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Characterization of the Most Odor-Active Compounds in an American Bourbon Whisky by Application of the Aroma Extract Dilution Analysis

LUIGI POISSON AND PETER SCHIEBERLE*

Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, D-85748 Garching, Germany

Application of the aroma extract dilution analysis (AEDA) on the volatile fraction carefully isolated from an American Bourbon whisky revealed 45 odor-active areas in the flavor dilution (FD) factor range of 32−4096 among which (*E*)-β-damascenone and δ-nonalactone showed the highest FD factors of 4096 and 2048, respectively. With FD factors of 1024, (3*S*,4*S*)-*cis*-whiskylactone, γ-decalactone, 4-allyl-2-methoxyphenol (eugenol), and 4-hydroxy-3-methoxy-benzaldehyde (vanillin) additionally contributed to the overall vanilla-like, fruity, and smoky aroma note of the spirit. Application of GC-Olfactometry on the headspace above the whisky revealed 23 aroma-active odorants among which 3-methylbutanal, ethanol, and 2-methylbutanal were identified as additional important aroma compounds. Compared to published data on volatile constituents in whisky, besides ranking the whisky odorants on the basis of their odor potency, 13 aroma compounds were newly identified in this study: ethyl (*S*)-2-methylbutanoate, (*E*)-2-heptenal, (*E*,*E*)-2,4-nonadienal, (*E*)-2-decenal, (*E*,*E*)-2,4-decadienal, 2-isopropyl-3-methoxypyrazine, ethyl phenylacetate, 4-methyl acetophenone, α-damascone, 2-phenylethylpropanoate, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, trans-ethylcinnamate, and (*Z*)-6-dodeceno-γ-lactone.

KEYWORDS: Bourbon whisky; aroma extract dilution analysis; aroma dilution analysis; ethyl (*S*)-2-methylbutanoate

INTRODUCTION

For whisky production, briefly, malt and/or ground cereals are mixed with water to yield a mash that is subsequently fermented with yeast. The resulting beer is then distilled to yield an alcoholic distillate, which is finally stored in barrels (*1*). It is generally accepted that barrel aging is among the key processing steps in the generation of the unique aroma of whisky.

Typical for American Bourbon whisky is (i) the high content of corn among the cereals used (>51%), (ii) the high alcohol content of the distillate (max. 80% by volume), and (iii) the storage of the distillate in new, heat-charred oak casks. While in the manufacturing of Scottish whisky, the filtered mash (wort) is processed, and the complete mash is fermented in the production of American whiskies. The mash is either fermented with yeast alone (sweet mash) or with a mixture of lactic acid bacteria, and yeast (sour mash) (*2*). A product stored for at least two years is called straight Bourbon whisky, but Bourbon whisky may not only originate from the Bourbon district in Kentucky but also originate from other areas, if it is manufactured according to the requirements given above (*2*).

For more than 40 years, investigations on the volatile components of whisky have been performed, and today, more than 300 compounds have been identified in several types of whisky (*3*–*5*). Already in 1963, Nykaenen and Suomalainen (*6*) identified several major components of the volatile fraction of American Bourbon as well as Scottish whisky, such as 2-methylpropanol, 3-methylpropanol, 2-phenylethanol, and acetaldehyde as well as acids and esters.

Steinke and Paulson (*7*) characterized various phenols in thermally processed grains, and they could show that 4-vinylphenol and 4-vinyl-2-methoxyphenol are formed during distillation by a decarboxylation of the precursors *p*-coumaric acid or ferulic acid, respectively, present in the cereals. The norisoprenoids α- and β-ionone were first detected in Bourbon whisky by LaRoe et al. (*8*), and α- and β-carotine were suggested as their precursors from corn. Another norisoprenoide, (*E*)-β-damascenone, was later identified in Bourbon whisky by Masuda and Nishimura (*9*), while Suomalainen and Nykaenen (*10*) identified the whisky lactone with a coconut-like smell for the first time.

In order to evaluate the contribution of individual volatile components to the aroma of whisky, Salo and co-workers (*11, 12*) were the first to calculate odor units for individual components on the basis of the ratio of their concentration in whisky and their odor thresholds in an ethanol/water mixture. The authors proposed in particular methylpropanal, butanal, 3-methylbutanal, pentanal, 2,3-butandione, and esters, such as ethyl acetate, ethyl hexanoate, and ethyl octanoate, as important aroma components.

* Corresponding author. Tel: +49 89 289 13265. Fax: +49 89 289 14183. E-mail: Peter.Schieberle@ch.tum.de.

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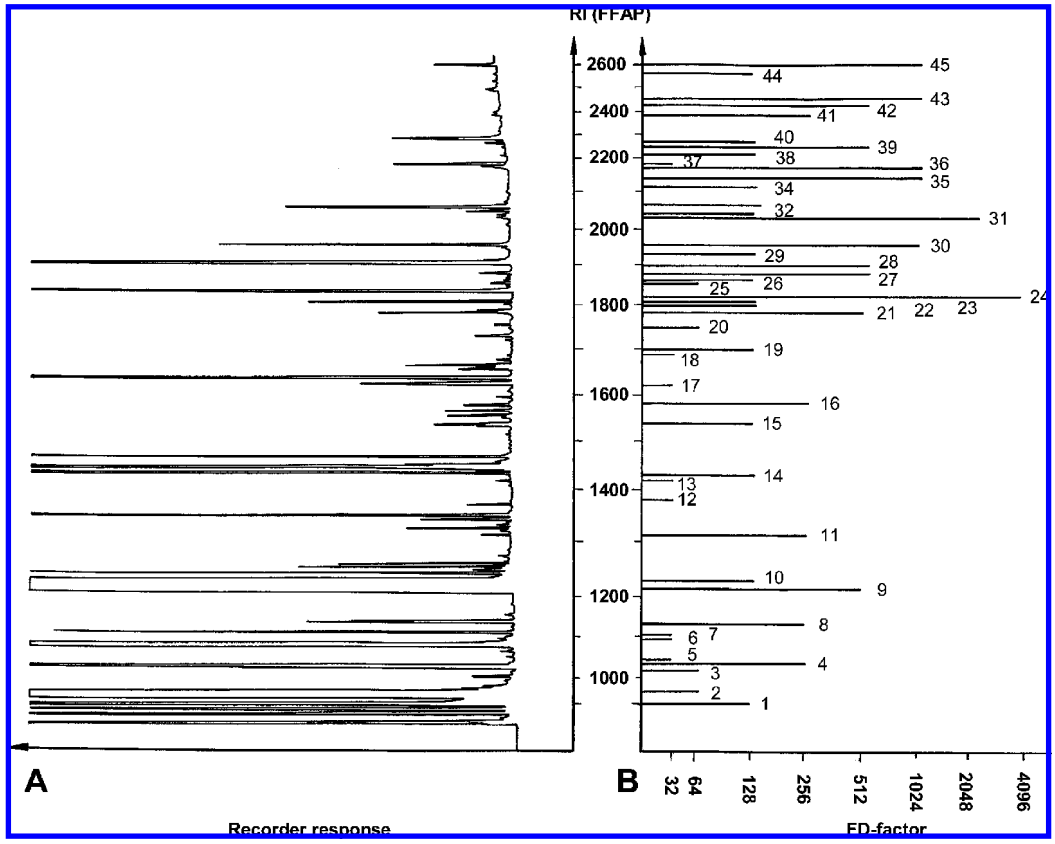

Figure 1. Gas chromatogram (**A**) and flavor dilution (FD) chromatogram (**B**) of the volatile fraction isolated from Bourbon whisky.

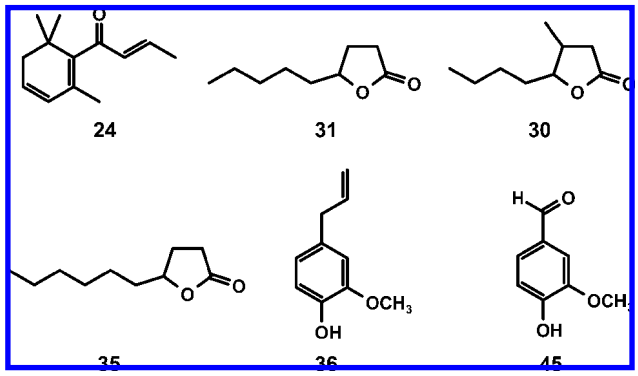

Figure 2. Structures of the most odor-active compounds (FD ≥ 1024) identified in Bourbon whisky (numbering refers to **Table 1**).

In addition, results obtained by preparing a model aroma mixture (*12, 13*) have led to the assumption that carbonyl compounds and esters are the most important odorants in whisky. Spiking of whisky with the respective reference compounds also revealed the importance of phenol, methylphenols, and 2-methoxyphenol as aroma-active constituents (*14, 15*).

On the basis of approaches using molecular sensory science, it has been shown for a considerable number of foods that the entire set of volatiles present in a food is not able to interact with human olfactory receptors (*16*). Instead, only a smaller number of the so-called key odorants is obviously detected by the human odorant receptors and is able to initiate a cascade of biochemical reactions finally leading to aroma perception in the brain.

An approach to separate odor-active volatiles from the bulk of odorless food volatiles is GC-Olfactometry (GC-O) or, more comprehensively, dilution to odor threshold techniques, such as aroma extract dilution analysis (*16*). However, only a very few studies using such techniques to unravel the key aroma compounds of whisky have been reported to date.

Only Connor et al. (*17*) applied GC-O on an extract obtained by SPME from Scotch whisky and particularly confirmed the importance of *cis*-whisky lactone and vanillin in the overall aroma of this type of whisky.

In order to systematically improve and/or modify the aroma of whisky by changing the recipe or the manufacturing technology, there is a need to reveal the influence of each technological step on aroma compound formation during whisky processing. But, although numerous investigations have been undertaken to follow changes in selected volatiles caused by processing, in particular, a comprehensive approach aimed at identifying the entire set of odor-active compounds in Bourbon whisky is still lacking. Because the knowledge on the key odorants in the final product is the prerequisite for studies on the influence of processing steps, the aim of the present study was to locate the potent odorants in an aroma extract from a commercial American Bourbon whisky by application of the aroma extract dilution analysis and to identify the most odor-active compounds by means of reference odorants.

MATERIALS AND METHODS

Whisky. The whisky under investigation was a Kentucky Straight Bourbon whisky which, according to the label, had been produced according to the sour mesh method and had been stored in new, heat-charred oak casks for at least three years. Several batches of the same product (same year of production) were purchased at a local supermarket. Mentioning of a brand name does not imply any research contact with the whisky manufacturer nor is it done for advertising purposes.

Chemicals. Reference compounds of the odorants identified were obtained from the commercial sources given in parentheses: 4-allyl-2-methoxyphenol, α-damascone, (*E*,*E*)-2,4-decadienal, γ-decalactone, 1,1-diethoxyethane, γ-dodecalactone, ethyl acetate, ethyl hexanoate,

Table 1. Most Odor-Active (FD ≥ 32) Volatile Constituents Identified in Bourbon Whisky

no.[a]	odorant[b]	odor quality[c]	fract.[d]	RI[e] on FFAP	RI[e] on DB-5	RI[e] on DB-1701	FD factor	earlier reported as volatile compound in whisky
1	1,1-diethoxyethane[f]	fruity	A	900	730	<800	128	(*6, 25*)
2	ethyl 2-methylpropanoate	fruity	A	958	756	811	64	(*10, 26*)
3	ethyl butanoate	fruity	A	1029	805	857	64	(*10, 27*)
4	ethyl (*S*)-2-methylbutanoate	fruity	A	1040	850	900	256	
5	ethyl 3-methylbutanoate	fruity	A	1049	849	906	32	(*3*)
6	2-methylpropan-1-ol	malty	C	1091	648	n.d.	32	(*6, 25*)
7	unknown	fruity	B	1102	988	n.d.	32	
8	3-methylbutyl acetate	fruity	B	1120	879	948	256	(*6, 25*)
9	3-methylbutan-1-ol	malty	C	1215	735	844	512	(*6, 25*)
10	ethyl hexanoate	fruity	B	1218	1000	1062	128	(*6, 10*)
11	(*E*)-2-heptenal	fatty, green	B	1311	959	1063	256	
12	nonanal	soapy	B	1380	1100	n.d.	32	(*28*)
13	2-isopropyl-3-methoxypyrazine	earthy	C	1413	1091	1145	32	
14	ethyl octanoate	fruity	B + C	1420	1197	1263	128	(*6, 25*)
15	(*E*)-2-nonenal	green	C + D	1533	1159	1275	128	(*29*)
16	(*E,Z*)-2,6-nonadienal[f]	green	C + D	1578	1153	n.d.	256	(*30*)
17	(*E*)-2-decenal	fatty	D	1624	1260	1370	32	
18	(*E,E*)-2,4-nonadienal	fatty	D	1691	1211	n.d.	32	
19	ethyl 2-phenylacetate	flowery	D	1695	n.d.	n.d.	128	
20	4-methylacetophenone	sweet, almond-like		1747	1186	1326	64	
21	α-damascone[f]	cooked apple	C	1779	1389	n.d.	512	
22	(*E,E*)-2,4-decadienal	fatty	B + C	1791	1315	1447	128	
23	2-phenylethyl acetate	flowery	B	1804	1256	1371	128	(*10, 25*)
24	(*E*)-β-damascenone	cooked apple	B	1813	1389	1487	4096	(*9*)
25	2-methoxyphenol	phenolic	C	1849	1089	1225	64	(*3, 31*)
26	2-phenylethyl propanoate	fruity		1860	n.d.	n.d.	128	
27	(3*S*,4*R*)-*trans*-whiskylactone	coconut-like	C	1866	1295	1487	512	(*31, 32*)
28	2-phenylethanol	flowery	D	1900	1113	1273	512	(*6, 25*)
29	β-ionone	violet-like	C	1929	1495	1620	128	(*8*)
30	(3*S*,4*S*)-*cis*-whiskylactone	coconut-like	C + D	1946	1328	1522	1024	(*31, 32*)
31	(*R*/*S*)-γ-nonalactone	coconut-like	D	2018	1365	1566	2048	(*26, 31*)
32	4-ethyl-2-methoxyphenol	phenolic, clove-like	D	2030	1284	1413	128	(*7, 31*)
33	δ-nonalactone	peach-like	D	2047	n.d.	1612	256	(*15, 26*)
34	*trans* ethyl cinnamate	fruity	D	2108	1461	n.d.	256	
35	γ-decalactone	peach-like	D	2132	1471	1690	1024	(*5*)
36	4-allyl-2-methoxyphenol	clove-like	C	2161	1357	1498	1024	(*31*)
37	4-ethylphenol	phenolic	C	2168	1168	1395	32	(*7, 31*)
38	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone[f]	seasoning-like	D	2206	1100	1362	128	
39	unknown	coconut-like	D	2243	1562	1805	512	
40	unknown	fruity	D	2265	n.d.	n.d.	128	
41	γ-dodecalactone	peach-like	D	2381	1685	1900	256	(*5*)
42	(*Z*)-6-dodeceno-γ-lactone	peach-like	D	2425	1680	n.d.	512	
43	unknown	flowery	A + E	2442	n.d.	n.d.	1024	
44	2-phenylacetic acid	flowery	E	2552	1275	n.d.	256	(*33*)
45	4-hydroxy-3-methoxy-benzaldehyde	vanilla-like	D	2600	1400	1632	1024	(*34*)

[a] Numbering refers to **Figure 1**. [b] The odorant was identified by comparing it with the reference compound on the basis of the following criteria: (i) retention indices on the capillaries detailed in the table, (ii) mass spectra obtained by MS-EI and MS-CI, and (iii) odor quality and odor threshold determined by GC-Olfactometry. [c] Odor quality perceived at the sniffing port. [d] The odorant was detected in the column chromatography fractions A−E. [e] Retention index; n.d. = not determined. [f] Because no unequivocal mass spectrum could be obtained, the odorant was identified by the remaining criteria given in footnote b.

4-ethyl-2-methoxyphenol, ethyl (*S*)-2-methylbutanoate, ethyl 3-methylbutanoate, ethyl octanoate, 4-ethylphenol, ethyl 2-phenylacetate, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, 2-isopropyl-3-methoxypyrazine, 4-methyl acetophenone, 2-methylbutanal, 3-methylbutanal, 3-methylbutanol, 3-methylbutyl acetate, 2-methylpropanal, (*E,E*)-2,4-nonadienal, (*E,Z*)-2,6-nonadienal, γ-nonalactone, δ-nonalactone, (*E*)-2-nonenal, phenylacetaldehyde, 2-phenylacetic acid, 2-phenylethyl acetate, 2-phenylethyl propanoate, *cis*/*trans*-whiskylactone, (*E*)-2-decenal, dimethyl sulfide, *trans*-ethyl cinnamate, ethyl butanoate, ethyl 2-methylpropanoate, ethyl pentanoate, ethyl propanoate, (*E*)-2-heptenal, 2-methylpropanol, nonanal, and 2-phenylethanol (Sigma-Aldrich Chemie, Taufkirchen, Germany); acetaldehyde, ethanol, 4-hydroxy-3-methoxybenzaldehyde, and 2-methoxyphenol (Merck, Darmstadt, Germany); β-ionone (Roth, Karlsruhe, Germany); (*E*)-β-damascenone was a gift from Symrise, Holzminden, Germany.

The following odorants were synthesized as reported in the literature given in parentheses: (*Z*)-6-dodeceno-γ-lactone (*18*) and (*Z*)-2-nonenal (*19*).

Diethyl ether and *n*-pentane (Merck, Darmstadt, Germany) were freshly distilled before use. Silica 60 (Merck, Darmstadt, Germany) was purified according to a procedure described in ref *20*.

Isolation of Volatiles. The volatiles present in whisky (25 mL) were isolated by extraction of a 1:1 dilution with tap water (saturated with NaCl) using diethyl ether (3 × 100 mL). The combined extracts were dried over anhydrous sodium sulfate and concentrated to 100 mL at 37 °C using a Vigreux column (50 cm × 1 cm i.d.). The nonvolatile compounds were removed by high vacuum distillation using the SAFE apparatus (*21*), and the distillate was concentrated to 100 μL by means of a Vigreux column (60 cm × 1 cm i.d.) followed by microdistillation (*16*).The distillate was separated into a fraction containing the neutral/basic volatiles (NBF) and the acidic volatiles (AF) by treatment with an aqueous sodium bicarbonate solution (*22*).

Fractionation of the Volatiles by Column Chromatography. The NBF fraction obtained from whisky (200 mL) was concentrated to 1 mL and was applied onto a water-cooled glass column (30 cm × 1 cm) filled with silica 60 (30 g) in *n*-pentane. To yield five fractions containing compounds of increasing polarity, the distillate was separated

Table 2. Most Odor-Active Volatile Constituents in the Headspace above the Bourbon Whisky

odorant[a]	odor quality[b]	RI on SE-54[c]	vol. [mL][d]	RDF-factor[e]	earlier reported as volatile compound in whisky
acetaldehyde	solvent-like	<600	0.5	20	(*6*)
dimethylsulfide	canned maize	<600	10.0	1	(*35*)
ethanol	ethanolic	<600	0.25	40	(*6*)
ethyl acetate	fruity	<600	2.5	4	(*6*)
2-methylpropanal	malty	562	5.0	2	(*26*)
3-methylbutanal	malty	650	0.1	100	(*26*)
2-methylbutanal	malty	660	0.5	20	(*26*)
ethyl propanoate	fruity	711	2.5	4	(*27*)
1,1-diethoxyethane	fruity	726	0.5	20	(*6*)
3-methylbutanol	malty	733	0.5	20	(*6*)
ethyl 2-methyl propanoate	fruity	754	0.25	40	(*26*)
unknown	fruity	757	0.25	40	
unknown	sulfury	771	0.5	20	
ethyl butanoate	fruity	803	1.0	10	(*36*)
unknown	earthy	818	1.0	10	
ethyl (*S*)-2-methylbutanoate	fruity	846	0.5	20	
ethyl 3-methylbutanoate	fruity	848	5.0	2	(*3*)
3-methylbutyl acetate	fruity	890	2.5	4	(*6*)
ethyl pentanoate	fruity	904	5.0	2	(*26*)
unknown	roasty	926	5.0	2	
unknown	green	958	5.0	2	
unknown	fruity	971	2.5	4	
ethyl hexanoate	fruity	1000	2.5	4	(*6*)

[a] See **Table 1**. [b] See **Table 1**. [c] Retention index. [d] Smallest headspace volume in which the odorant was detectable. [e] The relative flavor dilution factor (RDF) was calculated by dividing the largest headspace volume analyzed (10 mL) by the smallest volume in which the odorant was detectable by GC-O.

using five *n*-pentane/diethyl ether mixtures (100 mL each; fraction A, 100:0; fraction B, 95:5; fraction C, 90:10; fraction D, 80:20; fraction E, 50:50 by vol.). Each fraction was dried over anhydrous sodium sulfate and concentrated to 1 mL as described above.

High Resolution Gas Chromatography−Olfactometry (HRGC-O) and Mass Spectrometry (MS). HRGC was performed by means of a gas chromatograph type 8160 (Fisons Instruments, Mainz, Germany) with helium as the carrier gas at a pressure of 70 kPa. Samples were applied by the cold-on-column-injection technique onto either capillary DB-5 (CP-Sil 8CB) (Chrompack, Frankfurt, Germany), DB-FFAP, or DB-1701 (J &W Scientific; supplied by Agilent Technologies) (Waldbronn, Germany). The dimensions for all three columns were 30 m length, 0.32 mm i.d., and 0.25 μm film thickness. For GC-Olfactometry, the end of the capillary was connected to a deactivated Y-shaped glass splitter dividing the effluent of the column into two equal parts, which was then transferred via two deactivated but uncoated fused silica capillaries (50 cm × 0.25 mm) to a sniffing port and an FID, respectively. The sniffing port, which was mounted on a detector base of the GC, was held at 180 °C, and the FID was operated at 240 °C. On-column injection of the samples (0.5 μL) was performed at an oven temperature of 35 °C. After 2 min, the temperature was raised by 40 °C per min to 50 °C, held for 2 min, and raised by 6 °C per min to 180 °C. Then, the temperature was raised by 20 °C per min to 240 °C and finally held for 10 min. During a GC run, a panelist placed his/her nose close to and above the top of the sniffing port and evaluated the odor of the chromatographic effluent. All compounds detected during the evaluation of the extract by four trained panelists were considered relevant, and no anosmic panelist was used.

Linear retention indices (RI) of the compounds were calculated from the retention times of *n*-alkanes (C-8 to C-22) (*22*). Mass spectra were recorded using a gas chromatograph 5890 series II (Hewlett-Packard, Waldbronn, Germany) connected to a sector field mass spectrometer type MAT 95 S (Finnigan, Bremen, Germany). Mass spectra in the electron ionization mode (MS-EI) were recorded at 70 eV ionization energy and mass spectra in the chemical ionization mode (MS-CI) at 115 eV using isobutane as the reactant gas.

Aroma Extract Dilution Analysis (AEDA). For AEDA, fractions containing the neutral/basic (NBF) and acidic volatiles (AF) were stepwise diluted using diethyl ether as the solvent to obtain dilutions of 1:1, 1:2, 1:4, 1:8, 1:16... 1:4096 of the original extracts (*16*). Sniffing of both series of dilutions was continued until no odorant could be detected by GC-O. Each odorant was thus assigned a flavor dilution factor (FD factor) representing the last dilution in which the odorant was still detectable. The evaluation of the original extract was performed by four panelists, in particular, to agree upon the odor-active areas detectable and also upon their odor qualities. Odor qualities were assigned on the basis of a flavor language developed in our group by an evaluation of the odor quality of reference odorants (Czerny et al., unpublished work). The AEDA of both fractions was finally performed by two panelists in separate runs, and the FD factors determined were averaged. Training of the panelists was done by GC-O using at least 40 odor-active reference compounds in a concentration 5 times above their odor thresholds in air.

Static Headspace-Olfactometry (SHO) and Aroma Dilution Analysis (ADA). Aliquots of the whisky (5 mL) were pipetted into septum-sealed vessels (200 mL) and equilibrated for 30 min at room temperature. Decreasing volumes (10 to 0.25 mL) of headspace samples were taken from separate vessels using a gastight syringe (Hamilton, Australia) and were analyzed by HRGC-O as described previously (*22*).

Determination of Enantiomeric Ratios. The enantiomeric ratios were determined by two-dimensional gas chromatography−mass spectrometry using a chiral column (BGB-176, 30 m × 0.25 mm, film thickness 0.25 μm; BGB Analytik AG, Rothenfluh, Switzerland) in the second dimension. The intensities of the molecular ions (MS-CI) were recorded for the calculation of the enantiomeric ratio (*22*).

RESULTS AND DISCUSSION

Identification of Odor-Active Compounds. The Bourbon whisky under investigation exhibited on overall vanilla-like fruity aroma with additional smoky, malty odor qualities. By solvent extraction and SAFE distillation (*21*), a volatile fraction was obtained, which elicited the typical aroma of the whisky when sniffed by a group of 10 panelists on a strip of filter paper in an ethanol/water mixture (4:6 by vol.) equal to the original whisky. Thus, the method of volatile isolation was proven to be appropriate for aroma isolation from whisky.

To obtain insight into the odorants contributing to the overall aroma, an aliquot of the distillate representing the total volatiles of 200 μL of whisky were separated by GC, and the effluent was evaluated by GC-Olfactometry. A total of 45 odor-active

areas were detected, and a quite intense cooked-apple-like odor was smelled at position **24** (**Figure 1**). In addition, several coconut-like odors, **27**, **31**, and **42**, appeared with high intensities. By application of the aroma extract dilution analysis (AEDA), the cooked-apple-like odorant **24**, followed by the coconut smelling compound, **31** appeared with the highest flavor dilution (FD) factors (**Figure 1**). In addition, the vanilla-like smelling odorant **45**, a flowery smelling odorant **43**, the clove-like smelling odorant **36**, the peach-like smelling odorant **35**, and the coconut-like smelling odorant **30** showed somewhat lower FD factors.

To identify the compounds responsible for these odors, first, the retention indices of the odor-active areas and the respective aroma attributes were compared to data available in an in-house database containing about 500 compounds previously identified by our group as key food odorants. This procedure suggested a structure for most of the aroma compounds. To confirm these structures, a distillate obtained from 200 mL of whisky was separated into five fractions by column chromatography on silica gel, the odor-active areas were relocated in the fractions by GC-O and, then mass spectra in the electron impact mode (MS-EI) and in the chemical ionization mode (MS-CI) were recorded. The data were finally compared to data obtained by the analysis of the respective reference compounds.

Following this procedure, compound **24** with the highest FD factor was identified as (*E*)-β-damascenone (**Figure 2**) exhibiting an intense aroma like that of cooked apples. With compounds **21** and **29** (FD factors of 512 and 128, respectively), two further norisoprenoids were identified, namely, α-damascone, likewise exhibiting a cooked apple-like smell, and β-ionone exhibiting a violet-like odor (**Table 1**). Compound **31** with the second highest FD factor of 2048 was identified as γ-nonalactone (**Figure 2**). The two possible enantiomers were separated on a chiral cyclodextrin stationary phase, and the enantiomeric distribution was determined to be 56% *R*-γ-nonalactone and 44% *S*-γ-nonalactone (data not shown). Two further compounds with a coconut-like odor could be identified as *cis*- and *trans*-whiskylactones (β-methyl-γ-octalactone; **27** and **30**, **Table 1**). Whiskylactone (**30**; **Figure 1**) has two chiral centers at positions 3 and 4 resulting in 4 stereoisomers. Mosandl and co-workers (*23, 24*) have previously shown that only the (3*S*,4*S*)-*cis*-whiskylactone and the (3*S*,4*R*)-*trans*-whiskylactone occur in whisky. Thus, by comparison of the retention indices with published data (*23*), compound **27** was identified as (3*S*,4*R*)-*trans*-whiskylactone and compound **30** as (3*S*,4*S*)-*cis*-whiskylactone. With their high FD factors of 1024 and 512, respectively (**Table 1**), both enantiomers are likely to contribute to the overall whisky aroma.

Compound **36** with a clove-like, phenolic odor and a high FD factor of 1024 was identified as 4-allyl-2-methoxyphenol (**Figure 2**). Further odorants showing phenolic odor attributes, but with somewhat lower FD factors, were identified as 4-ethyl-2-methoxyphenol (**32**; **Table 1**) and 2-methoxyphenol (**25**; **Table 1**). Among these compounds having the same ortho-methoxy-hydroxy substitution at the aromatic ring, which were probably extracted from the degraded lignin of the oak cask, 4-hydroxy-3-methoxybenzaldehyde (Vanillin; **45**; **Figure 2**) with a vanilla-like aroma showed the highest FD factor.

The largest peak in the gas chromatogram (**9**; **Figure 1**) showing a malty smell was identified as 3-methylbutanol, and the aroma-active area **28** exhibiting a flowery aroma note was identified as 2-phenylethanol. For both alcohols, well known as metabolites of amino acid degradation by yeasts, an FD factor of 512 was determined.

Various esters exhibiting fruity or flowery aroma notes, were identified in the whisky distillate (**Table 1**), namely, ethyl 2-methylbutanoate (**4**), 3-methylbutyl acetate (**8**), ethyl hexanoate (**10**), ethyl methylpropanoate (**2**), ethyl butanoate (**3**), 2-phenylethyl acetate (**23**), 2-phenylethyl propanoate (**26**), *trans*-ethyl cinnamate (**34**), and ethyl 2-phenylacetate (**19**). Ethyl 2-methylbutanoate (**4**) was shown to be present as a nearly pure (*S*)-enantiomer (98%) when analyzed on a chiral cyclodextrin phase (data not shown). Ethyl (*S*)-2-methylbutanoate (**4**), ethyl 2-phenylacetate (**19**), and *trans*-ethyl cinnamate (**34**) were all identified for the first time in whisky.

Several aldehydes, exhibiting fatty or green odor notes, were also identified for the first time in the volatile fraction of whisky, among them were (*E*)-2-heptenal and (*E*)-2-decenal (**Table 1**). The results of the identification experiments in combination with the FD factors are summarized in **Table 1**, which also shows, whether a compound has previously been reported as constituent of whisky.

The extraction and distillation/concentration process used in the AEDA experiments, may lead to losses in highly volatile compounds. To overcome this gap, static headspace-olfactometry (SHO) was applied to the whisky sample. The results (**Table 2**) revealed 23 aroma-active areas, and the aroma contribution of these highly volatile components was evaluated by analyzing decreasing volumes of the headspace above the whisky. In the largest volume of 10 mL, 23 odor-active areas were detected. By decreasing the volume down to 100 μL, only the malty smelling 3-methylbutanal could finally be detected, corresponding to a relative FD factor of 100. Further odorants with a high FD factor were identified as ethanol and ethyl 2-methylpropanoate.

Although in our study no experiments were performed to identify precursors of the key odorants detected in whisky, several suggestions on their origin are given on the basis of literature data reported for other alcoholic beverages: (*E*)-β-damascenone, which appeared with the highest FD factor has already been detected in various alcoholic beverages, for example, rum, beer, and wine (*37–39*). In whisky, it has been reported for the first time by Masuda and Nishimura (*9*). In studies on the formation of (*E*)-β-damascenone, Roberts and Acree (*40*) suggested the acid-catalyzed formation of (*E*)-β-damascenone from 3-hydroxy-7,8-dihydro-β-ionol as the key precursor. This precursor has also been identified in wine (*41, 42*). On the basis of these findings, it can be assumed that cereals may contain similar precursors leading to the generation of (*E*)-β-damascenone during fermentation and/or distillation.

Whiskylactone was first identified in whisky by Nishimura and Masuda in 1971 (*31*) and has been the subject of investigation regarding whisky flavor ever since. Mosandl et al. (*23, 24*) reported that both enantiomers (3*S*,4*S*)-*cis*- and (3*S*,4*R*)-*trans*-whiskylactone are derived from the wood of American white oak (*Quercus alba*). Previously 3-methyl-4-(3,4-dihydro-5-methoxybenzoyloxy)-octanoic acid has been identified in extracts from *Quercus alba* and has been postulated as the precursor of the lactones during charring of barrels (*43, 44*). Consequently, Connor et al. (*17*) found that whisky lactone is present in significantly higher amounts in whisky that was stored in new, heat-treated oak casks as compared to whisky that was aged in re-used casks.

(*E*,*Z*)-2,6-Nonadienal was previously identified in malt whisky by Wanikawa et al. (*30*). During the process of malting, linoleic and linolenic acid present in cereals are suggested to be oxidized by lipoxygenases into hydroperoxides, which might be decomposed into unsaturated aldehydes such as (*E*)-2-nonenal. Linoleic

acid can also be formed during yeast fermentation (*45*) or can be released from oak wood (*46*). (*E*,*E*)-2,4-Nonadienal, (*E*)-2-decenal, and (*E*)-2-heptenal, identified here for the first time in whisky, may be generated by similar mechanisms of lipid oxidation.

In the present study, a total of 13 aroma compounds were newly detected in the volatile fraction of Bourbon whisky, namely, (*S*)-ethyl 2-methylbutanoate, (*E*)-2-heptenal, (*E*,*E*)-2,4-nonadienal, (*E*)-2-decenal, (*E*,*E*)-2,4-decadienal, 2-isopropyl-3-methoxypyrazine, ethyl phenylacetate, 4-methylacetophenone, α-damascone, 2-phenylethyl propanoate, 3-hydroxy-4,5-dimethyl-2(^{5}H)-furanone, *trans*-ethyl cinnamate, and (*Z*)-6-dodecenoγ-lactone. Previous studies have emphasized the importance of straight-chain ethyl esters, such as ethyl acetate, ethyl butanoate, ethyl hexanoate, and ethyl octanoate for whisky aromas as these are present in whisky in high concentrations (*47*). Our investigations have, however, shown that, in particular, branched ethyl esters such as ethyl (*S*)-2-methylbutanoate, ethyl 3-methylbutanoate, and ethyl 2-methylpropanoate contribute to the fruity attribute in whisky flavor among which ethyl (*S*)-2-methylbutanoate is suggested as the most important fruity smelling odorant.

To confirm the aroma contribution of the compounds listed in **Table 1**, quantitative studies and aroma recombination experiments are underway (*48*). However, the compounds identified can already be suggested as indicators to assess the odor quality of Bourbon whisky.

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