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

Wenqi Zhu, Keith R. Cadwallader

Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, 1302 West Pennsylvania Avenue, Urbana, IL 61801, USA

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.
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 isolates 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 scarce or expensive products. Aged distilled liquor is a good 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, Jingzi Liquor Co., Ltd., Shandong, China.) and a mid-range strong aroma liquor Gu Jing Gong Jiu (GJJG, Gujinggong Liquor Co., Ltd. Bozhou, China.). These liquors represent high and mid-range 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 purchased 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: \( [\text{2H}_4] \)-propionic acid (Cambridge Isotope Laboratories, Inc., Andover, MA, USA); \( [1,2,3\text{C}_2] \)-phenylacetic acid and \( [1,2,3\text{C}_2] \)-butanoic acid (Isotec, Miamisburg, OH, USA); \( [\text{3H}_2] \)-pentanoic acid; \( [2,2,3\text{H}_2] \)-3-methylbutanal (CDN Pointe-Claire, Quebec, Canada). \( [\text{3H}_2] \)-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-nonadienone (Guth & Grosch, 1989); \( [1,2,3\text{C}_2] \)-2-phenylethanol (Schuh & Schieberle, 2006); bis(2-methyl-3-furyl) disulfide (Hofmann, Schieberle, & Grosch, 1996); \( [\text{13C}_2] \)-sotolon (Blank, Lin, Fumeaux, Welti, & Fay, 1996); 4-hydroxy-3-[\( \text{2H}_2 \)]-methoxybezaldehyde (Schneider & Roland, 1992); \( [\text{2H}_4] \)-β-damascenone (Kotseridis, Baumes, & Skouroumounis, 1998); 2-methyl-[2,3,3\text{H}_2 \]-propan-1-ol, [2,3-\( \text{2H}_2 \)]-methylpropanal and [3,4-\( \text{3H}_2 \)-2-methylbutanal (Wu & Cadwallader, 2019).

2.2.1. Synthesis of \( [3,3,4,4,\text{3H}_2] \)-hexanoic acid

3-Hexyn-1-ol was deuterated to \( [3,3,4,4,\text{3H}_2] \)-hexan-1-ol using a previously described method (Hausch, Lorjaeroenphon, & 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 \( [3,4-\text{3H}_2 \)-methylbutyric acid. MS-EI, m/z (%) of \( [3,3,4,4,\text{3H}_2] \)-hexanoic acid (purity 98.3%): MS-EI, m/z
2.2.3. Synthesis of 4-hydroxy-3-[\text{H}_3]-5-dimethoxy benzaldehyde (3\text{H}_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, 10 mmol of organic acid; 200 µL of [\text{H}_2]-dimethylsulfate and allowing the reaction to stir ∼ pH 1 and then it was extracted with ethyl acetate (1 × 10 mL, 4 × 5 mL). The ethyl acetate layer was removed the pentane layer was washed again with aqueous saturated NaCl (2 × 5 mL) and then dried over anhydrous Na$_2$SO$_4$. The solvent was removed by a rotovap. The crude product was stored in a glass bottle equipped with a PTFE-lined cap. GC-MS and their counterpart SAFE-DIST isolates.

3. Methods

3.1. Direct distillation of liquor (SAFE-DIST)

Liquor sample (100 mL) was fed directly into the SAFE apparatus as previously described (Rotschatchkul, Chaiseri, & Cadwallader, 2007). The system was maintained under high vacuum (2–5 × 10$^{-5}$ 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 PFTE 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 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 300 °C.

Standards and Technology (NIST) Mass Spectral Library. Selected ions used for determination of peak areas of labeled and un-labeled compounds were compared against those determined for a homologous series of n-alkanes (from C7 to C28) 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 log3 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 isopropylated 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 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 compounds are listed in Table 15.

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.
Concentration analyte Rf Peak area ratio mass labeled analyte
its mean peak area ratio of selected ions of labeled and unlabeled target
analyzed in triplicate and the mass ratio of each point was plotted against
mass ratios from 1:5 to 5:1 (unlabeled:labeled). Each point was ana-
was determined by a

3.10. Calibration

The response factor (Rf) 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 ana-
alyzed 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 Rf of each labeled analyte was calculated by using the following
equation:

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

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

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

3.11. Thermal stability of selected disulfides

Since the aroma extracts were analyzed by split/splitless inlet in-
jection, 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-(methylthio) 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 com-
pared 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-MFT, 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:

\[
\text{Degradation (\%)} = 1 - \frac{\text{Peak area ratio (Disulfide internal standard)} \times 100}{\text{Peak area ratio (Internal standard)} \times \text{by on column injection}}
\]

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 ≤ 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 ≤ 0.05). Panelists had less correct re-
ponses 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 di-
ference between the peak areas of ethyl 2-

Table 1

| Liquor | Panel response (no. correct/no. total assessments) |
|--------|--------------------------------------------------|
| Clear liquor products | (2 SAFE – 1 (Neat)) Interal standard | (2 Neat – 1 SAFE) Internal standard | Total | Sign. | Diff. |
| MT<sup>a</sup> | 4/12 | 3/12 | 7/24 | N |
| GJJ<sup>a</sup> | 5/12 | 7/12 | 12/24 | N |
| YPJ<sup>a</sup> | 5/12 | 3/12 | 8/24 | N |
| Brown liquor products | (2 SAFE – 1 Neat) | (2 SAFE – 1 Neat) | | |
| DJT<sup>b</sup> | 4/12 | 6/12 | 10/24 | N |
| EWW<sup>b</sup> | 2/12 | 10/12 | 12/24 | N |
| AER<sup>b</sup> | 4/12 | 7/12 | 11/24 | N |

<sup>a</sup> 2 SAFE-1 Neat: Triangle test of 1 original liquor and 2 of their corre-
sponding SAFE distillate.
<sup>b</sup> 2 Neat-1 SAFE: Triangle test of 2 original liquor and 1 of their corre-
sponding SAFE distillate.
<sup>c</sup> Significant difference: N = not significantly different (p ≤ 0.05).

Table 2

| Liquor | Panel response (no. correct/no. total assessments) |
|--------|--------------------------------------------------|
| Clear liquor | (2 SAFE – 1 (Neat)) Interal standard | (2 Neat – 1 SAFE) Internal standard | Total | Sign. | Diff. |
| MT<sup>a</sup> | 4/12 | 3/12 | 7/24 | N |
| GJJ<sup>a</sup> | 5/12 | 7/12 | 12/24 | N |
| YPJ<sup>a</sup> | 5/12 | 3/12 | 8/24 | N |
| Brown liquor products | (2 SAFE – 1 Neat) | (2 SAFE – 1 Neat) | | |
| DJT<sup>b</sup> | 4/12 | 6/12 | 10/24 | N |
| EWW<sup>b</sup> | 2/12 | 10/12 | 12/24 | N |
| AER<sup>b</sup> | 4/12 | 7/12 | 11/24 | N |

<sup>a</sup> 2 SAFE-1 Neat: Triangle test of 1 original liquor and 2 of their corre-
sponding SAFE distillate.
<sup>b</sup> 2 Neat-1 SAFE: Triangle test of 2 original liquor and 1 of their corre-
sponding SAFE distillate.
<sup>c</sup> Significant difference: N = not significantly different (p ≤ 0.05).

Table 1

| 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) |
| Clear liquor | (2 SAFE – 1 (Neat)) | (2 Neat – 1 SAFE) | Total | Sign. | Diff. |
| MT<sup>a</sup> | 4/12 | 3/12 | 7/24 | N |
| GJJ<sup>a</sup> | 5/12 | 7/12 | 12/24 | N |
| YPJ<sup>a</sup> | 5/12 | 3/12 | 8/24 | N |
| Brown liquor | (2 SAFE – 1 Neat) | (2 SAFE – 1 Neat) | | |
| DJT<sup>b</sup> | 4/12 | 6/12 | 10/24 | N |
| EWW<sup>b</sup> | 2/12 | 10/12 | 12/24 | N |
| AER<sup>b</sup> | 4/12 | 7/12 | 11/24 | N |

<sup>a</sup> 2 SAFE-1 Neat: Triangle test of 1 original liquor and 2 of their corre-
sponding SAFE distillate.
<sup>b</sup> 2 Neat-1 SAFE: Triangle test of 2 original liquor and 1 of their corre-
sponding SAFE distillate.
<sup>c</sup> Significant difference: N = not significantly different (p ≤ 0.05).

Table 2

| Table 2 | 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) |
| Clear liquor | (2 SAFE – 1 (Neat)) | (2 Neat – 1 SAFE) | Total | Sign. | Diff. |
| MT<sup>a</sup> | 4/12 | 3/12 | 7/24 | N |
| GJJ<sup>a</sup> | 5/12 | 7/12 | 12/24 | N |
| YPJ<sup>a</sup> | 5/12 | 3/12 | 8/24 | N |
| Brown liquor | (2 SAFE – 1 Neat) | (2 SAFE – 1 Neat) | | |
| DJT<sup>b</sup> | 4/12 | 6/12 | 10/24 | N |
| EWW<sup>b</sup> | 2/12 | 10/12 | 12/24 | N |
| AER<sup>b</sup> | 4/12 | 7/12 | 11/24 | N |
identical for the neat liquor and the SAFE-DIST isolate, with the exception of only 5 odorants (nos. 27, 37, 47, 59 and 59) which differed by no more than 1 log3FD 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 log3FD 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-(methylthio) 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) splitless 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 compounds 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
| No. | Compound | Odor description | Retention Index | Log$_3$FD factor MT | Log$_3$FD factor EWW |
|-----|----------|------------------|-----------------|---------------------|---------------------|
|     |          |                  | Stabilwax Rxi-5ms | Neat SAFE-DIST      | Neat SAFE-DIST      |
| 1   | 2-methylpropanal | malty            | < 800           | 4 4 4               | – –                |
| 2   | acetal    | fruity, painty   | 938             | 7 7 5 5             | 4 4 4               |
| 3a,b| 2-/3-methylbutanal | malty            | 947             | 6 6 6 6             | 1 1                 |
| 4   | ethyl propanoate | fruity          | 972             | 5 5 4 4             | 4 4                 |
| 5   | ethyl 2-methylpropanoate | fruity, berry | 979             | 9 9 8 8             | – –                 |
| 6   | 2,3-butanedione | buttery, creamy | 993             | < 700 2 2 2 2 2 2 2 | – –                 |
| 7   | 2-methylpropyl acetate | solvent        | 1015            | 2 2 3 3             | – –                 |
| 8   | ethyl butanoate | fruity          | 1042            | 8 8 6 6             | 4 4                 |
| 9   | ethyl 2-methylbutanoate | fruity, berry | 1057            | 10 10 8 8 4 4 4     | – –                 |
| 10  | ethyl 3-methylbutanoate | blueberry      | 1079            | 8 8 6 6             | 3 3                 |
| 11  | ethyl pentanoate | fruity, berry   | 1142            | 6 6 5 5             | 1 1                 |
| 12  | ethyl 2-methylpentanoate | fruity, berry | 1142            | 4 4 4 4             | – –                 |
| 13  | propyl 2-methylbutanoate | fruity        | 1183            | – 4 4 4             | – –                 |
| 14  | ethyl 4-methylpentanoate | fruity, berry | 1195            | 6 6 4 4 0 0         | – –                 |
| 15a,b| 2-/3-methyl-1-butanol | malty          | 1213            | 4 4 2 2             | – –                 |
| 16  | ethyl hexanoate | fruity          | 1240            | 2 2 5 5             | 4 4                 |
| 17  | 2-/3-methyl-1-butanol | fruity      | –                | 3 3 4 4             | – –                 |
| 18  | ethyl 4-methylbutanoate | fruity, berry | 1296            | 3 3 2 2             | – –                 |
| 19  | ethyl 4-methylpentanoate | fruity, berry | 1312            | 1 1 1 1 3 3         | – –                 |
| 20  | ethyl 3-methylpentanoate | fruity, berry | 1344            | 1 1 0 0 3 3         | – –                 |
| 21  | dimethyl trisulfide | cabbage     | 1385            | 5 5 5 5             | 5 5                 |
| 22  | 2-/3-methyl-1-butanol | fruity     | 1392            | 4 4 2 2             | – –                 |
| 23  | dimethyl trisulfide | cabbage     | 1463            | 5 5 3 3             | – –                 |
| 24  | 2-methylpentanoate | fruity          | 1486            | 3 3 4 4             | – –                 |
| 25  | ethyl 3-methylpentanoate | fruity, berry | 1518            | 1 1 1 1 3 3         | – –                 |
| 26  | (E)-2-nonenal | metallic, fatty | 1551            | 3 3 4 4             | – –                 |
| 27  | 2-/3-methyl-1-butanol | fruity     | 1592            | 1 1 2 2             | – –                 |
| 28  | 2-methylpentanoate | fruity          | 1632            | 5 5 3 3             | – –                 |
| 29  | ethyl heptanoate | fruity          | 1663            | 3 3 4 4             | – –                 |
| 30  | butanoic acid | cheesy          | 1683            | 3 3 4 4             | – –                 |
| 31  | methional | cabbage           | 1713            | 5 5 3 3             | – –                 |
| 32a,b| 2-/3-methyl butanoic acid | cheesy, sweaty | 1766            | 3 3 4 4             | – –                 |
| 33  | 2-/3-methyl(1-methylthio) furan | vitamin    | 1811            | 5 5 3 3             | – –                 |
| 34  | 3-/3-methyl-3,4-nonenal | sweaty, rosy | 1849            | 2 2 3 3             | – –                 |
| 35  | 2-methylpentanoate | cheesy          | 1879            | 3 3 4 4             | – –                 |
| 36  | methional | cabbage           | 1919            | 2 2 4 4             | – –                 |
| 37  | ethyl phenylacetate | cheesy       | 1959            | 2 2 3 3             | – –                 |
| 38  | 2-/3-methyl benzoic acid | cheesy, sweaty | 1999            | 3 3 4 4             | – –                 |
| 39  | 2-methyl(3-methylthio) furan | vitamin | 2039            | 5 5 3 3             | – –                 |
| 40  | 2-/3-methyl-1-butanol | fruity      | 2079            | 3 3 4 4             | – –                 |
| 41  | 2-methylpentanoate | cheesy          | 2115            | 3 3 4 4             | – –                 |
| 42  | 2-/3-methyl-1-butanol | fruity      | 2151            | 3 3 4 4             | – –                 |
| 43  | 2-methylpentanoate | cheesy          | 2181            | 3 3 4 4             | – –                 |
| 44  | 2-/3-methyl-1-butanol | fruity      | 2211            | 3 3 4 4             | – –                 |
| 45  | 2-/3-methyl-1-butanol | fruity      | 2241            | 3 3 4 4             | – –                 |
| 46  | 2-/3-methyl-1-butanol | fruity      | 2271            | 3 3 4 4             | – –                 |
| 47  | 2-/3-methyl-1-butanol | fruity      | 2301            | 3 3 4 4             | – –                 |
| 48  | 2-/3-methyl-1-butanol | fruity      | 2331            | 3 3 4 4             | – –                 |
| 49  | 2-/3-methyl-1-butanol | fruity      | 2361            | 3 3 4 4             | – –                 |
| 50  | 2-/3-methyl-1-butanol | fruity      | 2391            | 3 3 4 4             | – –                 |
| 51  | 2-/3-methyl-1-butanol | fruity      | 2421            | 3 3 4 4             | – –                 |
| 52  | 2-/3-methyl-1-butanol | fruity      | 2451            | 3 3 4 4             | – –                 |
| 53  | 2-/3-methyl-1-butanol | fruity      | 2481            | 3 3 4 4             | – –                 |
| 54  | 2-/3-methyl-1-butanol | fruity      | 2511            | 3 3 4 4             | – –                 |
| 55  | 2-/3-methyl-1-butanol | fruity      | 2541            | 3 3 4 4             | – –                 |
| 56  | 2-/3-methyl-1-butanol | fruity      | 2571            | 3 3 4 4             | – –                 |
| 57  | 2-/3-methyl-1-butanol | fruity      | 2601            | 3 3 4 4             | – –                 |
| 58  | 2-/3-methyl-1-butanol | fruity      | 2631            | 3 3 4 4             | – –                 |
| 59  | 2-/3-methyl-1-butanol | fruity      | 2661            | 3 3 4 4             | – –                 |
| 60  | 2-/3-methyl-1-butanol | fruity      | 2691            | 3 3 4 4             | – –                 |

* 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.
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 because this method has little tolerance for “dirty” aroma extracts.

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

| No. | Compound | Neat MT<sup>a</sup> | MFT-MFT<sup>b</sup> | MFT-MFT<sup>c</sup> |
|-----|----------|----------------------|----------------------|----------------------|
| 50  | MFT-MFT  | Peak area ratio      | Degradation (%)      | Degradation (%)      |
|     |          | (mean ± STD)         |                      |                      |
|     |          | 6.658 ± 0.022        | 0.0                  | 0.0                  |
|     |          | 5.544 ± 0.027        | 16.7                 | 0.0                  |
|     |          | 5.294 ± 0.033        | 20.5                 | 0.0                  |
|     |          | 4.547 ± 0.038        | 31.7                 | 0.0                  |
|     |          | 3.848 ± 0.038        | 42.2                 | 0.0                  |

<sup>a</sup> MFTMFT: bis(2-methyl-3-furyl) disulfide.  
<sup>b</sup> MFT-MT: 2-methyl-3-(methylthio)furan.

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 | MT SAFE-DIST<sup>a</sup> | MT SAFE-DIST<sup>b</sup> | MT SAFE-DIST<sup>c</sup> | MT SAFE-DIST<sup>d</sup> | MT SAFE-DIST<sup>e</sup> |
|-----|----------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| 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-methylbutan | 17.70 ± 0.17 | 17.67 ± 0.29 | 99.8 | 0.308 ± 0.017 | 0.3051 ± 0.0041 | 100 |
| 3b  | 3-methylbutan | 37.11 ± 0.49 | 37.10 ± 0.36 | 99.9 | 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-phenylpropanoate | 38.64 ± 0.76 | 38.55 ± 0.27 | 99.8 | – | – | – |
| 64  | acetic acid | 72.58 ± 0.033 | 72.54 ± 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 | – | – | – |
| 25  | pentanoic acid | 4.383 ± 0.037 | 4.384 ± 0.0096 | 100 | – | – | – |
| 57  | hexanoic acid | 12.19 ± 0.037 | 12.10 ± 0.088 | 99.3 | – | – | – |
| 43  | phenylacetic acid | 20.44 ± 0.027 | 14.20 ± 0.016 | 69.5 | – | – | – |
| 58  | 2-phenylethanol | 20.77 ± 0.15 | 19.11 ± 0.10 | 92.0 | 28.97 ± 0.012 | 27.05 ± 0.19 | 93.4 |
| 67  | vanillin | – | – | – | 2.802 ± 0.015 | 0.4619 ± 0.0018 | 17.1 |
| 53  | syringaldehyde | 0.0962 ± 0.0010<sup>f</sup> | 0.08478 ± 0.00050 | 88.2 | – | – | – |
|     | sotolon | 0.0971 ± 0.015<sup>f</sup> | – | – | – | – | – |

<sup>a</sup> Average concentration (mg/L) ± standard deviation (n = 3).  
<sup>b</sup> Concentration determined by addition of isotope solution to the neat liquor followed by direct solvent extraction and fractionation without SAFE.  
<sup>c</sup> 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(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 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 (isotope added before SAFE distillation) were nearly 88%, which could explain why the log(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. Assayed 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 isolates have to be added before SAFE distillation to guarantee accurate quantitation results. Compared with the traditional 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 isolates. This approach could potentially be applied for the study of the flavor chemistry of wine, beer, fruit pulps and other aqueous food products.

Acknowledgement

Partial support for this project was provided by the National Institute of Food Agriculture, U.S. Department of Agriculture (ILLU-698-366).

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.foodchx.2019.100038.

References

Belitz, H.-D., Grosch, W., & Schieberle, P. (2009). Food Chemistry (pp. 364–365). (4th ed.). Berlin: Springer-Verlag.

Blank, I., Lin, J., Fumeaux, R., Welti, D. H., & Fay, L. B. (1996). Formation of 3-hydroxy-4,5-dimethyl-2(3H)-furanone (sotolon) from 4-hydroxy-L-isoleucine and 3-amino-4,5-dimethyl-3,4-dihydro-2(3H)-furanone. Journal of Agricultural and Food Chemistry, 44(7), 1851–1856.

Chen, S., Wang, D., & Xu, Y. (2013). Characterization of odor-active compounds in sweet-type Chinese rice wine by aroma extract dilution analysis with special emphasis on sotolon. Journal of Agricultural and Food Chemistry, 61(40), 9712–9718.

Comer, J. M., Paterson, A., & Pigott, J. R. (1993). Changes in wood extracts from oak cask staves through maturation of Scotch malt whiskey. Journal of the Science of Food and Agriculture, 62(2), 169–174.

Engel, W., Bahr, W., & Schieberle, P. (1999). Solvent assisted flavour evaporation – a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. European Food Research and Technology, 209, 237–241.

Fan, W., Xu, Y., & Qian, M. C. (2012). Identification of aroma compounds in Chinese “moutai” and “langjiu” liquors by normal phase liquid chromatography/fractionation followed by gas chromatography/olfactometry. In W. Fan, Y. Xu, & M. C. Qian (Eds.), Flavor Chemistry of Wine and Other Alcoholic Beverages, ACS Symposium Series 1104 (pp. 303–338). Washington D.C.: American Chemical Society.

Franzitt, L., Granvogl, M., & Schieberle, P. (2016). Characterization of the key aroma compounds in two commercial rums by means of the Sensomics approach. Journal of Agricultural and Food Chemistry, 64(3), 637–645.

Gabrielli, M., Buca, A., Fracassetti, D., Stander, M., Tirelli, A., & Wessel, J. (2015). Determination of sotolon content in South African white wines by two novel HPLC–UV and UPLC–MS methods. Food Chemistry, 169, 180–186.

Grosch, W. (2001). Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. Chemical Sensors, 26(5), 533–545.

Guth, H., & Grosch, W. (1989). Intense odour compound formed during flavour reversion of soya-bean oil. Fat Science and Technology, 91, 225–230.

Guth, H., & Grosch, W. (1994). Identification of the character impact odorants of steamed beef juice by instrumental analyses and sensory studies. Journal of Agricultural and Food Chemistry, 42(12), 2862–2866.

Hausch, B. J., Lorjarenphong, Y., & Cadwallader, K. R. (2015). Flavor chemistry of lemon–lime carbonated beverages. Journal of Agricultural and Food Chemistry, 63(1), 112–115.

Hofmann, T., Schieberle, P., & Grosch, W. (1996). Model studies on the oxidative stability of odor-active thiols occurring in food flavors. Journal of Agricultural and Food Chemistry, 44(1), 251–255.

Kotseridis, Y., Baumes, R., & Skouroumounis, G. K. (1998). Synthesis of labelled [2H4]-damasconene, [2H2]-2-methoxy-3-Isobutylpyrazine, [2H3]- ionone, for quantification of grape aroma components. Journal of Agricultural and Food Chemistry, 46(1), 71–78.

Lahne (2010). Aroma Characterization of American rye whiskey by chemical and sensory assays(M.S. thesis). Urbana, IL, USA: University of Illinois.

MacNamara, K., & Hofmann, A. (1998). Gas chromatographic technology in analysis of distilled spirits. Elsevier Sciences B.V.303–346.

Meižgalis, M., & Civile, G. V. (2007). Sensory evaluation techniques (4th ed.). Boca Raton, FL: CRC Press/ Taylor & Francis.

Mottram, D. S., & Whitfield, F. B. (1995). Maillard-lipid interactions in nonaqueous systems: Volatiles from the reaction of cysteine and ribose with phosphatidylcholine. Journal Agricultural Food Chemistry, 43(5), 1302–1306.

Poisson, L., & Schieberle, P. (2008). Characterization of the most odor-active compounds in an American Bourbon whisky by application of the aroma extract dilution analysis. Journal of Agricultural and Food Chemistry, 56, 5813–5819.

Rostatchakul, P., Chaiseri, S., & Cadwallader, K. R. (2007). Identification of characteristic aroma components of Thai fried chili paste. Journal of Agricultural and Food Chemistry, 55(2), 528–536.

Schnieder, S., & Roland, C. (1992). One step synthesis of vanillin. Journal of Labelled Compounds and Radiopharmaceuticals, 31(6), 92–95.

Schuch, C., & Schieberle, P. (2006). Characterization of the key aroma compounds in the beverage prepared from Darjeeling black tea: Quantitative differences between tea leaves and infusion. Journal of Agricultural and Food Chemistry, 54(3), 916–924.

van Den Dool, H., & Kratz, P. D. (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. Journal of Chromatography A, 11, 463–471.

Weerawatnakorn, M., Wu, J., & Pan, M. (2015). Reactivity and stability of selected flavor compounds. Journal of Food and Drug Analysis, 23(2), 176–190.

Wu, T., & Cadwallader, K. R. (2019). Identification of characterizing aroma compounds of roasted chicory “coffee” brews. Journal of Agricultural and Food Chemistry (published online, 4477), 1851–1856.

Zhang, R., Wu, Q., & Xu, Y. (2014). Lichenysin, a cyclooctapeptide occurring in Chinese liquor Jiannanchun reduced the headspace concentration of phenolic odor-flavors via hydrogen-bond interactions. Journal of Agricultural and Food Chemistry, 62(33), 8392–8397.

Zhang, R., Wu, Q., Xu, Y., & Qian, M. C. (2014). Isolation, identification, and quantification of lichenysin, a novel nonvolatile compound in Chinese distilled spirits. Journal of Food Sciences, 79(10), C1907–C1915.

Zhu, S., Lu, X., Ji, K., Guo, K., Li, Y., Wu, C., & Xu, G. (2007). Characterization of flavor compounds in Chinese liquor Moutai by comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry. Analytica Chimica Acta, 597(2), 340–348.