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Streamlined approach for careful and exhaustive aroma characterization of aged distilled liquors

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ABSTRACT

Solvent-assisted flavor evaporation (SAFE) is considered to be the best overall method to produce a "clean" aroma extract to avoid the loss of labile aroma compounds or the formation of thermally generated artifacts during gas chromatographic (GC) analysis. However, SAFE is both time consuming and labor intensive, especially when applied repeatedly for quantitation by stable isotope dilution analysis (SIDA), which requires the addition of isotopes within specific mass ratio ranges relative to target analytes. The streamlined approach described herein allows for accurate quantitation of odor-active components in liquor products with a single SAFE operation. The quantitative results achieved by this method are nearly identical for most odor-active components, except for specific semi-volatile constituents not recovered well by SAFE (e.g., vanillin and syringaldehyde in oak-aged liquors). The streamlined approach provides a simple and convenient way to expedite the careful and exhaustive study of the flavor chemistry of aged liquors.

1. Introduction

Study of the flavor chemistry of distilled spirits and liquors by GC has a long history of several decades. The accurate identification and quantitation of the aroma components of liquor products is of significant importance in determining product quality and authenticity and for optimization of brewing and blending technology (MacNamara & Hoffmann, 1998). Among the various techniques applied for volatile compound isolation and quantitation, solvent-assisted flavor evaporation (SAFE) combined with gas chromatography-olfactometry (GC-O) and stable isotope dilution analysis (SIDA) is considered the state-of-the-art approach, which has been widely applied in recent studies on the flavor chemistry of liquor products (Franitza, Granvogl, & Schieberle, 2016; Lahne, 2010; Poisson & Schieberle, 2008). SAFE generates authentic "clean" extracts suitable for careful on-column GC analysis, a procedure which minimizes losses of labile aroma compounds or generation of thermally generated artifacts (Engel, Bahr, & Schieberle, 1999). In the case of aged liquors the use of SAFE is essential if on-column GC analysis is to be used since the solvent extract may contain nonvolatile compounds, such as oak-derived nonvolatile compounds in the case of aged whiskeys. Even some liquors that are aged in pottery, e.g. Chinese liquors (baijius), may contain some nonvolatile compounds. A case in point is Moutai, a traditional Chinese baijiu, which is known to contain lichenysin, a nonvolatile cyclooctapeptide produced by *Bacillus licheniformis* during fermentation (Zhang, Wu, & Xu, 2014; Zhang, Wu, Xu, & Qian, 2014).

Meanwhile SIDA, which makes use of a deuterium or carbon-13 labeled analogue of a target analyte as the internal standard for quantitation, corrects for extraction bias – which occurs to some extent with all volatile isolation techniques including SAFE – thus providing highly accurate results. Due to the great similarity of the isotopes and their corresponding analytes with respect to their physical and chemical properties, their mass ratios remain essentially unchanged before and after extraction and during subsequent sample workup protocols. For the above reasons, SAFE and SIDA serve as the gold standards for volatile isolation and quantitation, respectively.

A typical procedure for the flavor analysis of a food product by using SAFE combined with SIDA is as follows (Fig. 1A): isotopes are added to the known amount of product and equilibrated followed by solvent extraction; The solvent extract is then subjected to SAFE and the peak area ratios of the isotopes and target analytes are determined by GC–MS to provide guidance for the adjustment of isotope addition if needed. To establish the ratio of each isotope to its corresponding target analyte within the linear range of the standard curve, this "addition-extraction-adjustment" procedure is repeated several times before finally the correct level of isotope addition is determined. The whole procedure is tedious and time consuming, and expensive if the isotopes and target product are costly, and possibly impractical if the target

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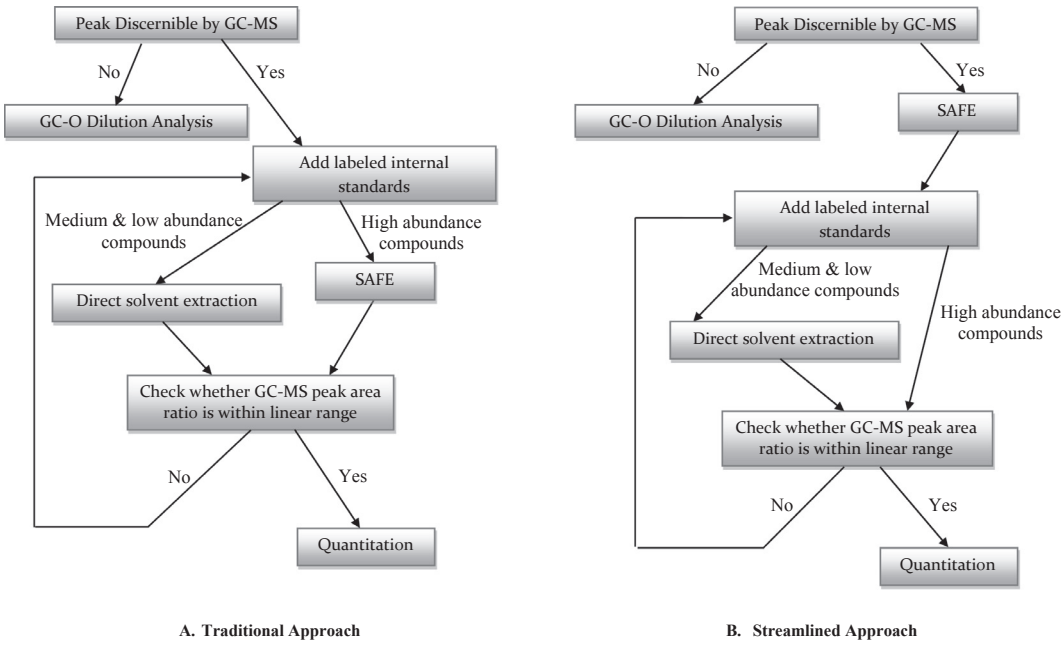

Fig. 1. Flowcharts of traditional (A) and streamlined (B) approaches used for stable isotope dilution analysis (SIDA).

product is scarce or otherwise in limited supply. Thus, optimizing the efficiency of this quantitative analysis method is essential for reducing the time and cost, as well as to maintain the accuracy of this state-of-the-art methodology.

In the SAFE procedure, odor-active components are distilled under high vacuum, thus, the extraction bias during SAFE is based on volatility rather than polarity, like in the case of solvent extraction. If volatile losses during SAFE are negligible the direct SAFE distillate could be subjected to SIDA to allow the adjustment of isotope addition performed in a small scale to reduce the number of SAFE operations and the time and costs associated with isotopes and potentially limited sample quantities.

In the present study, isotopes were added after SAFE extraction was completed instead of before any extraction procedures as in the standard protocol (Lahne, 2010; Poisson & Schieberle, 2008). To assess the feasibility of this streamlined procedure (Fig. 1B) and to identify potential pitfalls and limitations, a series of experiments were conducted. The standard protocol has limitations regarding quantitation of aroma components of scarce or expensive products. Aged distilled liquor is good a case in point and was selected as the target product in this study. To have a better assessment of the potential of the streamlined procedure, three clear liquors (Chinese baijius) aged in porcelain and three brown liquors (whiskey, tequila and rum) aged in oak barrels, were selected to assess and validate the procedure in the present study. Liquor samples were compared with their counterpart SAFE distillation isolates by sensory and instrumental methods to assess the advantages and limitations of the proposed streamlined approach.

2. Materials and methods

2.1. Materials

All the liquors used in this study were commercial products. The selected clear distilled liquors included the top soy sauce aroma liquor Moutai, which is also the national liquor of China (MT, Kweichow Moutai Co. Ltd. Guizhou, China.), the top sesame flavor liquor Yi Pin Jing Zhi (YPJZ, Jingzhi Liquor Co., Ltd., Shandong, China.) and a mid-range strong aroma liquor Gu Jing Gong Jiu (GJGJ, Gujinggong Liquor Co., Ltd. Bozhou, China.). These liquors represent top and medium level clear liquor products which are aged in pottery. For the oak aged (or brown) liquors, Evan Williams Kentucky Bourbon Whiskey (EWW, Old Evan Williams Distillery, Kentucky, U.S.A.), Don Julio Tequila (DJT, Tequila Don Julio, S.A. DE C.V. Jalisco, Mexico.) and Appleton Estate Jamaica Rum (Aged 12 years) (AER, J.Wray & Nephew LTD. Jamaica.) were selected to represent high and mid-range liquor products which are aged in oak barrels. Mention of brand name is not for advertisement or endorsement purposes and does not imply any research contract or sponsorship.

2.2. Chemicals

All authentic reference standards and reagent grade chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified. 2-Methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

The following isotopically labeled compounds were purchased from the suppliers listed in parentheses: [$^{2}H_{5}$]-propanoic acid (Cambridge Isotope Laboratories, Inc., Andover, MA, USA); [*1,2-*$^{13}C_{2}$]-phenylacetic acid and [1,2-$^{13}C_{2}$]-butanoic acid (Isotec, Miamisburg, OH, USA); [$^{2}H_{9}$]-pentanoic acid; [*2,2-*$^{2}H_{2}$]-3-methylbutanal (CDN Pointe-Claire, Quebec, Canada). [$^{2}H_{3}$]-acetic acid (CDN, Pointe-Claire, Quebec, Canada).

The following labeled and unlabeled compounds were synthesized according to procedures reported in the literature (in parentheses): 3-methyl-2,4-nonadione (Guth & Grosch, 1989); [*1,2-*$^{13}C_{2}$]-2-phenylethanol (Schuh & Schieberle, 2006); *bis*(2-methyl-3-furyl) disulfide (Hofmann, Schieberle, & Grosch, 1996); [$^{13}C_{2}$]-sotolon (Blank, Lin, Fumeaux, Welti, & Fay, 1996); 4-hydroxy-3-[$^{2}H_{3}$]-methoxybezaldehyde ([$^{2}H_{3}$]-vanillin) (Schneider & Roland, 1992); [$^{2}H_{4}$]-β-damascenone (Kotseridis, Baumes, & Skouroumounis, 1998); 2-methyl-[2,3-$^{2}H_{2}$]-propan-1-ol, [*2,3-*$^{2}H_{2}$]-methylpropanal and [*3,4-*$^{2}H_{2}$-2-methylbutanal (Wu & Cadwallader, 2019).

2.2.1. Synthesis of [3,3,4,4]-$^{2}H_{4}$-hexanoic acid

3-Hexyn-1-ol was deuterated to [*3,3,4,4-*$^{2}H_{4}$]-hexan-1-ol using a previously described method (Hausch, Lorjaroenphon, & Cadwallader, 2015). The deuterated alcohol was then oxidized to the corresponding acid using potassium permanganate as described previously (Guth & Grosch, 1994) for the synthesis of [3,4-$^{2}H_{2}$]-3-methylbutyric acid. MS-EI, *m/z (%)* of [*3,3,4,4-*$^{2}H_{4}$]-hexanoic acid (purity 98.3%): MS-EI, *m/z*

(%): 61 (1 0 0), 75 (39), 60 (29) , 44 (25), 43 (22), 45 (20), 62 (14), 74 (11).

2.2.2. Synthesis of [2,3-$^2H_2$]-2-methylpropanoic acid

2-Methyl-*[2,3-$^2H_2$]*-propan-1-ol was oxidized to the corresponding acid using potassium permanganate as described previously (Guth & Grosch, 1994) for the synthesis of [3,4-$^2H_2$]-3-methylbutanoic acid. MS-EI, *m/z(%)* of [*2,3-$^2H_4$*]-2-methylpropanoic acid (purity 99.8%): 61 (1 0 0), 75 (39), 60 (29) , 44 (25), 43 (22), 45 (20), 62 (14), 74 (11).

2.2.3. Synthesis of 4-hydroxy-3[$^2H_3$],5-dimethoxy benzaldehyde ($^2H_3$-syringaldehyde)

In a 50-mL screw-capped test tube (PTFE-cap) equipped with a stir bar, 3,4-dihydroxy-5-methoxybenzaldehyde (0.501 g; 3 mmol) was dissolved in aqueous 40% (w/v) KOH (5 mL). Then, under a gentle steam of nitrogen and over the course of 30 min, 0.35 mL (0.42 g, 3.2 mmol) of [$^2H_6$]-dimethylsulfate was added (5 to 6 drops every 5 min) to the reaction tube, after which the reaction mixture became yellow and cloudy. The vial was then capped and stirred for 2 h. The reaction was checked for completion by removing 5–6 drops of the reaction mixture, adding it to a vial containing 1 mL aqueous 1 N HCl and 0.5 mL ethyl acetate, and then analyzing the ethyl acetate layer by GC–MS. The reaction was continued by adding 0.08 mL (0.096 g, 0.73 mmol) of [$^2H_6$]-dimethylsulfate and allowing the reaction to stir overnight until nearly all starting material had been consumed. The reaction was stopped by acidifying the mixture to ~pH 1 and then it was extracted with ethyl acetate (1 × 10 mL, 4 × 5 mL). The ethyl acetate layer was washed with saturated NaCl and then dried over anhydrous Na_2SO_4. The solution was concentrated to ~10 mL using a vigreux column and the remaining solvent was then removed under a stream of nitrogen. The final product was weighed for a final yield of 0.5734 g (purity 98.1%). MS-EI, *m/z (%)* of [$^2H_3$]-syringaldehyde: 185 (100), 184 (66), 96 (20), 39 (15), 51 (14), 114 (14), 67 (13), 53 (12), 79 (12), 111 (11), 186 (11), 68 (10), 95 (10), 50 (10).

2.2.4. Synthesis of [$^2H_5$]-ethyl esters – general procedure

The following reagents were added to a 20-mL screw top test tube: 10 mmol of organic acid; 200 μL of [$^2H_6$]-ethanol (158 mg; 3 mmol) and 2 drops concentrated H_2SO_4. The test tube was sealed with PTFE-lined screw cap and placed in a large bottle or beaker to protect against breakage and then incubated in a 100 °C oven for 2 h. After cooling to room temperature, the mixture was diluted with pentane (10 mL) and then 5 mL of aqueous saturated Na_2CO_3 solution was added. The aqueous layer was removed the pentane layer was washed again with Na_2CO_3 (5 mL). (This step was necessary in order to remove any unreacted acid.). The pentane layer was washed with aqueous saturated NaCl (2 × 5 mL) and then dried over anhydrous Na_2SO_4. The solvent (pentane) was evaporated off to yield the ester, generally in high purity (> 99%). If needed, the ester was further purified by flash chromatography on silica gel using 100% pentane as elution solvent.

[$^2H_5$]-ethyl phenylacetate (purity 98.7%)

MS-EI, *m/z (%)*:91 (100), 169 (31, M+), 92 (14), 65 (11), 170 (5), 93 (5), 89 (5), 39 (4)

[$^2H_5$]-ethyl 3-phenylpropanoate (purity 99.7%)

MS-EI, *m/z (%)*: 104 (100), 107 (51), 105 (50), 183 (45, M+), 91 (45), 77 (19), 79 (18), 92 (15), 133 (13), 103 (13), 51 (10)

[$^2H_5$]-ethyl butanoate (purity 99.7%)

MS-EI, *m/z (%)*: 43 (100), 71 (88), 93 (62), 41 (35), 61 (27), 42 (24), 74 (23), 39 (15), 106 (10), 44 (10).

[$^2H_5$]-ethyl 3-methylbutanoate (purity 99.3%)

MS-EI, *m/z (%)*: 93 (100), 57 (70), 85 (59), 61 (50), 41 (48), 43 (28), 39 (23), 42 (23), 63 (16), 59 (14), 120 (13), 74 (12), 88 (12), 46 (12), 56 (11), 44 (10), 73 (10).

[$^2H_5$]-ethyl pentanoate (purity 98.8%)

MS-EI, *m/z (%)*: 93 (100), 85 (86), 57 (77), 61 (58), 74 (48), 41 (44), 106 (37), 55 (25), 56 (21), 63 (21), 39 (18), 73 (15), 42 (15), 64 (11), 49 (10).

[$^2H_5$]-ethyl hexanoate (purity 98.0%)

MS-EI, *m/z (%)*: 93 (100), 43 (64), 99 (46), 61 (41), 74 (34), 41 (33), 106 (29), 42 (26), 71 (22), 55 (22), 39 (15), 63 (14), 73 (10), 44 (10), 120 (10).

[$^2H_5$]-ethyl heptanoate (purity 99.8%)

MS-EI, *m/z (%)*: 93 (100), 43 (52), 61 (37), 41 (36), 74 (33), 113 (32), 106 (31), 55 (26), 42 (15), 63 (15), 120 (15), 56 (13), 39 (13), 57 (10).

[$^2H_5$]-ethyl octanoate (purity 99.8%)

MS-EI, *m/z (%)*: 93 (100), 106 (34), 57 (34), 74 (33), 41 (32), 61 (29), 55 (28), 127 (23), 43 (22), 42 (17), 63 (12), 56 (10).

[$^2H_5$]-ethyl decanoate (purity 97.4%)

MS-EI, *m/z (%)*:93 (100), 106 (44), 74 (31), 41 (28), 43 (25), 55 (24), 61 (20), 162 (14), 155 (11), 57 (11), 63 (11), 42 (10), 94 (10).

[$^2H_5$]-ethyl dodecanoate (purity 99.9%)

MS-EI, *m/z (%)*: 93 (100), 106 (52), 74 (27), 41 (27), 43 (25), 55 (25), 61 (14), 57 (14), 94 (13), 162 (10).

[$^2H_5$]-ethyl hexadecanoate (purity 92.8%)

MS-EI, *m/z (%)*: 93 (100), 106 (61), 43 (32), 55 (27), 41 (25), 74 (21), 57 (19), 94 (18), 162 (13), 69 (12), 61 (10).

3. Methods

3.1. Direct distillation of liquor (SAFE-DIST)

Liquor sample (100 mL) was fed directly into the SAFE apparatus as previously described (Rotsatchakul, Chaiseri, & Cadwallader, 2007). The system was maintained under high vacuum (2–5 × 102^{-5} Torr) and at 40 °C throughout the 2 h distillation process. The distillate was stored in a glass bottle equipped with a PTFE-lined cap.

3.2. Sensory methodology

Sensory testing was approved (protocol number 17507) by the Institutional Review Board (IRB) of the University of Illinois at Urbana-Champaign. Panelists (24), consisting of 16 females and 8 males, ranging in age from 21 to 55, were selected to participate in triangle difference testing to determine whether the overall aroma characteristics of the SAFE-DIST isolates differed from the original (neat) liquor products. SAFE isolates and liquor products (20 mL each) were transferred into 125-mL Teflon sniff bottles (Nalgene PTFE wash bottle without a siphon tube; Nalge Nunc International, Rochester, NY, USA) which were wrapped with aluminum foil to prevent visual bias and to protect the liquors from light exposure. For triangle difference testing (Meilgaard & Civille, 2007), samples were presented to panelists in two orders: one set consisted of two SAFE-DIST isolates and one original (neat) liquor product and the other consisted of one SAFE-DIST isolate and two original (neat) liquor products. Panelists were asked to assess the aroma properties of each sample and choose the odd sample.

3.3. GC-FID analysis

Original liquors and their counterpart SAFE-DIST isolates were analyzed using a 6890N GC-equipped with a flame-ionization detector (FID) (Agilent Technologies, Inc., Santa Clara, CA, USA). Separations were performed using an Rtx-Wax column (15 m × 0.53 mm i.d. × 1 μm film thickness; Restek Corp., Bellefonte, PA, USA). Analyses were conducted in triplicate to assure accurate and precise measurements. The samples were injected in hot split mode (1:10) with an inlet temperature of 250 °C. The carrier gas was helium at a flow rate of 2 mL/min. The oven temperature was programmed from 35 °C to 225 °C at a ramp rate of 10 °C/min with initial and final hold times of 5 and 20 min, respectively. Peak areas for selected compounds found in moderate to high abundance were compared across the original liquors and their counterpart SAFE-DIST isolates.

3.4. Gas chromatography–mass spectrometry-olfactometry (GC–MS-O)

One clear liquor (MT) and one brown liquor (EWW) were analyzed to compare the aroma profiles before and after SAFE distillation. Odor-active components in liquor products and their respective SAFE isolates were extracted by direct solvent extraction (DSE). SAFE distillate or neat liquor (7.5 mL) was pipetted into a 50-mL glass centrifuge tube containing 40 mL of odor-free deionized-distilled water and 0.5 mL of DCM. The mixture was shaken vigorously for 5 min and centrifuged at 4500 rpm for 10 min. The extraction procedure was repeated two more times. The pooled solvent extract was frozen overnight to remove excess water, then the extract was transferred into a 2-mL vial and condensed to 1 mL using a gentle stream of ultra-high purity nitrogen gas.

GC–MS-O was performed using a 6890N GC/5973N mass selective detector (MSD) system (Agilent Technologies, Inc.). Analyses were performed on both polar (Stabilwax, 30 m × 0.25 mm i.d., 0.25 μm film thickness; Restek Corp.) and nonpolar (Rxi-5ms, 30 m × 0.25 mm i.d., 0.25 μm film thickness; Restek Corp.) columns. Aroma extracts (2 μL) were injected using a CIS-4 (Gerstel, Germany) programmable temperature vaporizer (PTV) inlet in the cold-splitless mode (-50 °C initial temperature (0.1 min hold), ramped at 12 °C/s to 250 °C and held for 20 min). The carrier gas was helium at a flow rate of 1 mL/min. The oven temperature was programmed from 40 °C to 250 °C at a ramp rate of 3 °C/min with initial and final hold times of 5 and 30 min, respectively. Temperatures of MSD transfer line and olfactory port were set at 250 °C. Mass scan range was set as 33–300 amu with scan rate of 5.27 scans/s and electron energy was 70 eV. GC–MS data were analyzed by ChemStation Enhanced Data Analysis Software (Agilent Technologies, Inc.). For tentative compound identifications, mass spectra of the analytes were compared against those in 2008 National Institute of Standards and Technology (NIST) Mass Spectral Library.

3.5. On-column gas chromatography-olfactometry (GC-O)

GC-O was performed using a 6890 GC (Agilent Technologies Inc.) equipped with a flame ionization detector (FID), a sniff port (DATU Technology Transfer, Geneva, NY, USA) and a cool on-column injector (+3 °C, oven tracking). A selected SAFE-DIST (2 μL) was injected directly into either a polar (RTX-Wax, 15 m × 0.32 mm i.d., 0.5 μm film thickness; Restek Corp.) or nonpolar (RTX-5, 15 m × 0.32 mm i.d., 0.5 μm film thickness; Restek Corp.) column. The carrier gas was helium at a constant flow rate of 2 mL/min. The oven temperature was programmed from 40 to 250 °C at a ramp rate of 10 °C/min with initial and final hold times of 5 and 30 min, respectively. Column effluent was split equally between the sniff port and FID by using 0.15 mm i.d. deactivated capillary columns of equal length (1 m). FID and sniff port temperatures were maintained at 250 °C.

3.6. Compound identification

Retention indices (RI) were calculated based on comparing the retention times of analytes to those determined for a homologous series of n-alkanes (from C_7 to C_{28}) analyzed under the same analytical conditions (van Den Dool & Kratz, 1963). Odorants were identified by comparing their retention indices (RI) on both polar and nonpolar GC columns, mass spectra and odor properties to those of authentic standards. A compound was considered positively identified if all three of the above criteria matched those of a reference standard. However, in some cases, an odorant was considered tentatively identified when one or more of the above criteria could not be met, e.g. when no mass spectrum was available due to the compound being present at a trace level and below that of the detection limit of the MSD, or whenever an authentic standard was not available to confirm an RI, mass spectrum or odor properties. In the latter case, the compound was considered tentatively identified when its RI, mass spectrum and odor properties were in agreement with literature values or mass spectral library database entry.

3.7. Aroma extract dilution analysis (AEDA)

Relative potencies of odor-active compounds were determined by AEDA. DSE extracts were diluted stepwise at a ratio of 1:3 (v/v) in dichloromethane (DCM) and each dilution was analyzed by GC–MS-O as previously described. Flavor dilution (FD) factors of each odorant were determined as the highest dilution at which the odorant was last detected by GCO (Grosch, 2001). The FD factors are shown as $\log_3$ FD-factors for better comparison between each liquor product and its respective SAFE isolate.

3.8. Quantitation by stable isotope dilution analysis (SIDA)

SIDA was applied to accurately evaluate the recovery of volatile or semi-volatile components in SAFE-DIST. For this purpose, the concentrations of selected volatile components in MT and EWW and their respective SAFE-DIST isolates were determined by SIDA and compared.

Deuterated or carbon-13 labeled isotopes of selected analytes were dissolved in ethanol or DCM and spiked into 1 mL aliquots of neat liquor products or their SAFE-DIST isolates. Sample analysis was performed in the same manner as mentioned previously by using a Stabilwax column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Restek Corp.) and injections were made using the cold-split mode (split ratio 10:1; −50 °C initial temperature (0.1 min hold), ramped at 12 °C/s to 250 °C and held for 20 min). Samples were analyzed under simultaneous full scan (35–300 amu) and selected ion monitoring (SIM) modes. Selected ions used for determination of peak areas of labeled and unlabeled components are listed in Table 1S.

For quantitation of the Strecker aldehydes (2-methylpropanal, 2-methylbutanal and 3-methylbutanal) in liquor samples, headspace solid-phase microextraction (HS-SPME) was applied. MT (50 μL) or EWW (500 μL) were transferred to a 20-mL SPME vial containing 0.5 g of sodium chloride and 2 mL or 1.5 mL, respectively, of distilled odorless water. After spiking with a proper amount of isotope solution, samples were analyzed by HS-SPME-GC–MS using a 6890N GC/5973N MSD (Agilent Technologies, Inc.) equipped with an MPS2 autosampler (Gerstel) and CS4 injection port (Gerstel). A three phase SPME fiber (divinylbenzene/carboxen/polydimethylsiloxane; Supelco, Bellefonte, PA, USA) was used for volatile extraction. Vials were equilibrated at 60 °C for 20 min followed by a 10 min SPME headspace extraction. Volatiles were desorbed into the GC by hot splitless injection (260 °C; 4 min split valve-delay time). Separations were performed using a RTX-5ms column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Restek Corp.). Helium was used as the carrier gas at 1 mL/min. Oven temperature was programmed as follows: initial temperature, 30° C (5 min hold), ramp rate 6° C/min, final temperature, 225° C (30 min hold).

3.9. Quantitation of sotolon

A special case can be made for sotolon which could be generated in the hot inlet of the GC. Due to its low threshold of 10 ppb in wine and its relatively low concentration in liquor products (Gabrielli et al., 2015), the determination of concentration of sotolon generally requires extraction and compound class fractionation. To determine whether it is an artifact and also to determined its relative recovery by SAFE, sotolon was determined by SIDA using 3 different methods.

1) Isotope solution was added to 10 mL SAFE-DIST followed by direct solvent extraction using DCM (3 × 2 mL).
2) Isotope solution was added to 10 mL liquor product followed by direct solvent extraction using DCM (3 × 2 mL).
3) Isotope solution was added to 10 mL liquor product followed by SAFE distillation (SAFE was operated as mentioned previously) and the distillate underwent direct solvent extraction using DCM

Table 1
Triangle difference test comparison of perceived aroma attributes of neat versus SAFE-DIST isolates for selected liquors.

Liquor		Panel response (no. correct/no. total assessments)			
		(2 SAFE – 1 Neat)[a]	(2 Neat – 1 SAFE)[b]	Total	Sign. Diff.[c]
Clear liquor products	MT[d]	4/12	3/12	7/24	N
	GJGJ[e]	5/12	7/12	12/24	N
	YPJZ[f]	5/12	3/12	8/24	N
Brown liquor products	DJT[g]	4/12	6/12	10/24	N
	EWW[h]	2/12	10/12	12/24	N
	AER[i]	4/12	7/12	11/24	N

[a] 2 SAFE-1 Neat: Triangle test of 1 original liquor and 2 of their corresponding SAFE distillate.
[b] 2 Neat-1 SAFE: Triangle test of 2 original liquor and 1 of their corresponding SAFE distillate.
[c] Significant difference: N = not significantly different ($p \leq 0.05$).
[d] MT: Moutai.
[e] GJGJ : Gu Jing Gong Jiu.
[f] YPJZ: Yi Pin Jing Zhi.
[g] DJT: Don Julio Tequila.
[h] EWW: Evan Williams Kentucky Bourbon Whiskey.
[i] AER: Appleton Estate Jamaica Rum.

(3 × 2 mL).

In all of the above procedures, the DCM extract was washed (3 × 3 mL) with 5% (w/v) aqueous Na_2CO_3. Aqueous phase was then washed with DCM (3 × 5 mL). Then the aqueous phase was adjusted to pH 2 (1 N aqueous H_2SO_4) and then extracted with DCM (3 × 2 mL). Solvent phase was dried over anhydrous Na_2SO_4 (1 g) and condensed to 0.1 mL using a gentle stream of ultra-high purity nitrogen gas. Each method was conducted in triplicate and the extracts analyzed using the methods and instrumentation mentioned previously.

3.10. Calibration

The response factor (R*f*) for each isotope against its unlabeled target analyte was determined by a five-point standard curve with a range of mass ratios from 1:5 to 5:1 (unlabeled:labeled). Each point was analyzed in triplicate and the mass ratio of each point was plotted against its mean peak area ratio of selected ions of labeled and unlabeled target analytes.

The R*f* of each labeled analyte was calculated by using the following equation:

$$\frac{\text{mass (analyte)}}{mass\ (labeled\ analyte)} = \frac{area\ (analyte)}{area\ (labeled\ analyte)}\ (Rf)$$

The concentration of each target analyte was calculated by using the following equation:

$$Concentration\ (analyte) = \frac{Rf \times Peak\ area\ ratio \times mass\ (labeled\ analyte)}{Sample\ volume}$$

3.11. Thermal stability of selected disulfides

Since the aroma extracts were analyzed by split/splitless inlet injection, the volatile components were thus heated and condensed in the inlet which may cause the loss and/or formation of thermally generated artifacts, especially some sulfur compounds. Two sulfur compounds identified in MT, *bis*(2-methyl-3-furyl) (MFT-MFT) and 2-methyl-3-(methyldithio) furan (MFT-MT), were selected and analyzed using various methods to confirm the identification results. For this purpose, potential artifacts caused by different injection techniques were compared by using *n*-alkanes as internal standards which have boiling points close to those of the target analytes. Boiling point of MT-MFT and MFT-MFT are 210 °C and 280 °C, respectively, while the internal standards dodecane (IS for MT-MFT) and hexadecane (IS for MFT-MFT) have boiling points of 215–217 °C and 287 °C, respectively. The disulfides and alkanes were diluted in pentane, at concentrations of 10 μg/mL for MFT-MT, dodecane and MFT-MFT and 1 μg/mL for hexadecane. Solutions were analyzed by GC–MS as described previously using a SAC-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies, Inc.) and 3 different injection modes using a CIS4 PTV inlet (Gerstel) as earlier described, and by hot-splitless and cool on-column using the same inlet. For hot splitless injection the inlet was set to either 200 °C, 250 °C or 300 °C with split valve delay set to 0.5 min. Inlet temperature for on-column injection was 40 °C. The solution was analyzed in triplicate for each injection condition and the peak areas of each disulfide were compared with the peak areas of its corresponding alkane internal standard. Percent degradation was calculated using the following equation:

$$Degradation\ (\%) = 1 - \frac{Peak\ area\ ratio\left(\frac{Disulfide}{Internal\ standard}\right)}{Peak\ area\ ratio\left(\frac{Disulfide}{Interal\ standard}\right) by\ on\ column\ injection} \times 100\%$$

4. Results and discussion

4.1. Sensory comparison of neat liquors and SAFE-DIST isolates

Sensory difference testing was conducted to assess whether perceived aroma differences existed between the original liquor products (neat liquors) and their respective SAFE-DIST isolates. Out of 24 judges, 7–11 of them, depending on the specific liquors, were able to correctly identify the odd samples, which is still well below the minimal level of correct response of 13 needed for there to be a significant difference ($p \leq 0.05$) (Table 1) (Meilgaard & Civille, 2007). Thus, the results of sensory difference testing demonstrated that the overall perceived aroma profiles of the original liquor products and their respective SAFE-DIST isolates did not differ ($p \leq 0.05$). Panelists had less correct responses for the 3 clear liquors which were aged in pottery compared to the 3 brown liquors which were aged in oak barrels (Table 1). Even though there was no chance for visual bias (sample appearance was masked), there appeared to have been a context effect in the case of the brown liquors. That is, panelists found it easier to detect differences when 2 neat (brown) liquors were presented with 1 SAFE-DIST isolate. However, effect was not observed when 2 SAFE-DIST isolates were presented with 1 neat liquor. To account for such context effects, it is appropriate that both serving orders be considered in the final statistical analysis (Meilgaard & Civille, 2007).

4.2. Volatile profile comparison of neat liquors and SAFE-DIST isolates

An additional volatile profile comparison was made between neat liquors and their respective SAFE-DIST isolates. Eight compounds (2-methyl-1-propanol, 3-methy-1-butanol, ethyl hexanoate, tetramethylpyrazine, ethyl 2-phenylacetate, hexanoic acid, 2-phenylethanol and octanoic acid) possessing different functional groups (including 2 fusel alcohols, 2 fatty acids, 2 esters, 1 pyrazine and 1 phenolic compound) and different molecular weights and volatilities, were selected for analysis (Table 2). Among these 8 compounds, 2-methyl-1-propanol, 3-methy-1-butanol and ethyl hexanoate differed by no more than 2% between the original liquors and their respective SAFE-DIST isolates. For all 6 liquors, the difference between the peak areas of ethyl 2-phenylacetate and 2-phenylethanol were below 5% between the original liquor products and their SAFE-DIST isolates. However, for the 2 fatty acids analyzed, the differences between the original liquor products and their SAFE isolates were more significant for the brown

Table 2
GC-FID peak area[a] comparison for selected volatile components between various liquor products (neat) and their respective distillates prepared by direct solvent-assisted flavor evaporation (SAFE-DIST).

	2-Methy-1-propanol	3-Methy-1-butanol	Ethyl hexanoate	Tetramethyl-pyrazine	Ethyl-2-phenylacetate	Hexanoic acid	2-Phenyl ethanol	Octanoic acid
MT Neat	156	400	22.6	12.1	162	11.0	19.4	1.22
MT SAFE	156	402	22.5	8.12	154	10.8	18.7	1.22
% Diff.	0.00130	0.613	0.224	33.0[c]	4.89[b]	1.66	3.48[b]	0.0872
GJGJ Neat	101	288	314	–	–	777	2.98	1.22
GJGJ SAFE	101	283	293	–	–	759	2.86	1.22
% Diff.	0.303	2.00	1.64	–	–	2.31	4.12	0.0872
YPJZ Neat	172	794	–	–	44.6	136	40.2	1.87
YPJZ SAFE	171	794	–	–	43.5	134	39.0	1.79
% Diff.	0.919	0.0604	–	–	2.34[c]	1.47	3.04	0.0425
EWW Neat	224	1123	–	–	–	8.56	26.2	2.01
EWW SAFE	224	1121	–	–	–	7.31	25.0	0.964
% Diff.	0.0639	0.127	–	–	–	14.6[c]	4.36	52.0[c]
DJT Neat	403	736	–	–	–	2.49	6.88	2.71
DJT SAFE	395	729	–	–	–	2.44	6.78	2.70
% Diff.	1.99[b]	0.923	–	–	–	2.07	1.39	0.614
AER Neat	113	314	–	–	–	–	2.71	1.82
AER SAFE	113	314	–	–	–	–	2.70	1.04
% Diff.	0.168	0.0996	–	–	–	–	0.614	42.8[c]

[a] Average peak areas determined by GC-FID (n = 3).
[b] Peak areas differed between SAFE-DIST isolates and original (neat) liquor ($p < 0.05$).
[c] Peak areas differed between SAFE-DIST isolates and original (neat) liquor ($p < 0.01$).

liquors than for the clear liquors.

4.3. AEDA comparison of neat liquors and SAFE-DIST isolates

One clear liquor (MT) and one brown liquor (EWW) were selected for further study to evaluate potential differences with respect to the predominant odorants in neat liquors and their respective SAFE-DIST isolates. A combined total of 61 odorants were detected through application of GC–MS-O analysis and AEDA of the MT and EWW original liquors and their respective SAFE-DIST isolates, with 52 odorants detected in MT and 37 detected in EWW (Table 3).

As indicated in Table 3, 55 compounds were either positively or tentatively identified, while 5 were unidentified (unknown). The identified compounds included 18 esters (nos. 4, 5, 7–14, 16, 20, 22, 23, 37, 38, 42 and 49); 7 acids (nos. 29, 30, 32a, 32b, 35, 57 and 59); 7 sulfur-containing compounds (nos. 18, 21, 24, 26, 33, 36 and 50); 4 ketones (nos. 6, 19, 39 and 44); 4 aldehydes (nos. 1, 3a/3b, 27); 7 phenols/guaiacols (nos. 41, 45, 47, 51, 52, 55 and 58); 4 alcohols (nos. 15a/15b, 40 and 43); 1 pyrazine (no. 25); 1 lactone (no. 46); 1 acetal (no. 2), 1 furanone (no. 53) and 1 ether (no. 17).

As expected, MT and EWW differ with respect to their aroma profiles. Whiskey derives much of its aroma components a result of aging in oak barrels. Many of these oak-derived volatiles contribute to the unique aroma profile of whiskey; e.g., guaiacol, 4-ethylguaiacol, eugenol, syringol and vanillin (Conner, Paterson, & Piggott, 1993). On the other hand, MT is aged in pottery, an essentially inert container. There are of course other differences between MT and EWW caused by use of different raw materials, fermentation, distillation, etc. in their production.

All of the odorants identified in EWW have been previously reported in the literature (Lahne, 2010; Poisson & Schieberle, 2008). Most of the odorants in MT were previously reported (Chen, Wang, & Xu, 2013; Fan, Xu, & Qian, 2012; Zhu et al., 2007). However, several additional odorants are reported for the first time in this study, including 2-methyl-3-furanthiol (no. 18), ethyl cyclohexylcarboxylate (no. 22), (*E*)-2-nonenal (no. 27), 2-methyl-3-(methyldithio)furan (no. 33); dimethyltetrasulfide (no. 36) and *bis*(2-methyl-3-furyl) disulfide (no. 50).

The results AEDA are depicted as $\log_3$flavor dilution (FD) factors to allow for a clearer comparison between each of the neat liquors and their respective SAFE-DIST isolates. For MT, AEDA results were identical for the neat liquor and the SAFE-DIST isolate, with the exception of only 5 odorants (nos. 27, 37, 47, 55 and 59) which differed by no more than 1 $\log_3$FD factor between the two extracts. The AEDA results for EWW showed a similar trend, with only 1 odorant (vanillin, no. 59) being detected at a higher log3 FD factor in the neat liquor. In all cases where differences were observed, the odorants were detected at slightly lower $\log_3$FD factors in the SAFE-DIST isolates, possibly due to poor recovery of these compounds by SAFE.

4.4. Stability of selected sulfur compounds in MT

Seven sulfur compounds (nos. 18, 21, 24, 26, 33, 36 and 50) were detected in MT. Noteworthy among these were 2-methyl-3-furanthiol (MFT, no. 18), 2-methyl-3-(methyldithio) furan (MFT-MT no. 33) and *bis*(2-methyl-3-furyl) disulfide (MFT-MFT, no. 50), which have not been previously reported in MT and which possess intense savory (meaty and vitamin-like) aromas. It is known that MFT-MFT is formed by dimerization of MFT and, similarly, MFT-MT can be formed by reaction of MFT with methanethiol (Mottram & Whitfield, 1995; Weerawatanakorn, Wu, & Pan, 2015). It has also been reported that MFT can be oxidized during storage to its dimer MFT-MFT (Hofmann et al., 1996). MFT-MFT when exposed to an elevated temperature can be converted back to MFT, while MFT-MT is thermally stable (Belitz, Grosch, & Schieberle, 2009).

Based on the above observations, there was some concern over whether MFT (no. 18) was an artifact formed by the analysis conditions used in this study. Cold (cryo) spliltess injection using a PTV inlet was employed for GC–MS-O. During injection the temperature of the inlet was ramped from −50 °C to 250 °C. This means that for a brief period of time the volatile components were exposed to elevated temperatures as they were transferred from the inlet into the GC column. For this reason, cool on-column GC-O analysis was conducted to confirm the results obtained by cold-splitless GC–MS-O analysis (Table 4). By comparing the results from cool on-column GC-O analysis against the results of cold-splitless GC–MS-O analysis, it was found that 2-methyl-3-furanthiol (MFT), which has a FD factor of 243 by cold-splitless injection, was not detected at all by cool on-column GC-O analysis. This is an indication that MFT might be a thermally generated artifact. Therefore, an additional study was conducted to examine the relative stabilities of

Table 3
Comparative AEDA results for potent odorants ($log_3FD \geq 2$) in Moutai (MT) and Evan Williams Bourbon Whiskey (EWW) neat liquors and their respective distillates prepared by direct solvent-assisted flavor evaporation (SAFE-DIST).

No.	Compound	Odor description	Retention Index		Log_3FD factor MT[a]				Log_3FD factor EWW[b]	
			Stabilwax	Rxi-5ms	Stabilwax		Rxi-5ms		Neat	SAFE-DIST
					Neat	SAFE-DIST	Neat	SAFE-DIST		
1	2-methylpropanal	malty	< 800	< 700	4	4	4	4	–	–
2	acetal	fruity, painty	938	741	7	7	5	5	4	4
3a,b	2-/3-methylbutanal	malty	947	< 700	6	6	6	6	1	1
4	ethyl propanoate	fruity	972	720	5	5	4	4	4	4
5	ethyl 2-methylpropanoate	fruity, berry	979	768	9	9	8	8	–	–
6	2,3-butanedione	buttery, creamy	993	< 700	2	2	2	2	–	–
7	2-methylpropyl acetate	solvent	1015	792	2	2	3	3	–	–
8	ethyl butanoate	fruity	1042	806	8	8	6	6	4	4
9	ethyl 2-methylbutanoate	fruity, berry	1057	857	10	10	8	8	4	4
10	ethyl 3-methylbutanoate	blueberry	1075	862	8	8	6	6	3	3
11	ethyl pentanoate	fruity, berry	1142	902	6	6	5	5	1	1
12	ethyl 2-methylpentanoate*	fruity, berry	1142	944	6	6	4	4	–	–
13	propyl 2-methylbutanoate	fruity	1183	–	4	4	–	–	–	–
14	ethyl 4-methylpentanoate	fruity, berry	1195	971	6	6	4	4	0 [c]	0
15a,b	2-/3-methyl-1-butanol	malty	1213	749	4	4	2	2	5	5
16	ethyl hexanoate	fruity	1240	999	5	5	4	4	4	4
17	furfuryl ether *	ether like	1296	831	3	3	2	2	–	–
18	2-methyl-3-furanthiol	beefy, vitamin	–	872	–	–	5	5	–	–
19	1-octen-3-one*	mushroom	1312	1029	1	1	1	1	3	3
20	ethyl heptanoate	fruity	1341	1099	1	1	0	0	3	3
21	dimethyl trisulfide	cabbage	1385	969	5	5	3	3	5	5
22	ethyl cyclohexylcarboxylate *	fruity, berry	1430	1135	4	4	3	3	0	0
23	ethyl octanoate	waxy, plastic	1440	1197	3	3	2	2	3	3
24	2-furfurylthiol*	meaty, coffee	1447	912	1	1	4	4	–	–
25	2-ethyl-3,5-dimethylpyrazine	roasted	1463	1063	5	5	3	3	–	–
26	methional*	potato	1475	907	–	–	–	–	4	4
27	(*E*)-2-nonenal*	metallic, fatty	1518	1150	3	2	–	–	3	3
28	unknown	berry	1544	962	4	4	1	1	–	–
29	2-methylpropanoic acid	cheesy	1582	843	1	1	2	2	–	–
30	butanoic acid	cheesy	1637	872	3	3	0	0	–	–
31	phenylacetaldehyde	rosy	1663	1032	3	3	2	2	–	–
32a,b	2-/3-methyl butanoic acid	cheesy, sweaty	1676	–	4	3	–	–	1	1
33	2-methyl-3-(methyldithio) furan	vitamin	1681	1174	5	5	3	3	–	–
34	3-methyl-2,4-nonadione*	hay, fatty	1695	–	2	2	3	3	–	–
35	pentanoic acid	cheesy	1747	–	2	2	–	–	–	–
36	dimethyltetrasulfide	cabbage	1760	1214	4	3	2	2	–	–
37	ethyl phenylacetate	rosy	1801	1247	3	3	4	4	–	–
38	2-phenylethyl acetate	fruity, rosy	1829	–	1	1	–	–	4	4
39	β-damascenone	applesauce	1837	1384	5	5	5	5	4	4
40	geraniol	citrus	1859	–	3	2	–	–	2	2
41	guaiacol	smoky	1884	1091	3	2	0	0	5	5
42	ethyl 3-phenylpropanoate	fruity, grape	1898	1349	5	5	4	4	2	2
43	2-phenylethanol	rosy	1933	1130	5	5	1	1	5	5
44	β-ionone	floral	1954	–	1	1	–	–	2	2
45	4-ethylguaiacol	smoky, clove	2055	1281	1	1	–	–	3	3
46	γ-nonalactone	peachy	2062	1380	3	2	0	0	1	1
47	*p*-cresol	animal barn	2107	1106	3	3	0	0	–	–
48	unknown	hay, sweet	2131	–	–	–	–	–	2	2
49	ethyl cinnamate	rosy, fruity	2159	–	1	1	–	–	2	2
50	*bis*(2-methyl-3-furyl) disulfide	meaty	2178	1526	2	2	–	–	–	–
51	eugenol	spicy, clove	2188	–	–	–	–	–	6	6
52	4-ethylphenol	manure, stable	2205	–	3	3	–	–	0	0
53	3-hydroxy-4,5-dimethyl-furan-2(*5H*)-one (sotolone)	curry, spicy	2233	–	5	5	–	–	7	7
54	unknown	plastic	2266	–	3	2	–	–	1	1
55	syringol	bacon, smoky	2305	–	1	1	–	–	4	4
56	unknown	herbal	2386	–	1	1			2	2
57	phenylacetic acid	rosy	2603	1287	3	3	0	0	–	–
58	vanillin	vanilla	2624	–	1	2	–	–	7	5
59	3-phenylpropanoic acid	sweaty, rosy	2657	1349	2	2	–	–	–	–
60	unknown	cabbage	–	1057	–	–	3	3	–	–

* Compound was tentatively identified by matching its RI and odor properties with authentic standard compound.
[a] AEDA was conducted on Stabilwax and Rxi-5ms GC columns.
[b] AEDA was conducted on Stabilwax GC column.
[c] Indicates that compound was detected in the concentrated aroma extract only.

Table 4
Thermal degradation of selected odor-important disulfides as a function of GC injection method.

No.			Cool on-column	Cold-splitless	Hot splitless (inlet temperature)		
					200 °C	250 °C	300 °C
50	MFT-MFT[a]	Peak area ratio (mean ± STD)	6.658 ± 0.022	5.544 ± 0.027	5.294 ± 0.033	4.547 ± 0.038	3.848 ± 0.038
		Degradation (%)	0.0	16.7	20.5	31.7	42.2
33	MFT-MT[b]	Peak area ratio (mean ± STD)	0.5737 ± 0.0022	0.5745 ± 0.0012	0.5748 ± 0.0025	0.5733 ± 0.0015	0.5730 ± 0.0037
		Degradation (%)	0.0	0.0	0.0	0.0	0.0

[a] MFTMFT: *bis*(2-methyl-3-furyl) disulphide.
[b] MFT-MT: 2-methyl-3-(methyldithio) furan.

MFT-MFT and MFT-MT during typical GC analysis conditions.

The stabilities of MFT-MT and MFT-MFT and their potential to form MFT were tested under various injection scenarios, including hot splitless (200, 250 and 300 °C), cold splitless and cool on-column injection, using the same PTV inlet (modified for each injection method) and under the same GC–MS conditions. The results showed that MFT-MT was stable under all injection conditions tested, but MFT-MFT stability was highly dependent upon the injection conditions used (Table 4). MFT-MFT was most stable when cool on-column injection was used, but appreciable degradation (> 16%) occurred under either cold or hot splitless injection. Furthermore, as expected, hot splitless injection resulted in the greatest degradation of MFT-MFT, with around 42% degradation occurring when the inlet temperature was 300 °C. Even though on-column is an ideal method to avoid the degradation of MFT-MFT with subsequent formation of MFT, it is not a viable option for comparison between the neat liquor and its respective SAFE isolate because this method has little tolerance for "dirty" aroma extracts.

4.5. Quantitative comparison of selected aroma

Twenty-four odorants were selected for quantitation based on SIDA to provide a more accurate comparison between two neat liquors (MT and EWW) and their respective SAFE isolates (Table 5). Compounds chosen for analysis included 3 Strecker aldehydes (nos. 1, 3a and 3b), 11 esters (nos. 8, 10, 11, 16, 20, 23, 37, 42, 61, 62 and 63), 7 acids (nos. 29, 30, 35, 57, 64, 65 and 66), 1 alcohol (no. 43) and 3 semi-volatile components (nos. 53, 58 and 67). The quantitation results showed that highly volatile components like Strecker aldehydes, short chain fatty acids and low molecular weight ethyl esters had high SAFE-DIST recoveries from 98.1 to 100% for both MT and EWW. However, the recovery ethyl decanoate, ethyl dodecanoate and ethyl hexadecanoate were only 61.0% and 59.2% (for EWW) and 20.78% (for MT), respectively. These higher molecular weight esters are not very odor-active and thus do not contribute significantly to the overall aroma profile to the liquor products, which explains why even though their recoveries by SAFE were poor, the perceived aromas of the resulting SAFE-DIST isolates were still not significantly different from their respective neat

Table 5
Concentration comparison of selected potent odorants in original neat liquors of Moutai (MT) and Evan Williams Bourbon Whiskey (EWW) and their respective distillates prepared by direct solvent-assisted flavor evaporation (SAFE-DIST).

No.	Compound	Neat MT[a]	MT SAFE-DIST[a]	% recovery	Neat EWW[a]	EWW SAFE-DIST[a]	% recovery
1	2-methylpropanal	26.12 ± 0.66	25.74 ± 0.40	98.6	0.920 ± 0.022	0.911 ± 0.013	99.0
3a	2-methylbutanal	17.70 ± 0.17	17.67 ± 0.29	99.8	0.308 ± 0.017	0.3051 ± 0.0041	100
3b	3-methybutanal	37.11 ± 0.49	37.10 ± 0.46	100	0.3869 ± 0.0061	0.3799 ± 0.0078	98.2
8	ethyl butanoate	57.70 ± 0.41	57.36 ± 0.36	99.4	–	–	–
11	ethyl pentanoate	4.561 ± 0.034	4.539 ± 0.037	99.6	–	–	–
10	ethyl 3-methylbutanoate	11.32 ± 0.057	11.32 ± 0.038	99.9	–	–	–
16	ethyl hexanoate	17.11 ± 0.026	17.11 ± 0.083	100	2.691 ± 0.020	2.684 ± 0.015	99.6
20	ethyl heptanoate	1.250 ± 0.011	1.235 ± 0.013	98.4	–	–	–
23	ethyl octanoate	2.010 ± 0.015	2.005 ± 0.0054	99.5	10.36 ± 0.064	10.32 ± 0.085	99.6
61	ethyl decanoate	–	–	–	12.09 ± 0.15	7.38 ± 0.12	61.0
62	ethyl dodecanoate	–	–	–	5.61 ± 0.19	3.316 ± 0.046	59.2
63	ethyl hexadecanoate	19.25 ± 0.078	3.999 ± 0.041	20.8	–	–	–
37	ethyl phenylacetate	5.420 ± 0.011	5.357 ± 0.031	98.9	–	–	–
42	ethyl 3-phenylpropionate	38.64 ± 0.76	38.55 ± 0.27	99.8	–	–	–
64	acetic acid	7258 ± 0.033	7254 ± 0.031	99.9	2.499 ± 0.064	2.475 ± 0.041	99.2
65	propanoic acid	1229 ± 3.6	1229 ± 2.6	100	–	–	–
30	butyric acid	35.18 ± 0.25	35.06 ± 0.26	99.7	–	–	–
29	2-methylpropanoic acid	20.89 ± 0.19	20.50 ± 0.04	98.1	–	–	–
35	pentanoic acid	4.383 ± 0.037	4.384 ± 0.0096	100	–	–	–
66	hexanoic acid	12.19 ± 0.037	12.10 ± 0.088	99.3	–	–	–
57	phenylacetic acid	20.44 ± 0.027	14.20 ± 0.016	69.5	–	–	–
43	2-phenylethanol	20.77 ± 0.15	19.11 ± 0.10	92.0	28.97 ± 0.12	27.05 ± 0.19	93.4
58	vanillin	–	–	–	2.802 ± 0.015	0.4819 ± 0.0018	17.1
67	syringaldehyde	–	–	–	9.170 ± 0.045	0.4775 ± 0.0025	5.23
53	sotolon	0.0962 ± 0.0010[b] 0.097 ± 0.015[c]	0.08478 ± 0.00050	88.2	–	–	–

[a] Average concentration (mg/L) ± standard deviation (n = 3).
[b] Concentration determined by addition of isotope solution to the neat liquor followed by direct solvent extraction and fractionation without SAFE.
[c] Concentration determined by addition of isotope solution to the neat liquor followed by direct solvent extraction, SAFE distillation and fractionation.

liquors. However, these components may contribute to the mouthfeel of a liquor product and might induce a taste difference if eliminated. The recoveries of other semi-volatile components like vanillin and syringaldehyde (for EEW) were only 17.14% and 5.24%, respectively, which agrees with the AEDA results for vanillin, since the $\log_3$FD factor differed by 2 dilutions, roughly one ninth in concentration.

The quantitation results of sotolon by three different methods were in agreement in the case of MT, since the results of SIDA without SAFE and with SAFE (isotope added before SAFE distillation) were nearly identical. Therefore, sotolon is not an artifact but an odor-active component in this liquor. Results also show the recovery of sotolon by SAFE distillation was above 88%, which could explain why the $\log_3$FD factors of sotolon in the SAFE distillate and original liquor did not differ.

5. Conclusions

The proposed streamlined approach for quantitation of odor-active components in distilled liquors was evaluated by various methods, including sensory testing, semi-quantitative analysis (GC–MS-O AEDA) and advanced quantitation (SIDA). Among the selected 6 liquors examined, the aroma profiles did not differ between original liquors and those of SAFE-DIST. Assessed by AEDA, within selected clear and brown liquor samples, most potent odor-active components had the same FD factor in the extract of liquor products and their SAFE isolates. According to the SIDA results of odor-active components before and after SAFE, the difference in concentrations of most odor-active components between original liquors and their SAFE isolates were negligible, including Strecker aldehydes, short chain fatty acids and ethyl esters from butanoic acid to octanoic acid. Semi-volatile components, e.g. long chain ethyl esters, vanillin, and syringaldehyde, were not well isolated by the stream-lined approach due to poor recoveries by SAFE distillation. Thus, for the quantitation of semi-volatile compounds the isotopes have to be added before SAFE distillation to guarantee accurate quantitation results. Compared with the standard way to accomplish the quantitation of compounds using combined SAFE and SIDA, the "addition-extraction-adjustment" procedure to achieve reasonable isotope-to-target analyte ratios is significantly shortened by the proposed streamlined approach by allowing the researcher to quantitate more odorants without additional SAFE operations as long as the SAFE isolate is properly prepared and preserved. Furthermore, the direct SAFE-DIST isolate can be used for both identification and quantitation purposes which would not be feasible by following the traditional procedures. Thus this proposed approach not only allows quantitation of odor-active components with significant less time, effort, materials (especially important in the case of rare source materials) and isotopes. This approach could potentially be applied for the study of the flavor chemistry of wine, beer, fruit pulps and other aqueous food products.

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Declaration of Competing Interest

The authors declare no competing financial interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2019.100038.

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