

Abstract

Contributing characteristic porcine and jasmine-like aroma, 3-methyl Indole (3-MI), or skatole has been positively identified in an experimental test beer. To identify the source and levels of 3-MI, a HS-SPME-GC-SIM/MS method, with sub parts per billion detection levels was developed. This method was then applied to brewing raw materials, test mash samples, and finished beer. While beers typically contain levels of 3-MI below published threshold, brews containing a test batch of malted oats contributed significant amounts above human detection limits, contributing to undesirable beer quality. The results from this study highlight the presence of 3-MI in all beers and many raw materials used in the production of beer. Supplementing this study, cereal macronutrients and malting operations are discussed as areas of interest for maltsters to reduce the risk of 3-MI introduction to malted cereal grains.

GC Method and Validation

MS instrument outfitted with Agilent DB-FFAP column (60m, .250mm, 250um) operating with a split/splitless inlet at 240C. Inlet was set with a 15:1 split. The oven program was as follows: 40C initial and held for 2 minutes followed by a 2C/min ramp to 240 C, for a total run time of 70.67 min. A Combi-PAL autosampler was programmed to perform sample collection utilizing a PDMS/DVB 20 mm SPME fiber. All samples were kept in a 32 slot chiller tray held at 4C until sample collection. Samples were incubated at 35C and agitated for 2 mins (30 secs @ 500 rpm, 30 secs off) before headspace sampling. Fiber was desorbed in the inlet for 2 min.

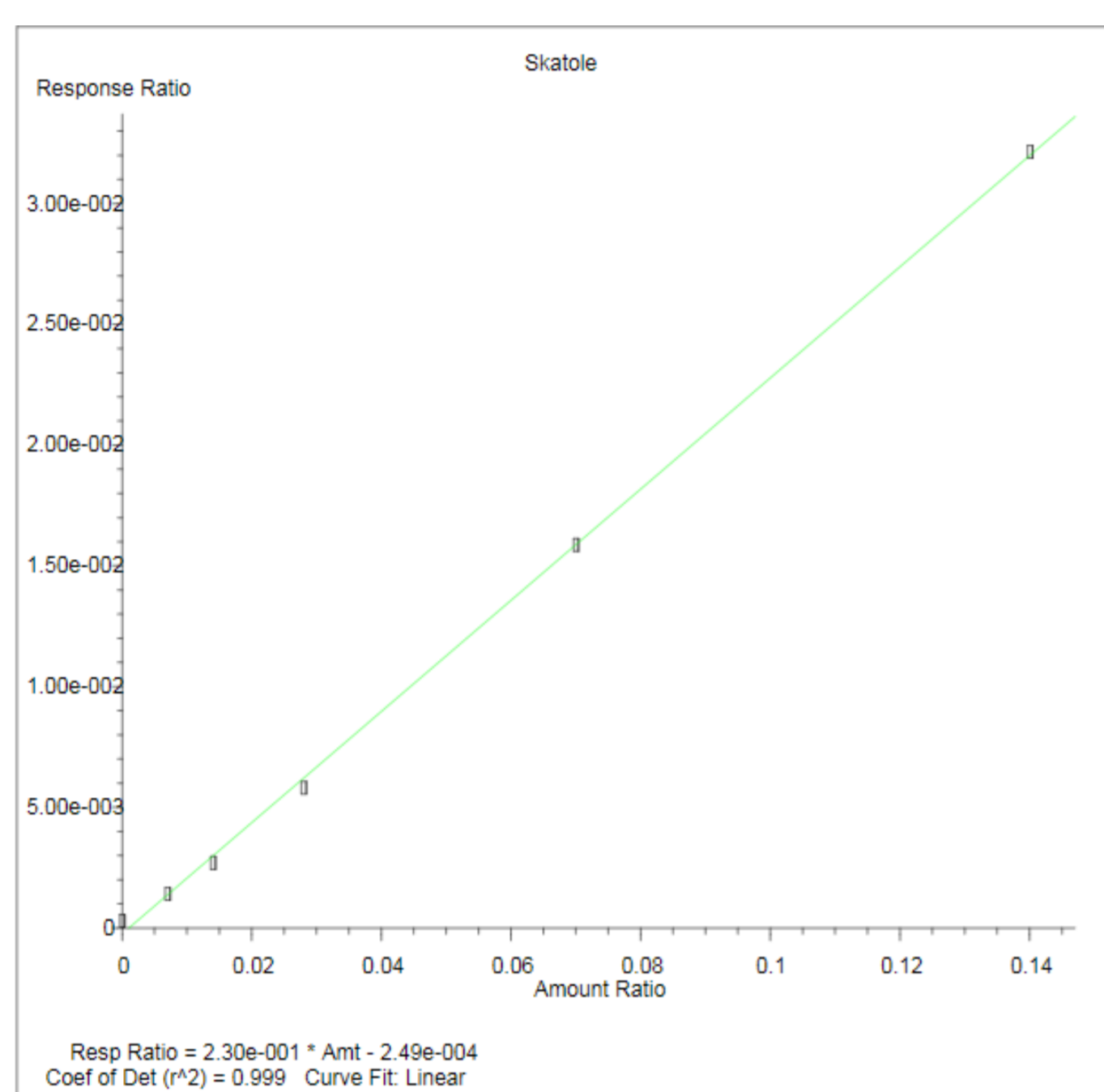
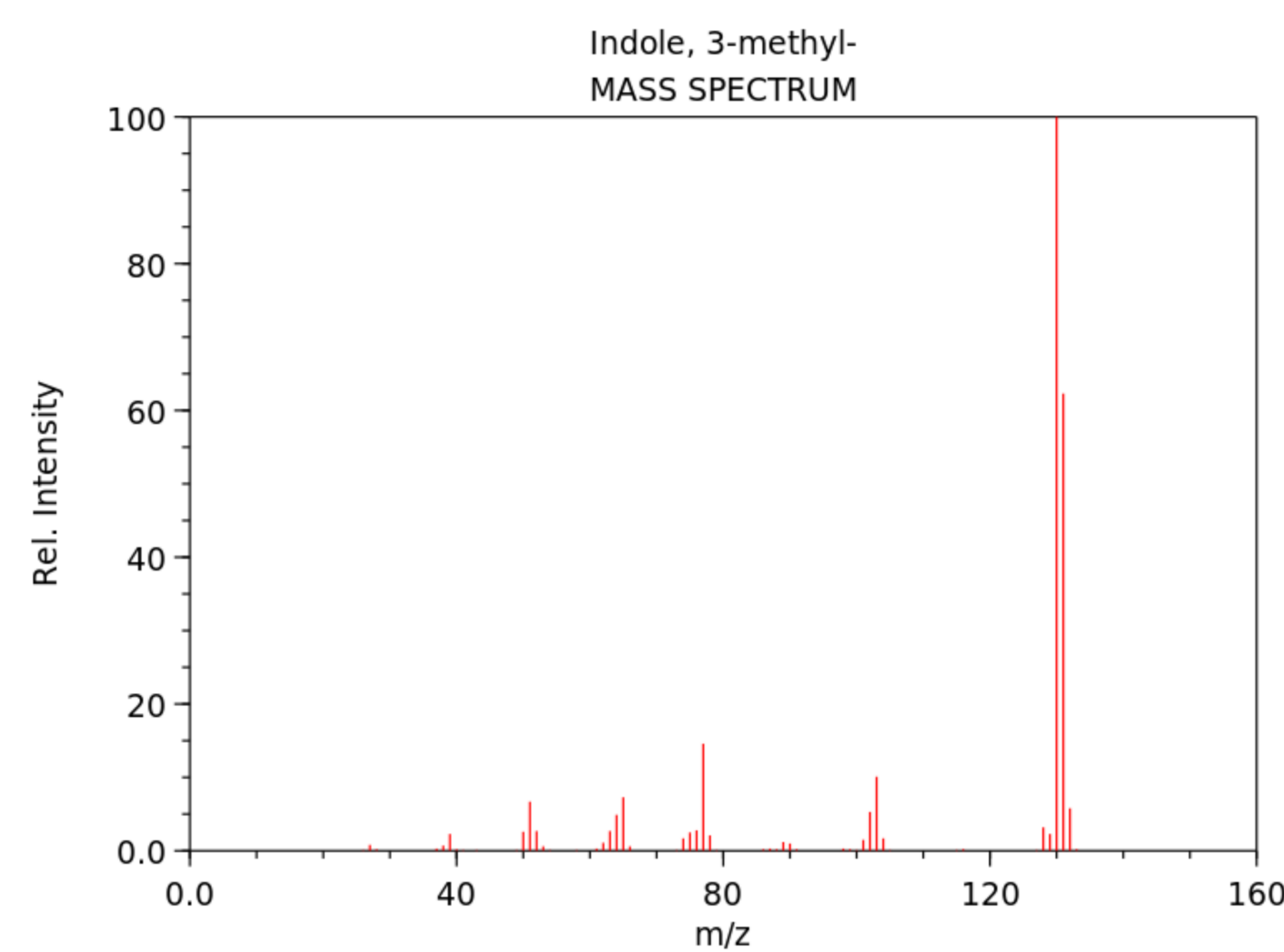


Figure 1: Calibration curve for 3-MI (Skatole). R-squared is 0.999.



NIST Chemistry WebBook (<https://webbook.nist.gov/chemistry>)

Figure 2: Electron Ionization Mass Spectrum of 3-MI from NIST

Table 1: Tryptophan and 3-MI quantitated values in commercial malt samples (modified hot steeps).

Key Code	Trp (nmoles)	3MI (ppb)
MOK	1.375	0.93
MSO	1.229	0.25
MSV5	1.466	0.25
MSS7	1.441	0.44
MS96	1.831	0.19
MS227	1.063	0.35
MS282	0.769	0.62
MSOM	1.457	0.43
MSOP	1.673	0.26
MOMO	1.123	0.23
MSCR	1.267	0.23
MSMB	1.429	0.65
MCP112	1.032	1.04
MCP01		0.27
MCR01		0.22
MCC01		0.25
MCU	1.625	0.15
MCM	0.989	0.58
MCW	1.854	0.25
MO1945	1.582	0.33
MCP77	1.223	0.68
MCP49	1.419	1.00
MOP5	1.527	0.22
MOP1	0.983	0.28
MOPO	1.183	0.29
MOCR	1.223	0.25
MOP2	1.513	0.30

Raw Material Survey

Working with our suppliers, we compiled a list of base and specialty malts that are commonly used in the brewing industry. In order to screen these materials for 3-MI, we utilized a modified ASBC hot steep method (ASBC Sensory 14) to collect clarified liquid that would be representative of only the selected grain. Each sample was weighed out to 52.0 grams, ground in a coffee grinder for 10 seconds and then reweighed to a mass of 50.0 g. The ground malt was placed into a 16oz thermos and had 400 mL of 65 degree C DDI water added to it. Bottles were shaken and let stand for 15 minutes. Grain was resuspended after by quickly swirling the thermos, and the entire contents was transferred to a pre-conditioned paper filter set up above a clean 500 mL jar. Once 100 mL of steep liquid had been collected, it was repoured into the funnel and collection was resumed. Collection of the liquid was stopped at 200 mL volume, purged with CO2 and capped and placed in the jar until sampling for analysis. Duplicate vials of each sample were prepared with 7mL of steep liquid and contained 2.5 grams of analytical grade sodium chloride.

Samples highlighted in Red (Table 1) contain 3-MI levels above threshold level and could produce beers with perceivable off-aroma characteristics of porcine and jasmine.

Work in Progress

Germination timing influence on 3-MI concentration: Working with industry partners, we have completed micro scale malting of oats. For this small trial, we have hypothesized that 3-MI creation and concentration will positively correlate with the time the oats were held in the germination stage of malting.

Commercial Beer Survey: Building on the knowledge that 3-MI is present in all brewing raw materials, we will gather data on the levels present in commercially available beers. This will also include a 3-MI survivability study to help understand how the levels are affected through the typical operations of a production brewery.

More results and discussion for these investigations will be presented at the 23rd Annual North American Barley Researchers Workshop and 43rd Barley Improvement Conference, September 2022, Davis, California.

Thank you to our industry partners who kindly provided a wide range of malts to help diversify the types of malts screened in this work.