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Establishment and practical comparison of methods to measure lactic acid and acetic acid in sour wort and sour beers

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Introduction

Sour beers are one of the fastest-growing categories in the beer market. The degree and character of tartness are key attributes. Sour beers may age for several months, or even years, to achieve the desired flavor. Several different bacteria including acetobacter, lactobacilli and pediococci, as well as yeast (*Dekkera*/*Brettanomyces*) are employed, alone or in tandem, to create the vast array of sour flavors. Lactic and acetic acids are among the key contributors responsible for the tartness in most sour beers.

An objective means of determining the degree of sourness would help assess the readiness of a given sour beer for packaging. One of the two existing official methods to measure acidity of sour beers is based on analytical titrations [(1)], providing useful information about the amount of acidity, but offering little insight into the character of tartness. The other method is an enzymatic assay specific for lactic acid (2), which provides quantitative information for lactic acid, but not for acetic acid. HPLC is among the few 'general purpose' laboratory techniques capable of quantitating both lactic and acetic acids in a single method.

Thereupon, this presentation outlines the development and optimization of an effective, efficient and robust HPLC-PDA (photodiode array detector) method for measuring lactic and acetic in sour wort and beer, such as Berliner weisse styles, made with various *Lactobacillus* species. In addition, a summary and practical comparison of available methods, including reflectrometric and titration-based methods, will be presented.

Experimental

Note: None of the methods evaluated distinguished lactic acid isomers. All references to lactic acid refer to a sum total of D- and L-lactic acid.

HPLC Conditions: The initial work was carried out using a PerkinElmer Series 200 HPLC system, including a quaternary pump, autosampler (20-uL sample loop), column oven, and photodiode array detector. Subsequently, a PerkinElmer Altus™ HPLC® system was used for spectra analysis and for the repeatability and linearity data presented in Figure 3.

HPLC Sample Preparation: All samples were chilled to 5 °C and centrifuged at 13,000 x g at room temperature (~22 °C) . 1-2 mL of each supernatant was then filtered through an 0.45-um PVDF syringe filter into an HPLC vial for analysis.

Titratable Acidity Method (TA): is a modified version of ASBC Beer-8A (Total Acidity By Titration of Diluted Beer with Phenolphthalein as Indicator). Exactly 10mL of sour wort or decarbonated (sonicated) sour beer was transferred to a 50-mL graduated cylinder, taken to 50mL total volume with deionized water, and transferred to a 125-mL Erlenmeyer flask, containing a magnetic stir bar. Five drops of a 1% phenolphthalein solution (Taylor # R-0638BR) were added and the solution was stirred constantly and titrated with 0.1N NaOH until the pink/purple color persists. The total volume (mL NaOH) of titrant required to reach the endpoint was multiplied by 0.1112 and the result was reported as "% titratable acidity".

Reflectometry (RFQ): A Reflectoquant RQflex plus 10 reflectometer (EMD Millipore #1.16955.0001) was used for direct measurement of lactic acid following the manufacturers instructions. Briefly, sour beer or wort samples were diluted to 0.5-1.0% v/v with deionized water. Then, simultaneously, 1) a test strip was immersed into the solution for 2 seconds and 2) a 5-minute timer is started on the RFQ apparatus. Subsequently, after allowing excess liquid to run off the test strip onto an adsorbent paper, the strip was immediately placed into the freshly calibrated RFQ apparatus. The lactic acid value was obtained at the end of the 5-minute countdown, the value being adjusted to account for the dilution factor. The results were expressed as ppm total lactic.

HPLC method development

Strategy:

1. Determine retention times of the analytes and the key interferents
2. Optimize the HPLC conditions to achieve well-resolved analyte peaks
3. Optimize integration parameters

Table 1: Conditions employed during HPLC method development

Method:	HPLC1	HPLC2	HPLC3	HPLC4	HPLC5	HPLC6	HPLC7
Column:	A	A	A	A	A	B	B
Mobile Phase:	20mM K-Phos pH 2.0	20mM K-Phos pH 2.0	0.1% formic pH 2.7	0.1% H3PO4 pH 2.2	0.2% H3PO4 pH 2.05	10-mM K-Phos pH 2.4	10-mM K-Phos pH 2.4
Flow Rate (pressure):	1mL/min (1430 psi)	1mL/min (1980 psi)	1mL/min (1910 psi)	1 mL/min (1940 psi)	1 mL/min (1860 psi)	1 ml/min (1400 psi)	1.5 ml/min (2900 psi)
Oven Temp:	35C	25C	25C	25C	25C	25C	25C

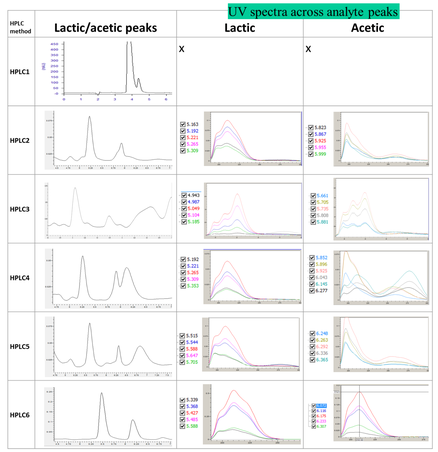

Figure 2: Qualitative assessment of HPLC peak purity

Table 2. Optimized HPLC conditions

Column: PerkinElmer® Brownlee Validated Aqueous C18, 5 µm, 4.6 x 220-mm (Part # N9303549)
Sample Prep: chill to 5C then centrifuge at 13,000 x g
Mobile Phase: isocratic; 10-mM K-phosphate buffer; pH 2.4
Analysis Time: 10.0 min.; wash/equilibration time = 5.0 min
Flow Rate: 1.0 mL/min. (~1400 psi)
Oven Temp.: 25 °C
UV Detection: 210 nm
Injection Volume: 20 µL
Sampling (Data) Rate: 5 pts./sec
Integration Parameters: Bunching factor =1; noise threshold = 100; area threshold = 1000

Reflectometry Method Precision

- Triplicate measurements on three different samples

Table 3: Replicate Lactic Acid measurements by RFQ.

	ppm lactic					
	test1	test2	test3	average	SD	RSD (%)
sample1	11460	6380	7000	8280	2771	33%
sample2	7400	6160	5880	6480	809	12%
sample3	4800	3840	3720	4120	592	14%

Per Table 3, overall the repeatability of the RFQ results were deemed adequate, but outliers (e.g. sample1, test1) appear to be sufficiently common that duplicate measurements have become standard practice, and results reported only if duplicates are within 20% of one-another. Otherwise, the measurement is repeated until two results within 10% of one-another are obtained.

Titratable Acidity Method Precision

- Replicate measurements on two different sour wort samples were carried out by a single operator

Table 4: Replicate Titratable Acidity measurements using method TA1.

				(TARGET=8.20)	calculation	ppm lactic summary		
sample	starting pH	caustic added (g)	caustic added (mL)	final pH	(as ppm lactic)	mean	SD	RSD (%)
1	3.16	8.148	8.12	8.21	7203.2	7306.2	122.2	1.67%
	3.2	8.41	8.38	8.35	7475.3			
	3.21	8.167	8.14	8.27	7232.1			
	3.25	8.233	8.20	8.17	7314.4			
2	3.03	11.605	11.56	8.43	10101.9	10269.8	144.5	1.41%
	3.08	11.965	11.92	8.46	10515.3			
	3.09	11.64	11.60	8.31	10182.8			
	3.08	11.817	11.77	8.54	10336.6			
	3.08	11.701	11.66	8.3	10274.2			
	3.08	11.728	11.68	7.93	10208.0			

Per Table 4, for sample1, extreme care was take to avoid over-shooting the endpoint. For sample2 the solution was rapidly titrated until a value of 8.0 first appeared on the pH meter at which time the solution was allowed to stabilize. The pH drifts downward during the stabilization period. If the pH is still below the pH 8.2 target after being allowed to stabilize, it was then carefully titrated back up to pH 8.2 to mark the endpoint of the titration, then allowed to stabilize once again, and the final pH value was noted. In both cases, the precision was deemed more-than-adequate.

HPLC Method Precision

- Figure 3A shows the overlay of 12 replicate injections of the level-4 calibrant (0.25% lactic acid/0.025% acetic acid) using method HPLC7, demonstrating high repeatability with respect to analysis of pure standards. The retention time precision was 0.08% RSD.
- Figure 3B and 3C shows the calibration results for the two analytes, with the calibrant levels ranging from 0.010 to 2.00 %. Both lactic acid and acetic acid standards exhibited an exceptional linear fit (R2 values > 0.9999 (n = 3 at each level)).

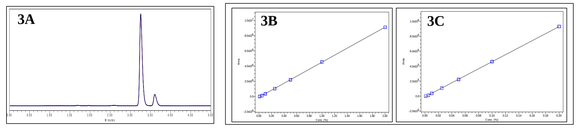

Figure 3 A) Overlay of 12 replicates of level-5 calibrant and results of 7-level calibration set for lactic (B) and acetic (C) acids , using method HPLC7

Comparison of Methods

Table 5: comparison of expected vs measured values in sour beer.

	measured in sour wort			expected values in sour beer		measured values in sour beer		
Batch#	TA	Lactic (RFQ)	pH	TA	Lactic (ppm)	TA	Lactic (RFQ)	pH
1	1.16%	6100	3.23	0.30%	1601	0.78%	2265	3.57
2	1.56%	15166	3.03	0.37%	3616	0.94%	2990	3.61
3	1.27%	9210	3.15	0.31%	2214	0.67%	2085	3.82

The linear correlation coefficient for expected vs. measured lactic acid in sour beer was 0.78, suggesting more work to be done to fully account for the lactic acid content in these beers.

Noteworthy/anecdotal findings:

- HPLC assay
  - Shikimic acid is a strong UV absorber, and it can coelute with these analytes under various conditions.
    - Shikimic acid is ubiquitous in plants and can be expected as a potential interferent
  - Two peaks were observed in a malic acid standard using methods HPLC2 and HPLC3. This is consistent with a report that separation of D- and L-malic acid isomers was achieved with this achiral column.
  - Lactic acid was fairly well-resolved under all conditions evaluated, whereas acetic acid co-eluted with an interferent under all but the last two test conditions (HPLC6 and HPLC7).
  - MRS broth contains high levels of acetate, which precludes the use of this method for optimization of culture conditions that employ MRS broth.
  - The key factors for integration optimization are bunching factor and area/noise threshold.
- Titratable Acidity assays
  - For the low-color samples evaluated in this study, the numeric endpoint of a pH meter did not offer any advantages compared with phenolphthalein color change endpoint.
- Reflectometric assay
  - This enzyme-based test is so sensitive that samples must be diluted at least 100-fold in order to register on the scale of the instrument. Care must be taken to avoid contamination with any lactic acid on skin. Timing is critical and any excess liquid must be carefully removed from the test strip prior to placing it in the instrument.

Summary/Conclusions

Three fundamentally different analytical methods for measuring tartness of sour beers and sour worts were investigated. The correlation of values obtained with any two methods was quite poor, but all of them appeared to provide more-than-adequate precision. Although this work is still ongoing, the pros and cons of each method are provided below.

method	pros	cons
TA	simple, affordable	low-throughput; poor choice for large numbers of samples
LA by RFQ	rapid, relatively inexpensive	provides no information about anything other than lactic acid
HPLC	provides quantitative information about both lactic and acetic acids; best for high-throughput optimization work;	high equipment cost; requires skilled operator; presence of acetate in MRS broth makes this method incompatible with MRS-based laboratory cultures

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