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Authors
Spevacek, Ann R
Benson, Katy H
Bamforth, Charles W
et al.

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Beer metabolomics: molecular details of the brewing process and the differential effects of late and dry hopping on yeast purine metabolism†

Ann R. Spevacek,[1] Katy H. Benson,[2] Charles W. Bamforth[2]* and Carolyn M. Slupsky[1,2]

The flavour of beer is complex, based upon changes at the molecular level in the key raw materials, notably grain, hops and yeast, as well as during the process stages that comprise malting and brewing. As analytical techniques evolve in their sophistication and sensitivity, there are opportunities to delve ever more deeply into the fate of small molecules in brewing. To this end, ^{1}H nuclear magnetic resonance (NMR) metabolomics was used to follow the progression of 76 metabolites in four different late or dry hopped beers (brewed in triplicate) at five time points throughout the brewing process. The majority of the metabolites identified, including sugars, amino acids and nucleotides, significantly decreased in concentration from the start of the boil to post-secondary fermentation, whereas energy-related and fatty acid associated metabolites significantly increased in concentration as wort nutrients were consumed by the yeast. Adenine was significantly higher in the dry hopped brews than in the late hopped brews after both primary ($p = 2.1 \times 10^{-6}$) and secondary ($p = 2.7 \times 10^{-9}$) fermentation, while 2′-deoxyadenosine (after primary, $p = 1.1 \times 10^{-2}$, after secondary, $p = 3.2 \times 10^{-5}$) and adenosine (after primary, $p = 2.6 \times 10^{-8}$; after secondary, $p = 3.1 \times 10^{-7}$)were significantly lower in the dry hopped beers at these time points. These results give molecular insight into the brewing process and the differential effects of hopping methods on yeast purine metabolism. **Copyright © 2015 The Institute of Brewing & Distilling**

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Keywords: metabolomics; nuclear magnetic resonance; hops; yeast

Introduction

Recently, metabolomics, or the study of small molecular weight compounds (metabolites that are generally <1000 Da) that are the reactants or products of metabolism, has been used to analyse beer. For example, metabolomics has been used for the following: to correlate concentrations of hop aroma compounds with sensory evaluation *(1)*; to determine the effect of storage conditions on the stability of beer *(2)*; and to study the metabolic outputs of various yeast strains *(3)*. Additionally, this technique has also helped distinguish between ales and lagers *(4)*, ales, lagers and non-alcoholic beers *(5)*, alcoholic and non-alcoholic beers *(6)*, malt varieties *(7)*, brewery locations *(7,8)* and brands of beer *(9)*. However, to date, few studies have used metabolomics to track changes in metabolites throughout the brewing process *(10)*.

^{1}H nuclear magnetic resonance (NMR) metabolomics was used to follow the metabolic profile of four beers (brewed in triplicate) through five time points. This technique requires very little sample preparation and simultaneously measures numerous metabolite concentrations in the micromolar to molar range, while providing absolute quantitation *(11)*. Also tested was the effect of late and dry hopping on the beer metabolomes by employing these hopping methods with either Cascade or Magnum hops. While late and dry hopping practices are well known to influence flavour, the interplay between hop compounds and yeast is poorly understood. *Saccharomyces cerevisiae* has been shown to biotransform several monoterpene alcohols commonly found in hops *(12,13)*. The abundance of these monoterpenoids differs between hop cultivars, and affects the concentration of flavour-active compounds present in the finished product *(14)*. Takoi et al. *(14)* showed that additional hop flavour molecules may be released by the glucoside hydrolases found in lager yeast. These studies provide evidence that yeast can alter the hop aroma profile of a beer, but to date, there is no published data indicating whether hop compounds affect yeast metabolism.

Here we show that ^{1}H NMR metabolomics is a powerful tool for tracking the molecular details of the brewing process. In addition, the results suggest that the two hopping methods differentially affect yeast metabolism during fermentation.

* Correspondence to: Charles W. Bamforth, Department of Food Science & Technology, University of California, Davis, CA 95616-8598, USA. E-mail: cwbamforth@ucdavis.edu

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[1] Department of Nutrition, University of California, Davis, Davis, CA 95616, USA

[2] Department of Food Science and Technology, University of California, Davis, CA 95616, USA

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Materials and methods

Beers

In total, four experimental beers were brewed – late hopped with Cascade hops, dry hopped with Cascade hops, late hopped with Magnum hops, and dry hopped with Magnum hops. All experimental beers were made using a 38 L brew system. The grist comprised two-row pale malt (4.2 kg) and Crystal-60 (0.5 kg). The malt was ground on a two-roll mill, and mashed in with strike water (24 L) at 45 °C. The mash followed a temperature-controlled regime with occasional manual agitation (Fig. 1).

The wort was lautered over a period of 60–80 min, incorporating a 10 min Vorlauf period, and subsequent sparging with water at 76 °C. Worts were boiled for 60 min. For all worts, a portion of whole-cone hops were added at the start of boil in a cheesecloth satchel for a target IBU of 40. For late-hopped beers a further 56.7 g was added using a 300 μm stainless-steel mesh vessel during the whirlpool stage (10 min). All worts were then cooled and transferred to foil-covered glass carboys, and the wort was aerated with sterile air, and pitched with two vials of California Ale Yeast (White Labs).

Fermentation in carboys took place for 14 days at room temperature (21–23 °C). During primary fermentation, dry-hopped beers were exposed to 56.7 g of whole-cone hops wrapped in sanitized cheesecloth between day 5 and day 11. At this time the beers were bottled with 113.4 g of corn sugar per brew and allowed to bottle condition for 14 days at room temperature.

Five time-point samples were collected through the process: before boil, after boil/whirlpool, after yeast pitch (immediately after pitch and suspension of yeast to wort), after primary fermentation (after 14 days in bottle), and after secondary fermentation (after 28 days in bottle, final beer). Samples collected were flash-frozen in liquid nitrogen at the time of sampling and stored at −80 °C prior to NMR analysis.

Sample preparation

Samples were removed from −80 °C storage and thawed on ice in preparation for NMR analysis. Aliquots (1 mL) were removed from each sample and centrifuged at 14,000 ***g*** for 5 min at 4 °C. The aqueous layer was decanted and applied to a 3000 molecular weight cut-off filter (Amicon Ultra-0.5; Millipore), which is composed of low-protein-binding regenerated cellulose that removes lipids and proteins. Sample (0.585 mL) was combined with internal standard (0.065 mL) containing 5 mM 3-(trimethylsilyl)-1-propanesulfonic acid-d_6 and 0.2% sodium azide in 99.8% D_2O (Chenomx). The pH of each sample was adjusted to 6.8 ± 0.1 by adding small amounts of NaOH or HCl and samples (0.6 mL) were subsequently transferred to a 5 mm Bruker NMR tube and stored at 4 °C until NMR acquisition (within 24 h of sample preparation).

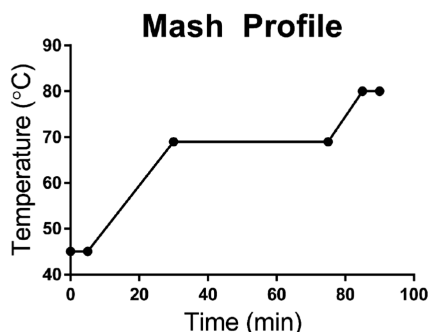

Figure 1. Profile of the temperatures used throughout the mashing process.

NMR and metabolite identification

^{1}H NMR spectra were acquired on a Bruker Avance 600-MHz NMR spectrometer equipped with a SampleJet autosampler using a NOESY-presaturation pulse sequence (noesypr) at 25 °C as previously described *(15)*. Water saturation was performed with a prescan delay of 2.5 s and a mixing time of 100 ms. Spectra were acquired with eight dummy scans and 32 transients over a spectral width of 12 ppm and a total acquisition time of 2.5 s. All acquired spectra were zero-filled to 128,000 data points, Fourier transformed with 0.5 Hz line broadening applied, and manual phased and baseline corrected using the NMR Suite v7.7 Processor (Chenomx). Metabolites were identified and quantified as previously described by identifying individual NMR spectral resonances with a combination of the 600 MHz library from the Chenomx NMR Suite v7.7 Profiler and an in-house library of metabolites and referencing the measured concentrations to the internal standard, in an approach known as 'targeted profiling' *(16)*. The final reported concentrations were obtained after correcting for dilution with the internal standard and water in the case of the low-volume samples (final sample volume, 0.65 mL, divided by the initial volume of beer filtrate, 0.585 mL). The measured metabolite concentrations ranged from the micromolar limit of detection of the NMR spectrometer *(11)* to millimolar, with an accuracy for the majority of the compounds within 10% of the actual concentration *(15)*.

Statistics

Statistical analysis was performed using a combination of Simca v13 (Umetrics), Prism v6.0 (GraphPad Software Inc.) and R v3.1.0. All concentrations were $\log_{10}$ transformed prior to use in principal component analysis (PCA) and in the linear mixed-effects models (LMM) described below.

Unsupervised PCA was used to explore the effect of brewing time point on the beer metabolome. To further investigate how brewing time point affected metabolite concentration, the lmer function (R package lmerTest) was used for the LMM with time, hop treatment (late vs dry), and brew batch modelled as interacting main effects and the brew sample as the random effect. Separate analyses were run on the first three time points and the last three time points to determine how metabolites were significantly affected before and after fermentation. The resulting *p*-values were adjusted for multiple comparisons by false discovery rate *(17)*. The late hopped vs dry hopped and brew batch means were compared at each time point using unpaired unequal variance two-tailed *t*-tests. Significant differences were defined as $p < 0.05$ for all statistical tests.

Results and discussion

Seventy-six metabolites were identified in samples taken at five points during the brewing process – start of the boil, after whirlpool, after yeast pitch, after primary fermentation (14 days post yeast pitch) and after secondary fermentation (28 days post yeast pitch; Table 1). These metabolites included 15 mono-, di- and oligosaccharides (1,6-anhydro-β-D-glucose, fructose, gentiobiose, glucose, isomaltose, isomaltotriose, kestose, kojibiose, maltose,

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Table 1. Effect of wort boiling/cooling and fermentation on metabolites measured throughout the brewing process by ^{1}H NMR spectroscopy[†]

	Pre-fermentation percentage change[§]	Pre-fermentation *p*-value	Post-fermentation percentage change[¶]	Post-fermentation *p*-value
Sugars				
1,6-Anhydro-β-D-glucose	14	0.41	−49	<0.001
Fructose	154	<0.0001	−88	<0.0001
Gentiobiose	26	<0.001	−27	<0.001
Glucose	34	<0.0001	−99	<0.0001
Isomaltose	19	0.01	−76	<0.0001
Isomaltotriose	23	<0.01	−81	<0.0001
Kestose	7	0.30	−96	<0.0001
Kojibiose	19	0.03	−60	<0.0001
Maltose	15	<0.0001	−94	<0.0001
Maltotriose	23	<0.01	−96	<0.0001
Maltulose	122	<0.0001	−94	<0.0001
Mannose	20	<0.001	−72	<0.0001
Melibiose	−14	0.30	−51	0.31
Sucrose	−17	<0.01	−98	<0.0001
Xylose	33	<0.0001	−69	<0.0001
Total sugars	21	<0.0001	−95	<0.0001
Amino acids and derivatives				
4-Aminobutyrate	27	<0.0001	11	<0.01
Alanine	22	<0.0001	−17	<0.0001
Asparagine	85	<0.0001	−90	<0.0001
Aspartate	32	<0.0001	−85	<0.0001
Betaine	17	<0.0001	−11	<0.0001
Glutamate	35	<0.001	−18	0.13
Glutamine	−63	<0.0001	−56	<0.0001
Histidine	0	0.82	−42	<0.0001
Isoleucine	22	<0.0001	−82	<0.0001
Leucine	18	<0.0001	−86	<0.0001
Lysine	21	<0.0001	−81	<0.0001
Methionine	21	<0.0001	−82	<0.0001
Phenylalanine	23	<0.0001	−72	<0.0001
Proline	20	<0.0001	−6	0.05
Pyroglutamate	127	<0.0001	−6	0.05
Threonine	20	<0.0001	−75	<0.0001
Tryptophan	17	<0.0001	−38	<0.0001
Tyrosine	31	<0.0001	−47	<0.0001
Valine	23	<0.0001	−51	<0.0001
Total amino acids	23	<0.0001	−36	<0.0001
Nucleotides and derivatives				
2′-Deoxyadenosine	−3	0.40	−20	<0.0001
2′-Deoxyguanosine	−21	0.04	−9	0.32
Adenine	31	0.01	0	<0.0001
Adenosine	17	<0.01	−47	<0.0001
ATP	14	0.29	−25	0.29
Cytidine	15	0.30	−12	<0.001
Cytosine	49	0.06	−53	<0.001
dCTP	28	0.04	−40	0.05
Guanosine	5	0.71	6	0.10
Inosine	8	0.12	21	0.21
Oxypurinol	−32	<0.01	162	<0.001
Thymidine	17	<0.0001	7	0.01
Uracil	32	<0.01	59	<0.001
Uridine	16	<0.0001	−11	<0.0001
Total nucleotides	9	0.09	−4	0.03

(continues)

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Table 1. *(Continued)*

	Pre-fermentation percentage change[§]	Pre-fermentation *p*-value	Post-fermentation percentage change[¶]	Post-fermentation *p*-value
Energy-related metabolites				
2-Methylglutarate	−4	0.46	17	<0.01
2-Oxoglutarate	37	<0.01	229	<0.0001
Acetone	72	<0.001	84	<0.001
Ethanol	233	<0.0001	26,111	<0.0001
Fumarate	34	<0.0001	14	<0.01
Lactate	25	<0.0001	105	<0.0001
Malate	147	<0.0001	63	<0.0001
Pyruvate	23	0.22	1322	<0.0001
Succinate	90	<0.0001	989	<0.0001
trans-Aconitate	133	<0.0001	71	<0.0001
Total energy metabolites	148	<0.0001	18,455	<0.0001
Fatty acid-associated metabolites			0	
Acetate	33	<0.0001	−36	<0.0001
Acetoacetate	43	<0.01	−22	0.02
Choline	25	<0.0001	−19	<0.0001
Ethanolamine	38	<0.01	12	0.05
Glycerol	−4	0.23	1090	<0.0001
Glycero-3-phosphocholine	20	<0.0001	3	0.29
Phosphocholine	31	<0.0001	15	0.11
Total fatty acids	14	<0.001	380	<0.0001
Vitamins				
4-Pyridoxate	−2	0.62	70	<0.0001
Niacinamide	−12	0.26	−51	0.29
Nicotinate	59	0.03	−38	<0.0001
Pyridoxine	7	0.03	27	<0.0001
Plant-associated metabolites				
Ferulate	87	<0.01	−58	<0.001
Myrcene	24	<0.01	−49	0.09
Trigonelline	52	<0.0001	12	0.01
Miscellaneous metabolites				
Acetoin	6	0.06	−18	0.23
Formate	44	0.03	−90	<0.0001
Methanol	-43	<0.001	112	0.05
Propylene glycol	17	<0.001	116	0.06

[†] *p*-Values are based on linear mixed-effects models, which tested whether brew metabolite concentrations significantly changed during the brewing process. Models included both late hopped and dry hopped brew samples ($n = 12$) at each time point. Pre-fermentation time points include start of boil, after whirlpool and after yeast pitch. Post-fermentation time points include after yeast pitch, after primary fermentation and after secondary fermentation. ATP, Adenosine triphosphate; dCTP, deoxycytidine triphosphate.

[§] Pre-fermentation percentage change was calculated by the following equation: $\frac{(\text{Metabolite concentration after yeast pitch} - \text{Metabolite concentration at the start of the boil})}{(\text{Metabolite concentration at the start of the boil})} \times 100$

[¶] Post-fermentation percentage change was calculated by the following equation: $\frac{(\text{Metabolite concentration after secondary fermentation} - \text{Metabolite concentration after yeast pitch})}{(\text{Metabolite concentration after yeast pitch})} \times 100$.

maltotriose, maltulose, mannose, melibiose, sucrose and xylose); 19 amino acids and derivatives (4-aminobutyrate, alanine, asparagine, aspartate, betaine, glutamate, glutamine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, pyroglutamate, threonine, tryptophan, tyrosine and valine); 14 nucleotides and derivatives [2′-deoxyadenosine, 2′-deoxyguanosine, adenine, adenosine, adenosine triphosphate, cytidine, cytosine, deoxycytidine triphosphate (dCTP), guanosine, inosine, oxypurinol, thymidine, uracil and uridine]; 10 energy-related metabolites (2-methylglutarate, 2-oxoglutarate, acetone, ethanol, fumarate, lactate, malate, pyruvate, succinate and *trans*-aconitate); seven fatty acid and associated metabolites (acetate, acetoacetate, choline, ethanolamine, glycerol, glycero-3-phosphocholine and phosphocholine); four vitamins (4-pyridoxate, niacinamide, nicotinate and pyridoxine); three plant-associated metabolites (ferulate, myrcene and trigonelline); and four miscellaneous metabolites (acetoin, formate, methanol and propylene glycol). NMR provides information on the atomic structure of molecules and therefore is a useful technique to simultaneously identify and quantify diverse sets of compounds. However, given the micromolar limit of detection of the NMR spectrometer, the metabolites reported in this study reflect a diverse subset of the total beer metabolome.

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The metabolic profile of beer changed throughout the brewing process

To explore the differences between the samples taken at the five time points during the brewing process, the multivariate statistical method unsupervised PCA was used. The resulting scatter plot showed that PC1, which explains 47.1% of the variance, clearly followed the progression of the brews as the wort samples clustered on the right side of the plot, whereas the fermented samples clustered on the left (Fig. 2A). This result is consistent with nutrient-rich wort being fermented by yeast. Further examination of the pre-fermentation samples revealed that the differences between the wort samples were subtle, with the boil causing the largest shift in the metabolic profile along PC1 (40.3% of the variance; Fig. 2B). In addition to sterilizing the wort, reducing wort pH and precipitating proteins, the boil concentrates wort sugars and initiates Maillard reactions between reducing sugars and amino acids *(18)*. Finally, a PCA plot of the post-fermentation samples showed a large degree of overlap, which indicates that there were modest differences in the small molecule composition between these samples (Fig. 2C). It is not surprising that the most dramatic change in metabolites occurred during primary fermentation, when the yeast cells were actively growing.

Molecular details of events during the boil

The most abundant sugars in wort were of course maltose, maltotriose, glucose, sucrose and fructose. However other sugars identified were gentiobiose, isomaltose, isomaltotriose, kojibiose, maltulose, mannose and xylose. Most sugars significantly increased in concentration as a result of concentration during the boil (Table 1). Sucrose increased in concentration from the start of the boil to after the whirlpool, but then decreased as soon as the yeast was pitched (Supporting Information Table 1). In contrast, 1, 6-anhydro-β-D-glucose, kestose and melibiose did not significantly differ in concentration from the start of the boil to post-whirlpool. Melibiose was one of the least abundant sugars and may have decreased during the boil due to Maillard reactions with amino acids.

All of the amino acids measured in this study, with the exception of histidine, significantly increased from the start of the boil to the

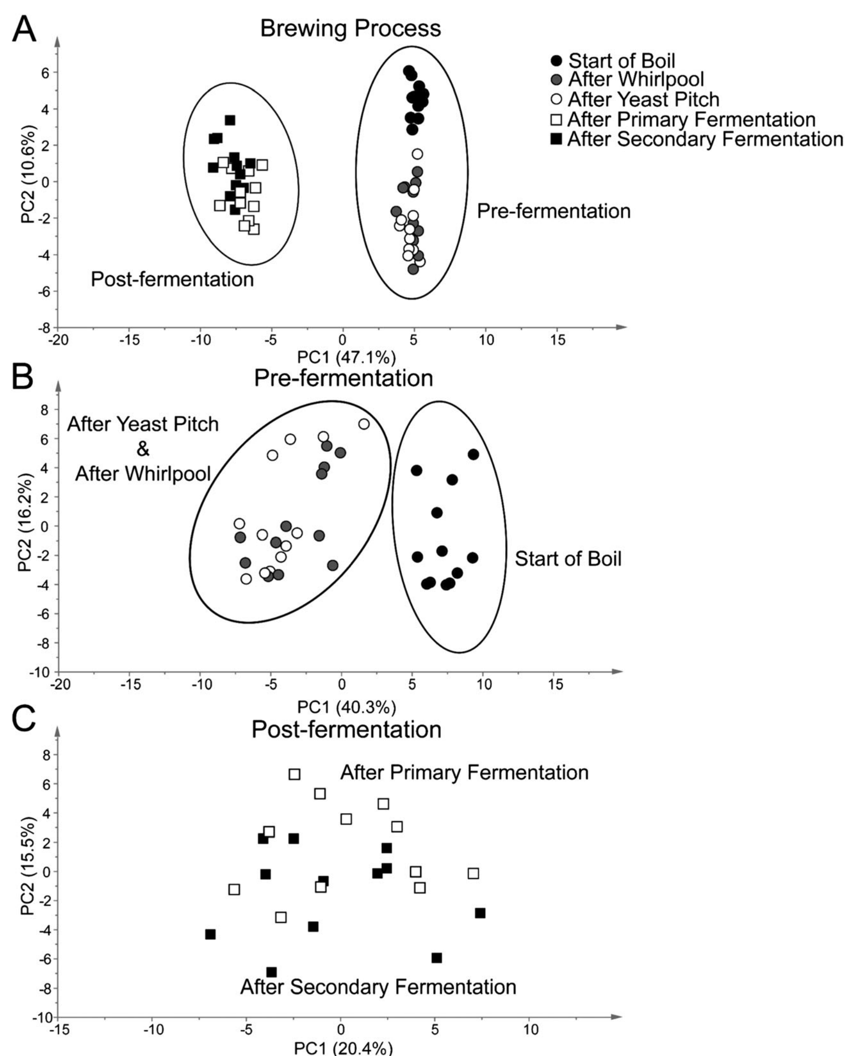

Figure 2. Principal component analysis scatter plots of metabolites throughout the brewing process. (A) PC1 separates the pre-fermentation samples [start of boil (black circles), after whirlpool (grey circles) and after yeast pitch (white circles)] from the post-fermentation samples [after primary fermentation (white squares), after secondary fermentation (black squares)]. (B) Principal component analysis (PCA) analysis of the pre-fermentation samples reveals that PC1 separates the start of the boil from after whirlpool and after yeast pitch. (C) PCA analysis of the post-fermentation samples shows little separation after primary and after secondary fermentation in PC2.

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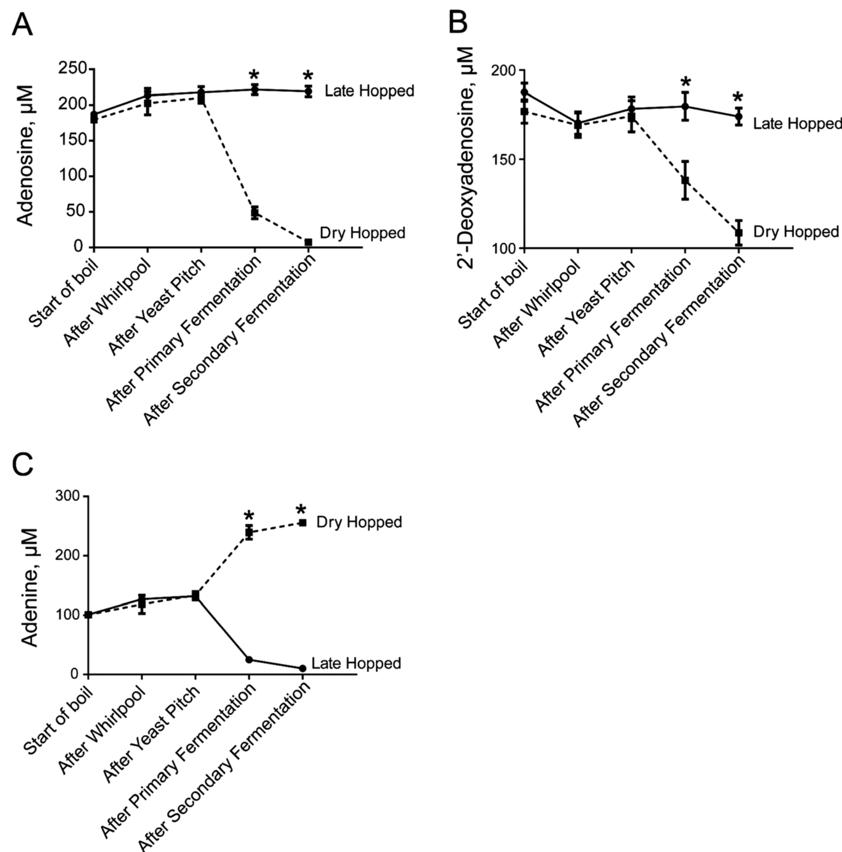

Figure 3. Beer metabolites affected by late vs dry hopping methods. Values are means ± SEM. (A) Adenosine and (B) 2′-deoxyadenosine decreased during primary and secondary fermentation in the dry hopped brews, but remained relatively constant in the late hopped brews. (C) Adenine increased in concentration in the dry hopped beers and decreased in the late hopped beers during fermentation. *Significant difference in mean concentrations ($n = 6$) between late and dry hopped brews at a specific time point, $p < 0.05$.

start of fermentation (Table 1). Similarly, most of the energy related and fatty acid and associated metabolites significantly increased in concentration during the boil. Most of the nucleotides and derivatives also increased during the boil, however, only 2′-deoxyguanosine, adenine, adenosine, dCTP, oxypurinol, thymidine, uracil and uridine were statistically significant.

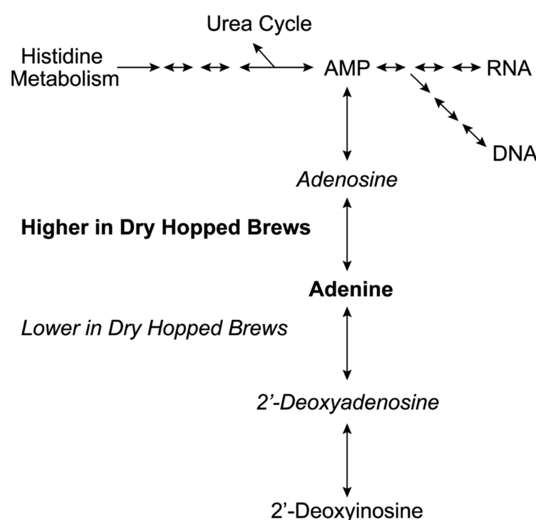

Figure 4. Adenine, adenosine, and 2′-deoxyadenosine in the purine metabolism pathway.

Certain vitamins (nicotinate and pyridoxine) and plant-associated metabolites (ferulate, myrcene and trigonelline) increased in concentration during the boil. Ferulate originates from malt, and is released during the mash most effectively at 45 °C *(19–21)*, which was the mash-in temperature used in this study. Ferulate has been characterized in hop extracts *(22)*; however, analysis of the aqueous fraction of hop extracts in this study revealed no measurable ferulate (data not shown) at the micromolar detection limit of NMR. Myrcene is one of most abundant molecules found in hop oil *(23,24)* and was the only hop aroma compound that was able to be identified in this work. Myrcene was recently reported to be present at 3 μM in beer *(25)*, which is at the detection limit of our technique. Therefore it is not surprising that it was not possible to identify more hop aroma compounds. Overall, all of the classes of metabolites increased in concentration during the boil due to evaporation.

Molecular details of fermentation

Glucose is the preferred carbon source for yeast as its presence represses the uptake of alternative sugars such as maltose *(26)*. Therefore glucose, together with sucrose and fructose, is depleted before yeast metabolizes maltose and maltotriose *(27)*. All of the sugar metabolites significantly decreased in concentration between yeast addition and post-secondary fermentation, with the exception of melibiose. The enzyme α-galactosidase cleaves melibiose into galactose and glucose but the gene encoding it is not found in ale strains such as the California Ale strain used here.

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Yeast assimilates amino acids other than proline under brewery fermentation conditions *(28)*. Proline is not used during fermentation because it requires a mitochondrial oxidase that is not active under anaerobic conditions *(29)*. In this study most of the wort amino acids significantly decreased in concentration during fermentation, with the exception of glutamate, proline and pyroglutamate (Table 1). Glutamate was not significantly utilized by the yeast during fermentation, which may be attributed to the specific behaviour of the ale yeast strain used.

Energy metabolites are by-products of yeast metabolism that also contribute to the organoleptic properties of beer. All of these metabolites significantly increased during fermentation (Table 1). Ethanol, naturally, was the most abundant energy metabolite measured in this study *(30)*. The second most abundant energy metabolite was the organic acid malate. Malate, along with citrate, fumarate and succinate, originates from the malt or from an incomplete citric acid cycle within yeast. These intermediates leak out of yeast cells and increase beer acidity, but help to maintain a neutral intracellular pH *(31)*. In addition to contributing to beer acidity, organic acids each have their own characteristic flavour, aroma and taste *(32–35)*, and may contribute to the perceived sourness of beer *(34,36)*. The yeast strain used, wort composition and fermentation conditions influence the types and abundance of organic acids in beer *(34,37–39)*.

Most of the fatty acid-associated metabolites decreased significantly during fermentation, including acetate, acetoacetate and choline (Table 1). In contrast, glycerol increased significantly from after yeast pitch to after secondary fermentation. This compound is formed during fermentation to maintain intracellular redox balance *(40)* and also plays a vital role in cellular osmoregulation *(41)*. Because of the large amount of glycerol produced during fermentation, the overall fatty acid metabolites increased post-fermentation.

Several metabolites differed between dry and late hopped beers

Because two different hopping methods were used in this study, we wanted to determine whether late or dry hopping affected any of the measured metabolites. Of the 76 compounds identified, only three were significantly affected by the hopping method while taking into account the brewing time point and brew batch – two nucleosides, one nucleobase. 2′-Deoxyadenosine, adenine and adenosine significantly changed over time in both dry and late hopped beers (Table 1), and were significantly affected by either late or dry hopping (hopping method effect: LMM, 2′-deoxyadenosine, $p = 1.0 \times 10^{-2}$; adenine, $p = 1.3 \times 10^{-7}$; adenosine, $p = 1.7 \times 10^{-7}$; Fig. 3). The interaction between time and hopping method was also significant for these metabolites (time-hopping method interaction: LMM, 2′-deoxyadenosine, $p = 2.0 \times 10^{-4}$; adenine, $p = 9.3 \times 10^{-11}$; adenosine, $p = 4.4 \times 10^{-10}$). These compounds are released from the malt during mashing by nucleotidases and nucleosidases, respectively *(42)*. The proportion of adenine, 2′-deoxyadenosine and adenosine has been shown to depend on mash temperatures in wort *(43)*. Adenine was significantly higher in the dry hopped brews than the late hopped brews after primary fermentation ($p = 2.1 \times 10^{-6}$) and secondary fermentation ($p = 2.7 \times 10^{-9}$), while 2′-deoxyadenosine (after primary, $p = 1.1 \times 10^{-2}$, after secondary, $p = 3.2 \times 10^{-5}$) and adenosine (after primary, $p = 2.6 \times 10^{-8}$; after secondary, $p = 3.1 \times 10^{-7}$) were significantly lower in the dry hopped beers at these time points. Adenine has been shown to decrease by 78% in the first 11 h of fermentation using *S. cerevisiae* strains *(43)*, whereas with the lager yeast *Saccharomyces pastorianus*, adenine concentrations dropped by 93% after one day of fermentation *(44)*.

The increase in adenine in the dry hopped brews suggests that hop compounds released during dry hopping affected yeast purine metabolism. Interestingly, both adenosine and 2′-deoxyadenosine are converted to adenine in this pathway (Fig. 4). A decrease in these nucleosides was observed in the dry hopped beers. Together these results suggest that dry hopping may liberate compounds that block adenine uptake, which the yeast cells then compensate for by converting 2′-deoxyadenosine and adenosine into adenine. The elevated adenine probably would not contribute to beer flavour *(45)*. However, redirecting the purine pathway to produce adenine may affect DNA and RNA synthesis, which could have repercussions for the health and vitality of the yeast for subsequent propagations.

In conclusion, it was shown that NMR metabolomics is a powerful technique for tracking the molecular details of the brewing process. In particular, it was shown that hop compounds released during dry hopping may affect yeast purine metabolism.

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