Title

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Authors

Martusevice, Paulina

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Fox, Glen

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Analysis of mousy off-flavor compound 2-Acetyl-tetrahydropyridine using Liquid Chromatography Mass Spectrometry with Electrospray Ionization in sour beer

Paulina Martusevice[a,b,c], Xueqi Li[a], Matt Hengel[d], Selina C. Wang[a], Glen Fox[a,*]

[a] Department of Food Science and Technology, University of California, Davis, CA, United States
[b] Lithuanian Research Centre for Agriculture and Forestry, Institute of Horticulture, Kaunas, Lithuania
[c] Vytautas Magnus University, Botanical Garden, Kaunas, Lithuania
[d] Department of Environmental Toxicology, University of California, Davis, CA, United States

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Quantitative analysis of 2-Acetyl-tetrahydropyridine using Liquid Chromatography Mass Spectrometry with Electrospray Ionization

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ABSTRACT

Mousy off-flavor describes N-heterocycles compounds related to spoilage in the brewing industry. It has also been identified in sour beers through sensory analysis. Therefore, preventing spoilage N-heterocycles development is essential to preserve end-products and obviate economic losses. To this day, no methods or protocols have been reported to identifying mousy off-flavor compounds in a beer matrix. The main objective of this work was to develop a standardized quantification method for 2-acetyl-3,4,5,6-tetrahydropyridine (ATHP) in beer matrix, by Liquid Chromatography Mass Spectrometry with Electrospray Ionization (LC-MS-ESI). Extraction of ATHP in the samples was performed using QuEChERS (quick, easy, cheap, effective, rugged, and safe) technique. Over a dozen different potentially mousy cask-aged sour beers including other spontaneously fermented beverages were provided, based on sensory analysis, to determine the variation in ATHP levels. Results indicated ATHP was found in all the samples, ranging from 1.64 ± 0.06 to 57.96 ± 2.15 μg L^{-1}. Herein, we described our detection method of mousy-off flavor compounds which enables future research to mitigate the occurrence of such defects in fermented beverages matrix.

- ATHP content in samples varied from 1.64 ± 0.06 to 57.96 ± 2.15 μg L^{-1}.
- The recovery range of ATHP using LC-MS-ESI varied from 71% to 97%.
- Basified QuEChERS salting-out procedure is applicable for ATHP extraction from beer and other fermented beverages matrices.

Specifications table

Subject area:	Food Science
More specific subject area:	Beer analytic and sample pre-treatment
Name of your method:	Quantitative analysis of 2-Acetyl-tetrahydropyridine using Liquid Chromatography Mass Spectrometry with Electrospray Ionization
Name and reference of original method:	N.A.

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* Corresponding author.
E-mail address: gpfox@ucdavis.edu (G. Fox).
Social media: (G. Fox)

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Resource availability:

Equipment:

- Agilent 1260 Infinity II mass spectrometer equipped with Agilent 6470 Triple Quad LC-MS (Agilent technoloigies, USA) and electrospray ionization Source (ESI),
- Kinetex (5 μm EVO C18 100 Å 150 × 2.1 mm) column conected with 2.1 mm i.d. guard column packed with the same material (Phenomenex, Lane Cove, NSW, Australia),
- Sonificator (TS – 6000 Digital Ultrasonic Cleaner, RayTech, USA),
- pH-meter (Benchtop pH-mV Meter 0–14 pH range, Sper Scientific, USA).

Software:

- Offline Method Editor and Mass Hunter Optimizer (Agilent, USA),
- Agilent MassHunter Workstation Data Aquisition, (Agilent, USA),
- Agilent MassHunter Quantative analysis (for QQQ) (Agilent, USA).

Reagents:

- Ammonium acetate HPLC Grade crystalline form (Fisher Chemical, USA) methanol (Fisher Chemicals, USA),
- Acetonitrile HPLC/UHPLC-grade (Fisher Chemicals, USA),
- Ammonium hydroxide reagent grade (Fisher Science Education, USA),
- Water for MS (Fisher Chemicals, USA),
- 2-Acetyl-3,4,5,6-tetrahydropyridine Hydrochloride (Technical Grade) (ATHP) standard (Toronto Research Chemicals, Canada).,
- 2-Acetyl-3,4,5,6-tetrahydropyridine-13C2 Hydrochloride (Technical Grade) ($^{13}C_2$ ATHP) internal standard (Toronto Research Chemicals, Canada). Standards were in crystalline form with hydrochloride,
- QuEChERS salts, which consisted of 4 g magnesium sulfate, 1 g sodium chloride (Agilent Technologies, US).

Introduction

Mousy off-flavor is a bouquet of N-heterocycles: 2-ethyl-1,3,4,5,6-tetrahydropyridine (enamine form); 2-ethyl-3,4,5,6-tetrahydropyridine (imine form) (ETHP), 2-acetyl-1,4,5,6-tetrahydropyridine (enamine form); 2-acetyl-3,4,5,6-tetrahydropyridine (imine form), acetyl-1-pyrroline (imine form) and 2-acetyl-2-pyrroline (enamine form) (APY) [1] which are related to the spoilage of beverage and commonly obtained in wild fermentation end-products, e.g., wine without sulfites, sour beer, ciders, kombucha and others [2,3]. Sensorial descriptions of mousy off-flavor include corny (sometimes referred to corn-based cheese flavor chips odor), cereal, fresh popcorn, sour bread odor, mouse urine and cracky off-flavor [4–6]. The threshold in water of ATHP, ETHP and APY is 1.6 μg kg^{-1} 150 μg kg^{-1} and 0.1 – 0.06 μg L^{-1}, respectively [7].In roasted barley tea, APY threshold was 0.053 μg kg^{-1} [8]. Although APY alone is described as having nutty and buttery flavor, in complex with ATHP and ETHP envelopes mousy off-flavor in wines [1,8,9]. There is little research reporting N-heterocycles using Gas Chromatography Mass Spectrometry (GS-MS) and Liquid Chromatography Mass Spectrometry (LC-MS) techniques [1,3]. However, identification using LC-MS is applicable for ATHP only. A previous study described an ATHP method via LC-MS using a tandem with atmospheric pressure ionization source (APCI) [3]. But analytical laboratories commonly have only an electrospray ionization source (ESI). ESI source covers a broader range of analytical compounds, reflecting the economic reasons why analytical laboratories choose to have it instead of APCI source [10].

The beer matrix is a complex mix of micro- and macro-components from grains and hops, which includes organic acids, carbonyl compounds, and over six hundred flavor compounds [11,12]. Also, over 160 beer styles are described in the American Brewers Guidelines, which refers to plenty of possible modifications of the beer brewing process [13]. Lager and ales are historically the popular styles, but sour beers, which are fermented in the cask and can involve wild environmental microbes inoculation, are gaining popularity among consumers. Nowadays people have a tendency to support local craft breweries, which encourages experimental craft brews as a point of differentiation. However, sour beer have a higher risk of developing mousy off -flavor resulting in decreased sales, which sometimes are crucial for smaller breweries.

Sour beers have an even more complicated matrix than regular beer due to long fermentation in the cask, microbial activity during fermentation, and their metabolite levels, especially increased organic acids and salt content [14–16]. Nonetheless, other grain-based spontaneously fermented beverages or kombucha, which have high microbial variation and population counts, may be applicable for detecting mousy-off flavor either [17,18].

The QuEChERS method is a liquid-liquid extraction procedure that directly analyses the solution extract in samples from different matrices such as food, effluents, and biological samples [19]. Main advantages of QuEChERS include its environmentally friendliness, low cost, high accuracy and rapidity [20]. Due to intermediate polarity acetonitrile can simultaneously remove ATHP from different beer samples. The basified (pH 9–10.5) salt-assisted extraction process (salting out) initiates the protonation of ATHP imine to enamine, which is more volatile and more detectable. In addition to promoting the separation of the organic phase, the resulting acetonitrile layer is available for direct analysis by LC, without the need to filter or dry the extract. The application of this method has already been effectively demonstrated in detecting pesticides, mycotoxins and bitter compounds in hops and beer [21,22]. However, the QuEChERS application for extracting ATHP is demonstrated here, along with LC-MS-ESI for quantitation, for the first time.

Table 1
Analytical data for ATHP and $^{13}C_2$ ATHP standards.

	Name	Abbreviation	Chemical formula	Molecular weight (g/mol)	Specific ions (*m/z*)	Comment
1.	2-Acetyl-3,4,5,6-tetrahydropyridine	ATHP	$C_7H_{11}NO$	125	56/84/98	standard
2.	2-Acetyl-3,4,5,6-tetrahydropyridine	$^{13}C_2$ ATHP	$C_7H_{11}NO$	127	56/84/100	internal standard (IS)

Note: Standards were in crystalline form with hydrochloride. The hydrochloride dissociates in the solvent and is not included in the molecular weight formula.

Method details

Beverage samples

Traditional commercially available lager and ale beers without ATHP and sixteen suspected mousy off-flavor sour beers were selected for developing the method and its validation. All suspected mousy beers were fermented in the cask. Three samples out of sixteen were more than five years old (samples 11, 13, and 14). Additionally, three samples were selected outside of the sour beer category. Hard kombucha sample (sample 18), spontaneously fermented non-alcoholic grain beverage (sample 19) and tea beer (sample 16). Kombucha and non-alcoholic grain beverage samples were fermented with a mixed-culture microbial population and suspected to have mousy off-flavor.

Standards and mobile phase preparation

Beer matrix involves complex compounds which affects mousy off-flavor compounds detection using spectrometer analysis[23]. To minimize the matrix affect an internal standard of ATHP was applied. Standards of main mousy off-flavor compound with analytical data are shown in Table 1. For LC-MS-ESI two standards: 2-acetyl-3,4,5,6-tetrahydropyridine (ATHP) and 2-acetyl-3,4,5,6-tetrahydropyridine $^{13}C_2$ ($^{13}C_2$ ATHP) were selected to optimize the method in sour beer samples. Also, ATHP derivative 2-ethyl-3,4,5,6-tetrahydropyridine (ETHP) was evaluated through LC-MS-ESI. However, the abundance was low compared to ATHP and thus not taken for further investigations in the beer matrix. APY was not selected because it is a volatile compound and requires the use of another instrument (i.e. GC)Moreover, APY without ATHP and ETHP do create favorable aroma [1,8,9].

Stock solutions of 0.1 mg mL^{-1} standard and internal standard was prepared in 100% methanol and stored in amber glass vial at -15 °C before using for the analysis. On the same day of analysis working solutions were prepared using UltraP water. Serial dilution technique was applied to reach a desired concentration. To compare the ATHP stability in water and methanol-based stock solutions same concentrations were prepared, and after a period analytical data was collected to evaluate peak area abundance. ATHP in water had lower stability than ATHP in methanol-based stock solutions (Fig. 1). In addition, experimental water-based stock solution was kept in the 4 °C and ATHP stability decreased 1.3-fold in three days and 1.7-fold after 7 days. In comparison, methanol-based stock solutions maintained stable ATHP and $^{13}C_2$ ATHP after 2-month period, which were kept in -15 °C.

Initially, for the mobile phase ammonium acetate 10 mM was used as solvent A. The pH correction to 9.0 was made with 20% NH_4OH. Methanol was selected as Solvent B. ATHP is polar compound and adaptation of 'like dissolves like' principle was implied. Overview of stock, working and mobile phase solvents are shown in Fig. 2.

0.1 mg mL^{-1} ATHP standard stock solution preparation: weighted 1 mg of ATHP was dissolved up to 10 mL with 100% methanol.

10 µg mL^{-1} ATHP standard working solution preparation: 1 mL of 0.1 mg mL^{-1} ATHP of stock solution was diluted up to 10 mL with UltraP water.

0.1 mg mL^{-1} $^{13}C_2$ ATHP standard stock solution preparation: weighted 1 mg of $^{13}C_2$ ATHP was dissolved up to 10 mL with 100% methanol.

10 µg mL^{-1} $^{3}C_2$ ATHP standard working solution preparation: 1 mL of 0.1 mg mL^{-1} $^{13}C_2$ ATHP of stock solution was diluted up to 10 mL with UltraP water.

10 mM ammonium acetate: weighted 0.77 g of ammonium acetate was dissolved with UltraP water up to 800 mL, the pH correction to 9.0 was made with 20% NH_4OH. The solution was diluted up to 1 L.

Stock solutions can be kept for at least 2 months (at -15 °C). Mobile phases and calibration curve can be made up in advance. However, on the day of analysis, it is important to make fresh internal standard and working solutions.

Equipment and conditions

LC-MS-ESI

Agilent 1260 Infinity II mass spectrometer equipped with Agilent 6470 Triple Quad LC/MS (Agilent technoloigies, USA) and electrospray ionization Source consisting of a binary pump, autosampler and column oven was used. Data aquisition and processing were performed using Agilent MassHunter software.

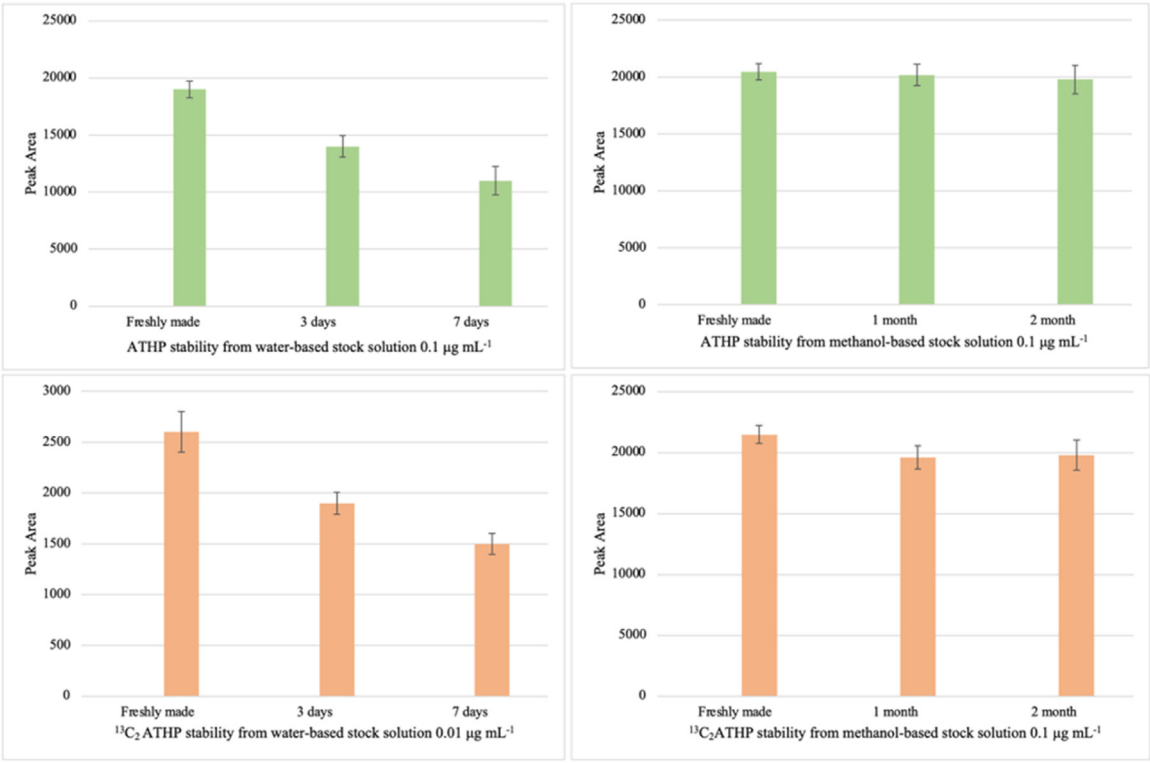

Fig. 1. Changes in peak areas of 2-acetyl-3,4,5,6-tetrahydropyridine ATHP and 2-acetyl-3,4,5,6-tetrahydropyridine $^{13}C_2$ ($^{13}C_2$ ATHP), as internal standard stability, in water and methanol-based stock solutions over a period.

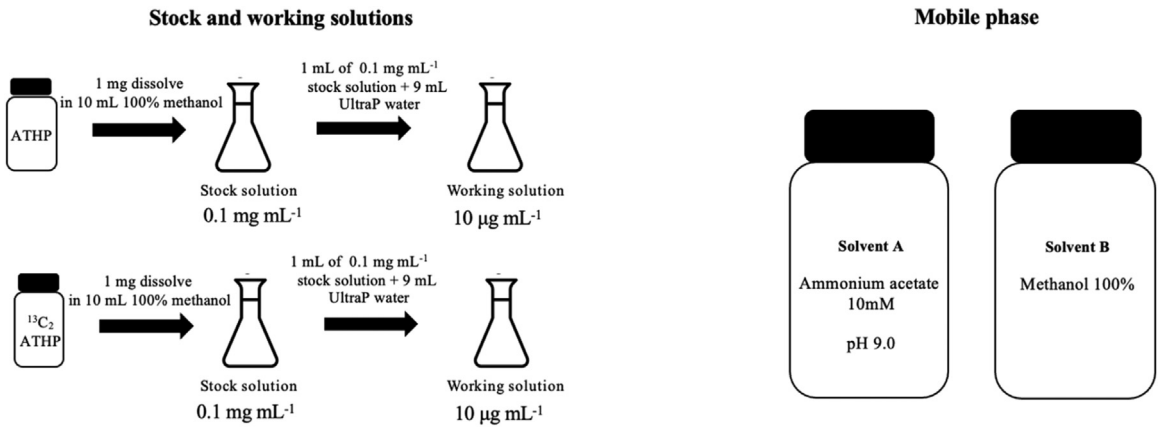

Fig. 2. Methanol-based stock solution and water-based working solution preparation and mobile phase solvent A and B.

LC separation

A 100 µl aliquot of sample was injected and chromatographed on a Kinetex (5 µm EVO C18 100 Å 150 × 2.1 mm) column connected with 2.1 mm i.d. guard column packed with the same material (Phenomenex, Lane Cove, NSW, Australia). 10 mM ammonium acetate (pH 9.0) was prepared as solvent A, and 100% methanol as solvent B. 20% ammonium hydroxide was used for pH corrections. For separation isocratic conditions were implemented: solvent A was selected at 40%, solvent B at 60%. A flow rate of 500 µl mL^{-1} and run time was set for 3 min for lager type beer and 6 min for ale type beer.

MS conditions

A positive ion mode in electrospray ionization was used to record generated multi-reactant-monitoring (MRM) of the ATHP ion. Negative ion mode was attempted but no useful ion transitions were identified. After different variations gas temperature was set for

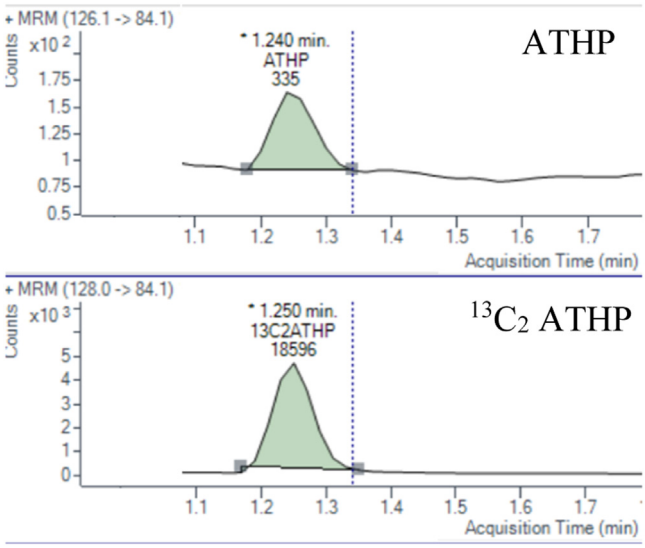

Fig. 3. ATHP of 0.0015 μg mL^{-1} in UltraP water with internal standard 0.1 μg mL^{-1} $^{13}C_2$ ATHP.

300 °C; gas flow 10 L min^{-1}; nebulizer 45 psi; shealth gas temparature 250 °C; shealth gas flow 1 L min^{-1}; capillary positive and negative voltage was set to 1500 V and nozzle positive and negative voltage was set to 500 V.

For quantification ATHP and $^{13}C_2$ ATHP were detected by multiple reaction monitoring (MRM). Fragmentation was set for 135 after tested coarse range from 20 to 200, and collision energy was set to 16 V. Mass transitions, *m/z* 126.1 → *m/z* 98.0 (qualifier), 84.0 (quantifier), and 56.0 (qualifier) for ATHP, and *m/z* 128.1→ *m/z* 100.0 (qualifier) and 84.0 (quantifier) and 56.0 (qualifier) for $^{13}C_2$ ATHP. For all transitions the cell accelerator voltage was set to 5 V.

Set of quatification and method detection limit

The limit of quantitation (LOQ) was defined as the lowest fortification level attempted, and the method detection limit (MDL) was calculated by the following equation: MDL=Student t-value x Standard Deviation [24].The 10 μg mL^{-1} ATHP working solution was serially diluted to make 1.5, 0.15, 0.0015 and 0.00015 μg mL^{-1} solutions. An internal standard spiking solution was prepared by taking a 100-μL aliquot of the 0.1 μg mL^{-1} $^{13}C_2$ ATHP working solution. LOQ were determined to be 0.0015 μg mL^{-1} in UltraP water (Fig. 3). The result of MDL was calculated to be 0.5 μg L^{-1}.

Calibration curve

Calibration curve was prepared with standard addition containing ATHP at 0.0015, 0.005, 0.01, 0.05, 0.1, 0.3, 0.5 μg mL^{-1} and $^{13}C_2$ ATHP at a constant concentration of 0.1 μg mL^{-1} A calibration curve was constructed by fitting a linear regression line (no weighting) to a set of calibration data with the ion response ratio (peak ratio) of ATHP and $^{13}C_2$ ATHP against ATHP concentration (Fig. 4).

Sample treatment and method validation

QuEChERS for extractions

Two decades ago, the QuEChERS methods were developed for analyzing pesticide residues and other chemical contaminants from agricultural materials, including food and beverages[19]. QuEChERS is a procedure based on principles involving intermediate polar solvent acetonitrile, contributing to salting-out extraction. Liquid-liquid extraction extracts metabolites and hydrophilic compounds to acetonitrile (ACN), which can be used for further sample clean-up or direct analysis [19,25]. Following the procedure, a freshly opened beer sample was sonicated with a de-gas function for 15 min. Further, 10 mL aliquot was basified with ammonium hydroxide to reach pH 9 (depending on the sample in the range between 100 and 175 μl). The sample was mixed with can and vortexed for 1 min. QuEChERS salts, consisting of 4 g magnesium sulfate and 1 g sodium chloride, were added to the beer and ACN solution. The mixture was vortexed for 1 min and centrifuged for 5 min at 10.000 rpm. The upper layer was collected for further ATHP analysis. 1 mL of upper layer extract was spiked with 0.1 μg mL^{-1} ATHP internal standard, basified with 10 μl of 20% NH_4OH and diluted up to 4 mL with UltraP water. The preparation flow diagram is shown in Fig. 5.

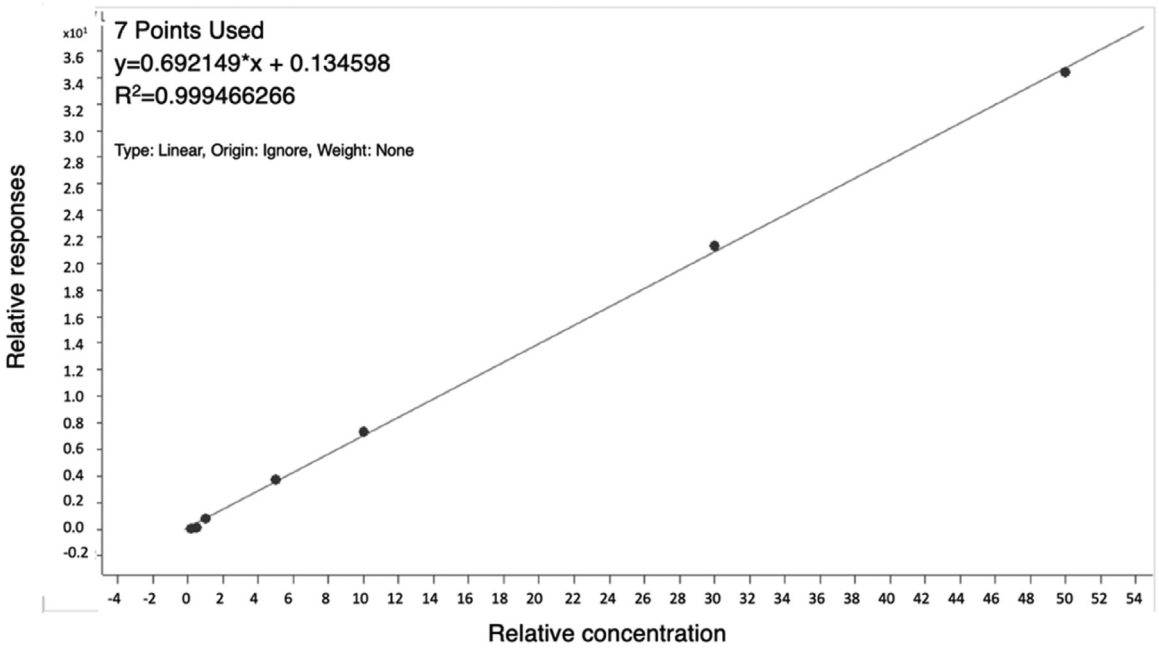

Fig. 4. Calibration curve of ATHP in UltraP water.

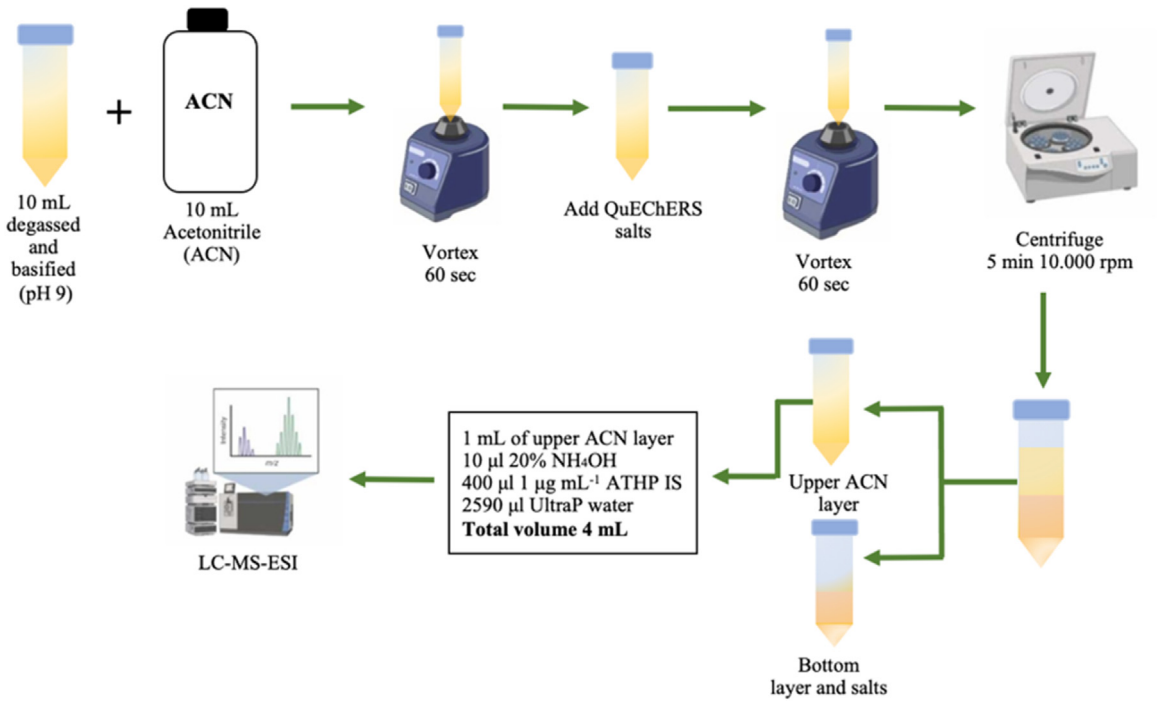

Fig. 5. Beer and other fermented beverages sample preparation using QuEChERS for ATHP analysis.

Method validation and effect of a beer matrix

A recovery test assessed the 'ethod's accuracy and reproducibility using lager and ale-type beer without ATHP. The pH of lager and ale beer was approximately 4–4.5. Before the salting-out procedure, beer samples were fortified by 0.0015 ($n = 7$), 0.015 ($n = 3$) and 0.15 ($n = 3$) µg mL^{-1} of ATHP and 0.1 µg mL^{-1} internal standard. The following equation calculated recovery of the ATHP: Recovery (%) = (RR_2 x 100)/ RR_1, where RR_1 – relative response of 0.0015 µg mL^{-1} standard from the calibration curve, RR_2 –

Table 2
ATHP recovery (%) for lager and ale type beer.

ATHP concentration, μg mL^{-1}	0.15 (n = 3)	0.015 (n = 3)	0.0015 (n = 7)
Lager	78.2 ± 3.9	80.2 ± 8.1	97.5 ± 27.8
Ale	71.4 ± 2.6	66.25 ± 10.1	84.7 ± 12.6

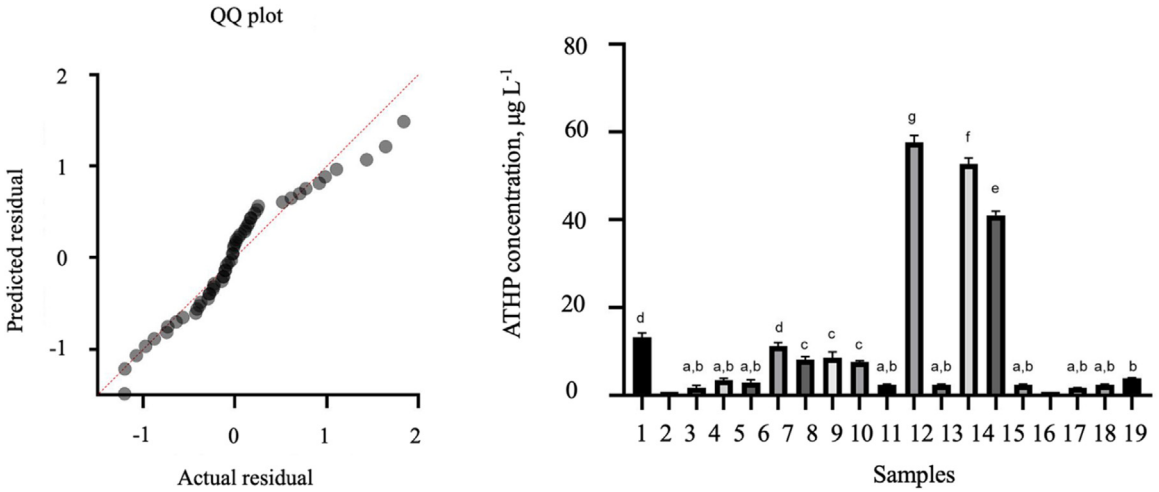

Fig. 6. Normality test (QQ plot) and ATHP concentration in tested samples. Different letters indicate significant differences (one-way ANOVA and Tukey's HSD test, $p < 0.05$).

Table 3
ATHP concentration in suspected mousy samples.

Sample	ATHP conc., μg L^{-1}	Sample	ATHP conc., μg L^{-1}	Sample	ATHP conc., μg L^{-1}	Sample	ATHP conc., μg L^{-1}
1	13.25 ± 0.98^{d}	6	11.16 ± 0.90^{d}	11	57.96 ± 2.15 g	16	< LOQ
2	< LOQ	7	8.07 ± 0.76^{c}	12	2.44 ± 0.17^{ab}	17	1.64 ± 0.06^{a}
3	1.75 ± 0.54^{a,b}	8	8.68 ± 1.87c	13	52.96 ± 1.92^{f}	18	2.44 ± 0.17^{a,b}
4	3.48 ± 0.45^{a,b}	9	7.55 ± 0.37^{c}	14	41.02 ± 1.39^{e}	19	3.70 ± 0.29^{b}
5	2.93 ± 0.61^{a,b}	10	2.42 ± 0.20^{a,b}	15	2.44 ± 0.17^{a,b}		

* Different superscript letters indicate significant differences (one-way ANOVA and Tukey's HSD test, $p < 0.05$).
Samples 1- 15 and 17 are suspected mousy sour beer samples; samples 16 - tea beer; sample 18 - hard kombucha; sample 19 - spontaneously fermented non-alcoholic grain beverage.

relative response of spiked beer sample. Results are presented in Table 2. Recovery of lager beer was from 78.2 to 97.5% and ale beer from 66.3% to 84.7%.

The results from the developed methods for ATHP using LC-MS-ESI are presented in Fig. 6 and Table 3. The normality test shows that selected samples vary in normal distribution. Two samples, one beer (sample 2) and non-beer (sample 16) did not reach LOQ while other potentially mousy samples had measurable amounts of ATHP ranging from 1.76 to 57.96 μg L^{-1} (samples 1–15, and 17). Samples (11, 13 and 14) with higher ATHP concentration >40 μg L^{-1} were older than five years old and continuously changed chemically in casks or bottles. The parameters that can impact the development of ATHP include a wide range: sugars, dissolved oxygen, amino acids, in particular lysine, proline, ornithine content, and microbial activity [4,26–28]. *Brettanomyces bruxelensis* is the most prevalent in the last stages of sour beer production in the cask and can be found in beer bottles after five years [18,29,30]. *B. bruxellensis* can partly develop ATHP through metabolic pathways L-lysine [29]. However, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) may also be responsible[4]. Compared to suspected mousy wines, the variation in results is similar. ATHP concentration in eight suspected mousy wine samples using LC-MC-APCI varied from 0.52 to 26.45 μg L^{-13}.

Limitations and conclusions

Beer has over 160 different styles and the varying matrices can differently suppresses ATHP [13]. Having a reliable and reproducible method to measure ATHP enables future research to track and mitigate its occurrence in fermented beverages matrix. Herein, we developed an extraction and quantification method of ATHP using LC-MS-ESI with a recovery range from 71% to 97%. The salting-out extraction showed promising application, and by incorporating additional clean-up stages, e.g., solid phase extraction (SPE), the recovery may be improved. The sample size of this work is modest, and more investigation may be needed for other spontaneously

fermented beverage matrices. Another limitation involves the unknown sensory threshold of ATHP, ETHP and APY in the beer matrix (i.e. higher concentrations may spoil beer, smaller concentrations can participate in favorable pallet of sour beer flavor), a study incorporating sensory panels along with analytical chemistry would help elucidate this relationship.

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Please declare any financial interests/personal relationships which may be considered as potential competing interests here.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Paulina Martusevice: Conceptualization, Methodology, Visualization, Writing – original draft. **Xueqi Li:** Software, Validation, Data curation, Conceptualization, Methodology, Project administration. **Matt Hengel:** Supervision, Software, Methodology, Validation, Data curation, Investigation, Resources. **Selina C. Wang:** Supervision, Writing – review & editing, Resources. **Glen Fox:** Supervision, Conceptualization, Resources, Writing – review & editing, Funding acquisition.

Data availability

The authors do not have permission to share data.

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References

[1] D. Kiyomichi, C. Franc, P. Moulis, L. Riquier, P. Ballestra, S. Marchand, S. Tempère, G. de Revel, Investigation into mousy off-flavor in wine using gas chromatography-mass spectrometry with stir bar sorptive extraction, Food Chem. 411 (2023) 135454, doi:10.1016/j.foodchem.2023.135454.
[2] A.A. Williams, Flavour research and the cider industry, J. Inst. Brew. 80 (5) (1974) 455–470, doi:10.1002/j.2050-0416.1974.tb06795.x.
[3] Y. Hayasaka, Quantitative analysis of mousy off-flavour compound 2-Acetyl Tetrahydropyridine in wine using liquid chromatography tandem mass spectrometry interfaced with atmospheric chemical ionisation, J. Chromatogr. A 1588 (2019) 108–114, doi:10.1016/j.chroma.2018.12.047.
[4] E.M. Snowdon, M.C. Bowyer, P.R. Grbin, P.K. Bowyer, Mousy off-flavor: a review, J. Agric. Food Chem. 54 (18) (2006) 6465–6474, doi:10.1021/jf0528613.
[5] L. Künzler, M.S. Pour Nikfardjam, Investigations into the formation of 2-Acetylpyridine and the mousy off-flavor in wine, Mitt. Klosterneubg. 63 (4) (2013) 87–198.
[6] R. Tressl, K.G. Grünewald, B. Helak, Formation of flavor components from proline and hydroxyproline with glucose and maltoseand their importance to food flavour, in: B. Peter Schreier (Ed.), Flavour '81: 3rd Weurman Symposium Proceedings of the International Conference, Munich April 28–30, De Gruyter, Boston, 1981, pp. 397–416. https://doi.org/10.1515/9783110844061-033.
[7] S. Tempère, B. Chatelet, G.de Revel, M. Dufoir, M. Denat, P.Y. Ramonet, S. Marchand, M. Sadoudi, N. Richard, P. Lucas, C. Miot-Sertier, O. Claisse, L. Riquier, M.C. Perello, P. Ballestra, Comparison between standardized sensory methods used to evaluate the mousy off-flavor in red wine, OENO One 53 (2) (2019), doi:10.20870/oeno-one.2019.53.2.2350.
[8] T. Sotaro, M. Yoshihide, N. Koichi, H. Thomas, S. Martin, Key odorants in Japanese roasted barley tea (Mugi-Cha)—Differences between roasted barley tea prepared from naked barley and roasted barley tea prepared from hulled barley, J. Agric. Food Chem. 68 (9) (2020) 2728–2737, doi:10.1021/acs.jafc.9b08063.
[9] X. Wei, D.D. Handoko, L. Pather, L. Methven, J.S. Elmore, Evaluation of 2-Acetyl-1-Pyrroline in foods, with an emphasis on rice flavour, Food Chem. 232 (2017) 531–544, doi:10.1016/j.foodchem.2017.04.005.
[10] K. Tobias, F. Oliver, Advances in structure elucidation of small molecules using mass spectrometry, Bioanal. Rev. 2 (1) (2010) 23–60, doi:10.1007/s12566-010-0015-9.
[11] K. Tang, Q. Li, 11 - Biochemistry of wine and beer fermentation, in: Current Developments in Biotechnology and Bioengineering, Elsevier, 2017, pp. 281–304, doi:10.1016/b978-0-444-63666-9.00011-x. Dussap, Ashok Pandey and Maria Ángeles Sanromán and Guocheng Du and Carlos Ricardo Soccol and Claude-Gilles, Ed.Part 2: Alcoholic Beverages.
[12] K. Habschied, I.J. Košir, V. Krstanović, G. Kumrić, K. Mastanjević, Beer polyphenols—bitterness, astringency, and off-flavors, Beverages 7 (2) (2021) 38, doi:10.3390/beverages7020038.
[13] Association, B. 2023 brewers association beer style guidelines. https://www.brewersassociation.org/edu/brewers-association-beer-style-guidelines/(accessed 2023-11-22).
[14] A. Dysvik, S.L.L. Rosa, G.D. Rouck, E.O. Rukke, B. Westereng, T. Wicklund, Microbial dynamics in traditional and modern sour beer production, Appl. Environ. Microbiol. 86 (14) (2020) e00520–e00566, doi:10.1128/aem.00566-20.
[15] F.A.W. Spitaels, The microbial diversity of traditional spontaneously fermented lambic beer, PLoS One 9 (4) (2014) 1–13, doi:10.1371/journal.pone.0095384.
[16] A. Ciosek, K. Fulara, O. Hrabia, P. Satora, A. Poreda, Chemical composition of sour beer resulting from supplementation the fermentation medium with magnesium and zinc ions, Biomolecules 10 (12) (2020) 1599, doi:10.3390/biom10121599.
[17] L. Sahu, S.K. Panda, Chapter 14 - Kefir, kombucha, and sour beers, in: Probiotic Beverages, Academic Press, 2021, pp. 287–307, doi:10.1016/b978-0-12-818588-9.00008-5. RussellSandeep K. Panda and Julie Kellershohn and Inge, Ed..
[18] M. Avramova, A. Cibrario, E. Peltier, M. Coton, E. Coton, J. Schacherer, G. Spano, V. Capozzi, G. Blaiotta, F. Salin, M. Dols-Lafargue, P. Grbin, C. Curtin, W. Albertin, I. Masneuf-Pomarede, Brettanomyces bruxellensis population survey reveals a diploid-triploid complex structured according to substrate of isolation and geographical distribution, Sci. Rep. 8 (1) (2018) 4136, doi:10.1038/s41598-018-22580-7.
[19] J.H. Matt, W.W. Jon, C.R. Zachary, R. Caitlin, L.W. Katryn, Analysis of pesticides in plant foods by QuEChERS and gas chromatography–mass spectrometry: an undergraduate laboratory experiment, J. Chem. Educ. 97 (1) (2020) 226–233, doi:10.1021/acs.jchemed.9b00476.

[20] N. Christina, O. Anna, H. David, H. Ester, L. Dimitra, QuEChERS—a green alternative approach for the determination of pharmaceuticals and personal care products in environmental and food samples, in: Green Chemistry and Sustainable Technology, Springer Singapore, Singapore, 2019, pp. 395–430, doi:10.1007/978-981-13-9105-7_14.
[21] J.M. González-Jartín, A. Alfonso, I. Rodríguez, M.J. Sainz, M.R. Vieytes, L.M. Botana, A QuEChERS based extraction procedure coupled to UPLC-MS/MS detection for mycotoxins analysis in beer, Food Chem. 275 (2019) 703–710, doi:10.1016/j.foodchem.2018.09.162.
[22] S.P.D. Marques, R.W. Owen, A.M.A. da Silva, M.L.A. Neto, M.T.S. Trevisan, QuEChERS extraction for quantitation of bitter acids and xanthohumol in hops by HPLC-UV, Food Chem. 388 (2022) 132964, doi:10.1016/j.foodchem.2022.132964.
[23] J.A. Peters, R. van Dam, Mycotoxin profiling of 1000 beer samples with a special focus on craft beer, PLoS One 12 (10) (2017) 1–27, doi:10.1371/journal.pone.0185887.
[24] J.A. Glaser, D.L. Foerst, G.D. McKee, S.A. Quave, W.L. Budde, Trace analyses for wastewaters, Environ. Sci. Technol. 15 (12) (1981) 1426–1435.
[25] J.H. Matt, S Takayuki, Method development and fate determination of pesticide-treated hops and their subsequent usage in the production of beer, J. Agric. Food Chem. 50 (12) (2002) 3412–3418, doi:10.1021/jf020089n.
[26] P. Moulis, C. Miot-Sertier, L. Cordazzo, O. Claisse, C. Franc, L. Riquier, W. Albertin, S. Marchand, G.D. Revel, D. Rauhut, P. Ballestra, Which microorganisms contribute to mousy off-flavour in our wines? OENO One 57 (2) (2023) 177–187, doi:10.20870/oeno-one.2023.57.2.7481.
[27] P.J. Costello, Formation of Mousy Off-Flavor in Wine by Lactic Acid Bacteria, The Australian Wine Research Institute, 1998.
[28] P.J. Costello, T.H. Lee, P. Henschke, Ability of lactic acid bacteria to produce N-heterocycles causing mousy off-flavour in wine, Aust. J. Grape Wine Res. 7 (3) (2001) 160–167, doi:10.1111/j.1755-0238.2001.tb00205.x.
[29] L. Conterno, T. Henick-Kling, 13 - Brettanomyces/Dekkera off-flavor and other microbial spoilage, in: Managing Wine Quality, 2nd ed., Woodhead Publishing Series in Food Science, Technology and Nutrition; Woodhead Publishing, 2022, pp. 427–476, doi:10.1016/b978-0-08-102065-4.00011-0. Reynolds, Andrew G., Ed..
[30] F. Spitaels, S.V. Kerrebroeck, A.D. Wieme, I. Snauwaert, M. Aerts, A.V. Landschoot, L.D. Vuyst, P. Vandamme, Microbiota and metabolites of aged bottled gueuze beers converge to the same composition, Food Microbiol. 47 (2015) 1–11, doi:10.1016/j.fm.2014.10.004.