-2-methylbutanol. Lower concentrations were determined for 2-methylbutanal, ethyl hexanoate, 3-methylbutanoic acid, 2-methylbutanoic acid, and 3-methylbutyl acetate. Again, a few compounds showed concentrations below 1 μg/L: (*E*)-*β*-damascenone, 4-ethylphenol, ethyl 3-phenylpropanoate, *cis*- and *trans*-whiskey lactone, sotolone, 4-methylphenol, and (*E*,*E*)-2,4-decadienal (Table 3).

To evaluate the contribution of each compound to the overall aroma, OAVs (ratio of concentration to odor threshold) were calculated. Odor thresholds determined in a mixture of ethanol/water (40/60 by vol) were used, except for ethanol, for which an odor threshold in water was used. In rum A, by far the highest OAV was calculated for ethanol (12900), followed by vanillin (42), ethyl (*S*)-2-methylbutanoate (40), (*E*)-*β*-damascenone (12), 3-methylbutanal (12), 2,3-butanedione (11),

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Table 3. Concentrations of the Most Important Aroma Compounds in Rums A and B

compound	concentration[a] (μg/L) in rum A	B
ethanol	322000000	309000000
acetic acid	55000	nd[b]
3-methylbutanol	19300	667
methylpropanol	6660	412
1,1-diethoxyethane	5310	4050
(*S*)-2-methylbutanol	4850	156
vanillin	912	2.05
cis-whiskey lactone	318	0.41[c]
2-phenylethanol	291	1.93[c]
decanoic acid	195	635
butanoic acid	172	629[c]
3-methylbutyl acetate	75.6[c]	26.8
ethyl butanoate	74.3	532
ethyl hexanoate	66.8	79.6
hexanal	43.5	4.31
3-methylbutanal	34.1	315
3-methylbutanoic acid	29.7	68.8[c]
2,3-butanedione	29.7	621
(*S*)-2-methylbutanoic acid	21.5[c]	28.0
trans-whiskey lactone	19.7[c]	0.37[c]
4-allyl-2-methoxyphenol	18.3	1.47
2-methoxyphenol	16.3	1.33
ethyl (*S*)-2-methylbutanoate	8.78	6.87
(*S*)-2-methylbutanal	8.20	61.2
(*R*)-2-methylbutanoic acid	6.90[c]	23.0
(*R*)-2-methylbutanal	6.39	27.4
ethyl 3-methylbutanoate	6.37	4.22
ethyl pentanoate	6.26[c]	26.5
4-ethyl-2-methoxyphenol	1.78	2.51
4-ethylphenol	1.72	0.55[c]
(*E*)-β-damascenone	1.67	0.96
sotolon	1.53[c]	0.33
4-methylphenol	1.50[c]	0.17[c]
ethyl 3-phenylpropanoate	0.52[c]	0.51
2-methoxy-4-propylphenol	0.37[c]	3.71
(*E*,*E*)-2,4-decadienal	0.13	0.11
ethyl cyclohexanoate	0.06[c]	1.20

[a]Mean values of triplicates, differing not more than ±12%. [b]nd, not detected. [c]Mean values of duplicates, differing not more than ±10%.

Table 4. Odor Activity Values (OAVs) and Orthonasal Odor Thresholds of the Most Important Aroma Compounds in Rums A and B

compound	odor threshold[b] (μg/L)	OAV[a] in rum A	B
ethanol	24900[c]	12900	12400
vanillin	22	42	<1
ethyl (*S*)-2-methylbutanoate	0.22	40	56
(*E*)-β-damascenone	0.14	12	7
3-methylbutanal	2.8	12	110
2,3-butanedione	2.8	11	220
ethyl butanoate	9.5	8	110
1,1-diethoxyethane	719	7	6
cis-whiskey lactone	67[d]	5	<1
ethyl 3-methylbutanoate	1.6	4	3
4-allyl-2-methoxyphenol	7.1	3	<1
ethyl pentanoate	3.0[e]	2	9
ethyl hexanoate	29.7	2	3
2-methoxyphenol	9.2	2	<1
acetic acid	75521[e]	<1	<1
2-methylbutanal[f]	33[g]	<1	3
2-methoxy-4-propylphenol	1.89[h]	<1	2
butanoic acid	1200[h]	<1	<1
(*E*,*E*)-2,4-decadienal	1.1	<1	<1
decanoic acid	2800[h]	<1	<1
ethyl cyclohexanoate	1.6[e]	<1	<1
4-ethyl-2-methoxyphenol	6.9	<1	<1
4-ethylphenol	173	<1	<1
ethyl 3-phenylpropanoate	14[h]	<1	<1
hexanal	87.9[g]	<1	<1
(*S*)-2-methylbutanoic acid	3500[g]	<1	<1
3-methylbutanoic acid	78[h]	<1	<1
(*S*)-2-methylbutanol	24000[g]	<1	<1
3-methylbutanol	56100	<1	<1
3-methylbutyl acetate	245	<1	<1
4-methylphenol	81.5[g]	<1	<1
methylpropanol	10069[e]	<1	<1
2-phenylethanol	2600	<1	<1
sotolon	24.2[e]	<1	<1
trans-whiskey lactone	790[d]	<1	<1

[a]Odor activity values were calculated by dividing the concentrations (cf. Table 3) by the respective odor thresholds. [b]Odor thresholds were determined in ethanol/water (40/60 by vol).[28] [c]Odor threshold was determined in water.[33] [d]Odor threshold previously reported in ref 34. [e]Odor threshold previously reported in ref 35. [f]Odor threshold was determined for the racemate. [g]Odor threshold was newly determined in ethanol/water (40/60 by vol). [h]Odor threshold previously reported in ref 36.

ethyl butanoate (8), 1,1-diethoxyethane (7), *cis*-whiskey lactone (5), and ethyl 3-methylbutanoate (4). Interestingly, many components were not present above their odor thresholds, resulting in an OAV below 1 (Table 4). These components were obviously overestimated by AEDA, because by application of any GC-O method the entire amount of a given odorant is volatilized, but without taking the influence of the matrix into consideration.

In rum B, calculation of OAVs also revealed ethanol (12400) as the most important aroma-active compound, followed by 2,3-butanedione (220), 3-methylbutanal (110), ethyl butanoate (110), and ethyl (*S*)-2-methylbutanoate (56). Ethyl pentanoate (9), (*E*)-β-damascenone (7), and 1,1-diethoxyethane (6) showed comparably lower OAVs. Similar to rum A, again lots of compounds showed an OAV below 1 (Table 4).

Comparison of the Odorants in Rums A and B. The results of the identification experiments revealed almost no differences in the qualitative pattern of odorants, except for ethyl 4-methylpentanoate (**11**), acetic acid (**18**), (*E*,*Z*)-2,6-nonadienal (**23**), and phenylacetaldehyde (**25**), which all were detected only in rum A. Main differences in FD factors were found for hexanal (**7**), methylpropanol (**8**), 2- and 3-methylbutanol (**12a** and **12b**), butanoic acid (**24**), 2- and 3-methylbutanoic acid (**26a** and **26b**), (*E*,*E*)-2,4-decadienal (**27**), 2-phenylethanol (**32**), *cis*-whiskey lactone (**33**), 4-allyl-2-methoxyphenol (**37**), 4-ethylphenol (**38**), sotolon (**39**), and vanillin (**42**), which were higher in rum A. By contrast, ethyl cyclohexanoate (**14**) showed a higher FD factor in rum B (Table 2).

Quantitations and calculation of OAVs revealed 14 odorants in rum A and 12 odorants in rum B to be above their respective

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odor thresholds. In both rums, ethanol showed the highest OAV. However, vanillin, *cis*-whiskey lactone, 4-allyl-2-methoxyphenol, and 2-methoxyphenol were present above their thresholds only in rum A. By contrast, 2-methylbutanal and 2-methoxy-4-propylphenol occurred above their odor thresholds only in rum B, and for 2,3-butanedione, 3-methylbutanal, and ethyl butanoate clearly higher OAVs were determined compared to rum A.

Aroma Simulation. To verify that all compounds contributing to the overall aroma of both rums were correctly identified and quantitated, reconstitution experiments were performed. Therefore, all aroma compounds showing OAVs ≥ 1 were added in their natural concentrations in a mixture of ethanol/water (40/60 by vol) to an odorless rum matrix prepared by several extraction steps and lyophilization from the respective rum. A trained sensory panel evaluated different single-odor attributes for the recombinate in comparison to the original spirits. The aroma recombinates revealed good similarities with the original spirit in their aroma profiles, and overall similarities of 2.8 for rum A (Figure 4) and of 2.7 for rum B (data not shown) were obtained.

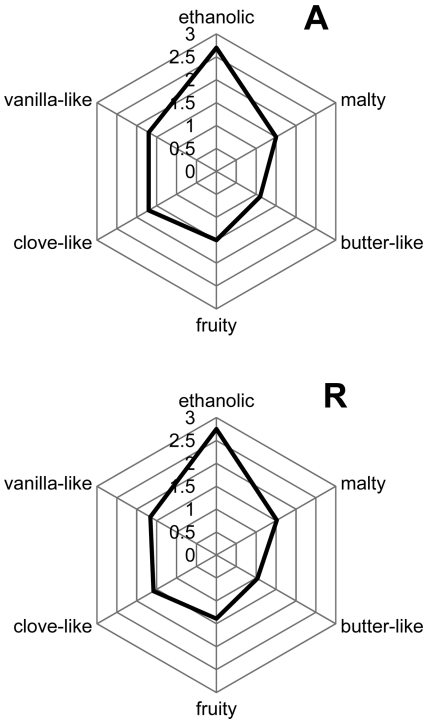

Figure 4. Aroma profile analyses of rum A (A) and of the corresponding aroma recombinate (R). The intensities of the selected descriptors were evaluated by 20 panelists and the results averaged.

Sources of Odorants and Suggestions for the Reasons for Differences. Besides ethanol, many odorants are known to be formed during microbial fermentation as byproducts, including ethyl esters or aldehydes or alcohols. During the fatty acid metabolism, ethyl esters are generated by the reaction of ethanol with acyl-CoA.[37] According to Nordström, different yeast strains are able to convert fatty acids to the corresponding ethyl esters in different yields.[38] In another study, Nordström described esterification of fatty acids with ethanol in dependence of the fatty acid composition.[39] Also, Vocke showed an increase of the concentrations of ethyl esters during fermentation, but esters such as ethyl 2-methyl butanoate, ethyl 3-methylbutanote, and 3-methylbutyl acetate did not increase before maturation.[40] All in all, ester formation is influenced by several factors leading to different ester concentrations in both rums due to varying manufacturing processes.

2,3-Butanedione is an important odorant generated by yeast metabolism via oxidative decarboxylation of (2*S*)-2-hydroxy-2-methyl-3-oxobutanoic acid, which is formed from pyruvate.[41,42] The different storage times of rum A (15 years) and rum B might be an explanation for the huge difference in OAVs of 2,3-butanedione (11 for rum A compared to 220 for rum B, respectively; Table 4). Its decrease during storage due to evaporation has already been reported for whiskey.[40] A further possibility leading to the higher concentration of 2,3-butandione in rum B might be due to the use of different yeast strains, which might also be the reason for the differences in (*R*)-2- and (*S*)-2-methylbutanal as well as 3-methylbutanal, all showing clearly higher OAVs in rum B. Although these so-called Strecker aldehydes are mainly generated by the influence of temperature, for example, during the distillation step, they might also be formed by yeasts following the Ehrlich pathway.[43]

1,1-Diethoxyethane, a key odorant in both rums, was reported as an important aroma-active compound in different spirits.[35,36,40] However, by means of a model distillation of an ethanolic solution containing acetic acid and acetaldehyde, no formation of 1,1-diethoxyethane was found, although the conditions for an acetalization were given. Thus, it can be assumed that 1,1-diethoxyethane might be formed from precursors or catalysts present in the mash.[40]

During storage, the origin of wood used for the barrels and the toasting level have a significant influence on the concentrations of several aroma compounds, which can be extracted during barrel aging, for example, 2-methoxyphenol, 4-allyl-2-methoxyphenol, and vanillin[3] as well as *cis*- and *trans*-whiskey lactone. These are characteristic aroma compounds for distilled spirits stored in oak casks enabling the proof of wood aging. Although four isomers of whiskey lactone are possible, only these two isomers were detected in distilled spirits up to now.[44] All of these phenolic aroma compounds were found in concentrations above their odor thresholds only in rum A due to its storage in a solera system of wooden barrels. In contrast, 2-methoxy-4-propylphenol and 4-ethyl-2-methoxyphenol were present in higher amounts in rum B, which indicates that phenolic compound formation might also be, of course to a much lesser extent, influenced by yeast. It was reported that different yeast strains released volatile phenols from a nonspecified precursor extract of grapes. In a must extract, which was fermented with different yeasts, the concentration of, for example, 2-methoxyphenol, differed between 0.55 and 1.07 μg/L.[45] Under acidic conditions during maturation, ethanol can react with lignin to an alcohol-soluble form and lead via coniferyl alcohol and coniferyl aldehyde by oxidation reactions to vanillin.[3]

Also, (*E*)-β-damascenone, either in the free state or as its glycoside, has been identified in fruits and in many alcoholic beverages,[46] for example, cognac,[35] pear brandy,[36] and whiskey,[40] and also in extracts of sugar cane molasses[47−49] and was found above its odor threshold in both rums. A heat-induced formation of (*E*)-β-damascenone in combination with an acidic pH, for example, in the fermented mash and during the distillation process, may explain its formation, which was already discussed for apple brandy.[50]

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In summary, clear differences in the amounts of odorants were found for rums A and B. The producer of rum A promises exceptional quality and exquisite flavor achieved by aging in oak barrels using the solera system. Rum B, clearly lower in price without any information about the manufacturing process, revealed a weaker overall aroma, and aroma compounds derived from the barrel aging were missing. Thus, the formation of aroma-active compounds in both rums was clearly influenced by the conditions during the production (and by the raw material), ending in a different final aroma.

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Notes

The authors declare no competing financial interest.

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