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Magnesium-Accelerated Maillard Reactions Drive Differences in Adjunct and All-Malt Brewing

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ABSTRACT

Magnesium impacts key processes in brewing including yeast metabolism and mash pH but is typically overshadowed in brewing studies, owing to the established centrality of calcium. Using flame atomic absorption spectroscopy (FAAS), a 33.7% average increase in magnesium concentration in commercially available beers brewed with 100% barley malt versus those brewed with adjunct grains was identified. Parallel analysis of brewing grains implicates rice in driving this discrepancy. Given the known catalytic properties of magnesium, its role in beer color development via Maillard chemistry using model systems and wort (unfermented beer) was investigated. Kinetic data were obtained by ultraviolet-visible spectrometry and reaction species were identified by electrospray ionization mass spectrometry. Magnesium accelerated Maillard chemistry in all systems in a dose-dependent manner. These findings reveal a divergence in outcomes of all-malt and adjunct brewing driven by magnesium-catalyzed color formation in the brewhouse. It is proposed that magnesium inhibits water mobility and serves as a Lewis acid catalyst to facilitate Maillard reactions.

KEYWORDS

Brewing; magnesium; Maillard reaction; malting

Introduction

Beer is traditionally brewed with malted barley because of its high enzymatic content, which enables the rapid conversion of starch to fermentable sugars that give rise to alcohol, carbon dioxide, and flavor compounds during fermentation by yeast. As the industry has evolved, brewers have introduced alternative or adjunct grains and derivatives such as corn, rice, sorghum, wheat, oats, corn syrup, and corn starch, which in the absence of malted barley are generally incapable of producing full starch conversion. Brews falling short of sufficient enzyme activity for starch conversion typically require exogenous enzyme addition; therefore, it is technically possible for a beer to contain a greater proportion of adjunct grains than malted barley. While certain of these materials serve cost efficiency motives, each contributes different textures and flavors to beer along with micronutrients required by yeast in fermentation.[1] In relation to flavors in beer, divalent cations such as calcium and magnesium are known to play key roles in pH, mouthfeel and bitterness in beer brewing.[2–6] Grains are the primary source of cations in beer and in the early stages of brewing are milled and mixed with hot water to trigger enzyme activity (Figure 1). Typically, brewers account for calcium shortfalls by adding calcium chloride and calcium sulfate, and the balance of these can impact bitterness and body in the finished product.[3,7–10] Despite the growing consensus among food scientists that magnesium plays an important role in Maillard chemistry,[11–13] gaps remain in our understanding of its impact on beer flavor development. A recent survey of standard strength North American and European beer brands measured magnesium concentrations from 61 to 119 ppm and found it to correlate with potassium concentrations in finished beer, implicating potash fertilizer as a possible vector.[14]

Magnesium concentration is known to have diverse impacts on quality outcomes of brewing.[6] Its impact on bitterness was documented half a century ago in a study demonstrating that at 158 ppm, magnesium drives humulone isomerization 200-fold above background levels in a model solvent system.[2] Likewise, Bastgen et al. recently showed that hop utilization, measure of the heat-induced isomerization of hop acids that serves as the main driver of bitterness in beer, is pushed beyond the 30–40% limit typically observed by brewers by increasing the concentration of magnesium by 40 ppm.[15] Magnesium was shown to promote the activity of proteinases during barley and sorghum germination, key steps in the malting process, indicating a potential parallel role in the low-temperature mashing steps that define many traditional and craft brewing operations.[16] Impacts of magnesium on yeast physiology have been explored.[17,18] Although one group found no effect of modifying the magnesium to calcium ratio on yeast fermentation performance,[19] Walker was able to demonstrate a protective effect of magnesium on yeast viability following ethanol and heat shock.[18] Magnesium was also found to

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counteract the agglomerating effect of calcium on yeast cells.[19]

While these studies illustrate the diversity of impacts that magnesium exerts on various qualities of beer, its effects on food science more generally are far-reaching. The acceleration of Maillard chemistry by magnesium is proposed to occur through reduction of water mobility.[20,21] An increase in the rate of browning (melanoidin pigment formation) using model Maillard reactions (e.g., xylose-glycine system) in the presence of magnesium has been reported.[12] Melanoidins exhibit both antioxidant and pro-oxidant properties, which contribute to the stabilization of color, aroma, flavor and foam.[22–30] While pale beers were assumed to have shorter shelf life than dark beers, given the relatively low concentration of melanoidin present in pale beers,[25,31] recent work indicates increased oxidative potential in beer containing darker malts owing to the heat-triggered release of bound iron during specialty malt processing.[32,33] It stands to reason that these impacts would extend to other foods (nuts, coffee, and meats, for example) that depend on development of Maillard reactions for sensory qualities.

During routine analysis of soluble metals concentration by staff at Phillips Brewing & Malting Co., high magnesium levels in brewhouse and finished beer samples prompted investigation into the source. As results failed to identify a source at the brewing facility or its onsite malting plant, the analysis broadened to include commercially available beers from four continents. The effect of magnesium on color formation during key brewing steps was then investigated by simulating Maillard chemistry under optimized reaction conditions. Model systems combined maltose with amino acids proline, phenylalanine, and leucine, subjected to increasing concentrations of magnesium. The Maillard reaction products were characterized using electrospray ionization mass spectrometry (ESI-MS). Ultraviolet-visible spectrometry (UV-Vis) was used to monitor changes in color during reactions. Results obtained from the model systems provided a benchmark for analysis of brewhouse wort, a grain extract comprising a complex assortment of sugars, amino acids and micronutrients. This work provides an updated industry snapshot of magnesium concentrations in finished beer, links 100% barley malt beers with increased magnesium content relative to their adjunct counterparts and describes chemical mechanisms that may underpin color formation driven by magnesium in both simplified reaction systems and simulated brewhouse chemistry.

Experimental

Chemicals and reagents

Maltose monohydrate (95%) and L-proline (99%) were purchased from Fisher Scientific (Ottawa, ON). L-leucine (⩾ 98%), D-phenylalanine (⩾ 98%), magnesium chloride ($MgCl_2$) and formic acid, HCl (37% w/v), lanthanum oxide (La_2O_3), and magnesium turnings were purchased from Sigma-Aldrich (Oakville, ON, Canada). All chemicals were used as received. Deionized water was obtained from a Millipore Milli-DI water purification system.

Materials and sample preparation

Adjunct grain products, defined as all nonbarley grains, were purchased from Beer Grains Supply Co. (Gatineau, QC, Canada). Raw barley grown in British Columbia, Canada and pale barley malt were supplied by Phillips Brewing & Malting Co. (Victoria, BC, Canada). Brewhouse wort, defined as mash fluid sampled downstream of mash filtration before reaching the boiling kettle (Figure 1), was supplied by Phillips Brewing & Malting Co. from an English style amber ale (16^0P, pH 5.5). Seventeen adjunct beers, defined as having been brewed with any quantity of nonbarley material as source of fermentable sugar, and 21 100% barley beers, defined as beers brewed entirely with barley malt and no adjunct materials, were obtained from local liquor retailers or generously supplied by breweries. Recipe information for the delineation of adjunct beers from 100% barley beers was obtained through direct communication with brewers, from product labels, and through publicly available information from producer websites. Beer samples

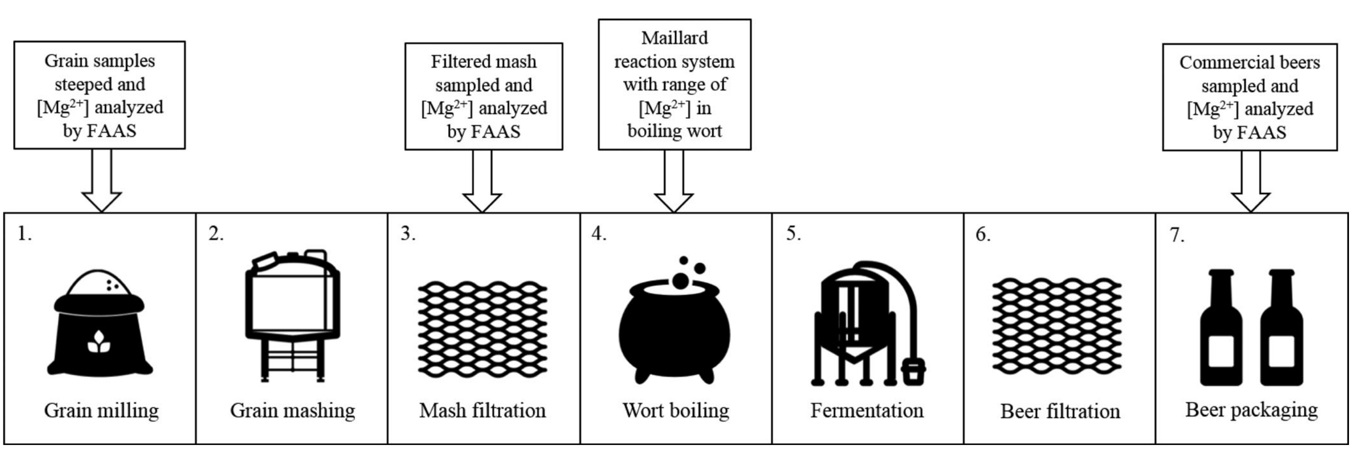

Figure 1. Sampling and experimental events associated with brewing process steps. To generate a nutrient-rich sugar solution suitable fermentation by brewing yeast, grain is milled to a flour consistency (1), mashed with water at approximately 65 °C (2), and filtered to remove grain particulates (3). At this stage the liquid is known as wort, which is boiled after the addition of hops for approximately 1 h (4) prior to chilling and fermentation by yeast (5). Upon completion of fermentation, solids including yeast and hops are removed by filtration (6) to generate finished beer ready for packaging (7). Arrows above processes indicate sampling points in this study.

were prepared for analysis by aseptically opening the package and transferring liquid directly into clean, rinsed glassware for degassing.[34] Grain samples were prepared as described in the American Society of Brewing Chemists Methods of Analysis, Malt-4, for dry basis, fine grind (DBFG),[35] which yields approximately 8°P original gravity for pale malted barley. Following the mashing regime, samples were filtered through fluted filter paper number 313 (VWR, Radnor, PA, U.S.A.). Control water samples were processed to avoid contamination of grain samples with equipment. Grain samples from a single batch were processed in triplicate, while triplicate packaged beer samples were processed independently.

Flame atomic absorption spectroscopy (FAAS)

A Perkin Elmer Analyst 200 flame atomic absorption spectrometer (Waltham, MA, U.S.A.) was used to determine the concentration of magnesium in liquid samples. The instrument was equipped with a multi-element hollow-cathode lamp (Ba, Ca, Sr, Mg) as the radiation source operating at 25 mA, 0.7 nm spectral bandwidth, and a wavelength of 285.2 nm for magnesium detection. The instrumental parameters were set according to the manufacturer's recommendations. The acetylene and air flow rates were set to 2.5 L/min and 10 L/min respectively, and the burner height was adjusted to optimize the maximum stable absorbance signal. After a 5 s read delay, absorbance intensities were recorded using a time-average integration setting where three readings measured over a 3 s integration time were averaged. FAAS measurements were carried out in triplicate.

Calibration for determination of Mg by flame atomic absorption spectroscopy

Calibration was performed according to the ASBC MOA Wort-15 and Beer-38,[35] in the linear range of 0.05 – 1 mg/L magnesium (Supplemental Online Figure S1). Calibration solutions were prepared with 0.1 g magnesium turnings dissolved in a minimum amount of concentrated HCl and volumetrically diluted with deionized water to a 1000 ppm stock solution from which a working solution of 10 ppm magnesium was prepared. Lanthanum oxide was employed as a releasing agent and prepared as a 5% w/v stock solution by hydration with deionized water, dissolution in a minimum amount of concentrated HCl, and dilution with deionized water. Beer and wort samples were diluted 200-fold prior to analysis. Lanthanum oxide stock solution was added to all analyzed standards, samples and blanks as described in ASBC MOA Wort-15 and Beer-38.[35]

Maillard reaction

Model systems of maltose-proline [Mal (10 mmol, 10 eq)/Pro (1 mmol, 1 eq)], maltose-phenylalanine [Mal (10 mmol, 10 eq)/Phe (1 mmol, 1 eq)], maltose-leucine [Mal (10 mmol, 10 e)/Leu (1 mmol, 1 eq)], and maltose-proline-phenylalanine-leucine [Mal (10 mmol, 10 eq)/Pro (1 mmol, 1 eq)/Phe (1 mmol, 1 eq)/

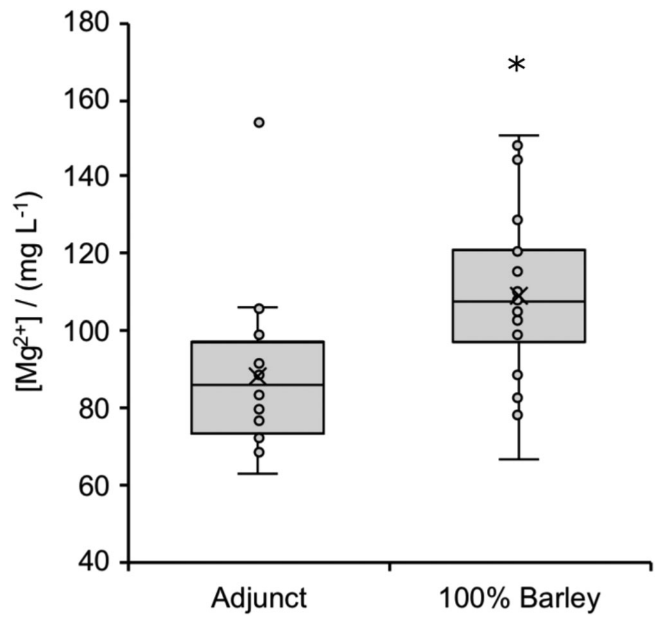

Figure 2. Magnesium content of commercial beers brewed with barley plus adjunct grains (17 beers) or 100% barley (21 beers). The top and bottom of each box represents the first and third quartiles, respectively, with the interior horizontal line representing the median (exclusive) distance between regions. The upper and lower whiskers represent the maximum and minimum, respectively, with calculated outliers positioned outside of the whiskers. The mean is indicated with a cross marker. Star denotes significant difference between groups ($p < 0.0001$).

Leu (1 mmol, 1 eq)] were prepared. Deionized water containing 0 ppm, 2 ppm, 5 ppm, 10 ppm, 20 ppm, 50 ppm, 100 ppm or 200 ppm $MgCl_2$ was added to the model systems and to brewhouse wort. Maltose was chosen as the reducing sugar given its predominance in brewing worts.[36] Amino acids were selected based on the ability of brewing yeast to produce them from exogenous sources, where proline is nonessential, leucine is important and phenylalanine is vital to yeast growth and a healthy fermentation.[37] However, each of these amino acids could also contribute to the flavor or visual quality of the beer.[38,39] The range of magnesium concentration (0 – 200 ppm) was selected to exceed the range of magnesium concentrations measured in commercial beers (Figure 2). All analytes were prepared in triplicate and refluxed at 130 °C for 5 min, 10 min, 15 min, 30 min, 45 min, 60 min, and 105 min; and cooled to room temperature.

UV-Vis spectroscopy

Prior to absorbance measurements, the cooled analytes were gravity filtered, and the filtrates were diluted with deionized water. Spectroscopy was performed using an ASEQ Instruments LR-1 compact spectrometer (version 2.1, Configuration B). Absorbance was measured at 430 nm for all analytes.

ESI-MS

Prior to ESI-MS analysis, the cooled analytes were gravity filtered; the filtrates were diluted to 0.001% v/v with deionized water, and 0.1% v/v formic acid was added to the analytes. The ESI-MS spectra were obtained by means of a quadrupole-time of flight (Q-TOF) SYNAPT G2-Si instrument (Waters Corp., Manchester, U.K.). Instrument parameters were set as follows: capillary voltage 3 kV, cone voltage 20 V, source offset 30 V, source temperature 100 °C, desolvation temperature 200 °C, cone gas flow rate 100 L/h, desolvation gas flow rate 100 L/h, nebuliser 2.5 bar, scan time 3 s. All analytes were fed into the mass spectrometer with a Hamilton GASTIGHT® syringe connected to PEEK tubing and a syringe pump at a flow rate of 10 μL/min. MS/MS experiments were performed with a trap collision energy between 2–20 V. Interpretation of mass spectra was facilitated using chemcalc.org.[40]

Statistical analysis

Magnesium concentrations of adjunct and 100% barley brewed commercially available beers were compared by two-tailed Student's t-test assuming equal variance using Microsoft Excel. Variances were compared using an F-test.

Results and discussion

The present study investigates differences in magnesium concentration between all-malt and adjunct beers, and the effect that this difference may have as brewers approach recipe formulations and process decisions with respect to Maillard product formation.

Magnesium content of commercial beers and associated brewing grains

Magnesium concentration has been shown to decline by approximately 5% from beginning to end of fermentation, owing largely to its sequestration by yeast cells.[41] This indicates that finished beer can serve to approximate starting magnesium concentration, and the authors noted that yeast health is directly proportional to its ability to sequester magnesium from the surrounding medium. The magnesium content of barley, adjunct grains, and commercially available beers was measured by FAAS (Table 1). Beers brewed with 100% barley contained 33% higher magnesium levels than beers brewed with adjunct grains (Figure 2, $p < 0.0001$). The highest magnesium measurement among adjunct beers of 154.4 ppm was in a brand listing barley malt and cane sugar as its sugar sources; nevertheless, pre-isomerized hop products (extract or pellets) could contribute some magnesium to beer.[2] Breweries often add cane sugar in small quantities (generally up to 5% of total fermentable sugars) to help diminish excess density and viscosity contributed by proteins and other grain constituents. In adjunct grains, the concentration of magnesium was generally higher than the magnesium content in barley; however, flaked rice stood out with considerably lower magnesium content than all other grains (Table 2). Note that to compare magnesium measurements between grain and finished beer samples, magnesium concentrations should be corrected by a factor of 1.5, as typical 5% alcohol by volume beer is produced from an approximately 12°P, while grain samples were prepared to 8°P. The variable magnesium content in different barley

Table 1. Concentration of Mg in commercial beer samples determined by FAAS.

Origin		Ingredients[a]	Magnesium (mg/L)[b]	
North America	Canada	B	148.1 ± 0.0025	(0.37)
		B	99.3 ± 0.0012	(0.22)
		B	102.4 ± 0.0035	(0.63)
		B	110.2 ± 0.0104	(1.79)
		B	82.7 ± 0.0032	(0.66)
		B	150.7 ± 0.0052	(0.75)
		B	115.1 ± 0.0045	(0.60)
		OB	144.7 ± 0.0025	(0.40)
		B, C	76.8 ± 0.0021	(0.51)
		B	105.3 ± 0.0040	(0.51)
	U.S.A.	B, C, W	68.8 ± 0.0021	(0.56)
		B	90.7 ± 0.0012	(0.25)
		S, Ri	62.6 ± 0.0021	(0.60)
		B, Ri, C	98.6 ± 0.0038	(0.80)
		B, L	88.9 ± 0.0021	(0.32)
		B, O, W	105.8 ± 0.0044	(0.55)
		B	121.3 ± 0.0017	(0.22)
	Mexico	B, C	92.2 ± 0.0006	(0.13)
		B, C	79.5 ± 0.0029	(0.73)
		B, C	90.0 ± 0.0035	(0.79)
Europe	England	B, W	83.4 ± 0.0015	(0.37)
		OB, H	88.7 ± 0.0006	(0.12)
		B[c]	66.8 ± 0.0021	(0.37)
		B, CS	154.4 ± 0.0056	(0.70)
	Ireland	B[c], W	77.3 ± 0.0006	(0.16)
		B	99.7 ± 0.0040	(0.77)
	Italy	B, C	91.3 ± 0.0012	(0.23)
	Czech	B	116.7 ± 0.0032	(0.52)
		B	120.6 ± 0.0012	(0.21)
	Germany	B	104.1 ± 0.0012	(0.21)
		B	106.5 ± 0.0020	(0.37)
		B	77.8 ± 0.0021	(0.50)
	Netherlands	B	112.3 ± 0.0038	(0.64)
	Austria	B	129.1 ± 0.0035	(0.51)
	Belgium	B, O, W	72.4 ± 0.0044	(0.68)
Asia	Japan	B, St, C, Ri	68.4 ± 0.0025	(0.68)
	China	B, Ri	105.9 ± 0.0021	(0.37)
Oceania	New Zealand	B	107.9 ± 0.0035	(0.62)

FAAS, flame atomic absorption spectroscopy.
[a]B, barley malt; C, corn (in most cases, corn syrup); CS, cane sugar; H, honey; L, lactose (nonfermentable and not considered an adjunct for the purpose of this study); O, oats; OB, organic barley; Ri, rice; S, sorghum; St, corn starch; W, wheat.
[b]Average measured values ($n = 3$) ± standard deviation (SD). Coefficients of variance (CV) in brackets. Mg^{2+} concentration is normalized to 5% alcohol by volume for all products to account for differences in original gravity.
[c]Export recipes may be subject to modifications that were not confirmed by industry representatives.

Table 2. Concentration of Mg determined by FAAS in brewing grains.

	Ingredient[a]	Magnesium (mg/L)	
Barley			
	Pale Malted Barley	27.7 ± 0.0017	(1.10)
	Organic Malted Barley (A)	4.8 ± 0.0012	(2.14)
	Flaked Barley (B)	51.2 ± 0.0012	(0.44)
	Toasted Barley Flakes (A)	76.8 ± 0.0021	(0.55)
	Toasted Barley Whole	31.7 ± 0.0017	(0.98)
	Dark Munich Malt	99.6 ± 0.0012	(0.24)
	Chocolate Malt	42.1 ± 0.0006	(0.26)
Adjunct			
	Malted Wheat (B)	111.5 ± 0.0007	(0.13)
	Malted Rye	109.3 ± 0.0026	(0.50)
	Flaked Oats	16.5 ± 0.0017	(1.62)
	Flaked Oats (B)	87.2 ± 0.0062	(1.46)
	Flaked Rice (C)	8.5 ± 0.0017	(2.44)
	Flaked Wheat (B)	53.2 ± 0.0021	(0.76)
	Flaked Corn (B)	87.6 ± 0.0040	(0.94)
	Flaked Rye (C)	79.9 ± 0.0036	(0.92)

FAAS, flame atomic absorption spectroscopy.
[a]Corresponding letters indicate ingredients obtained from same suppliers.

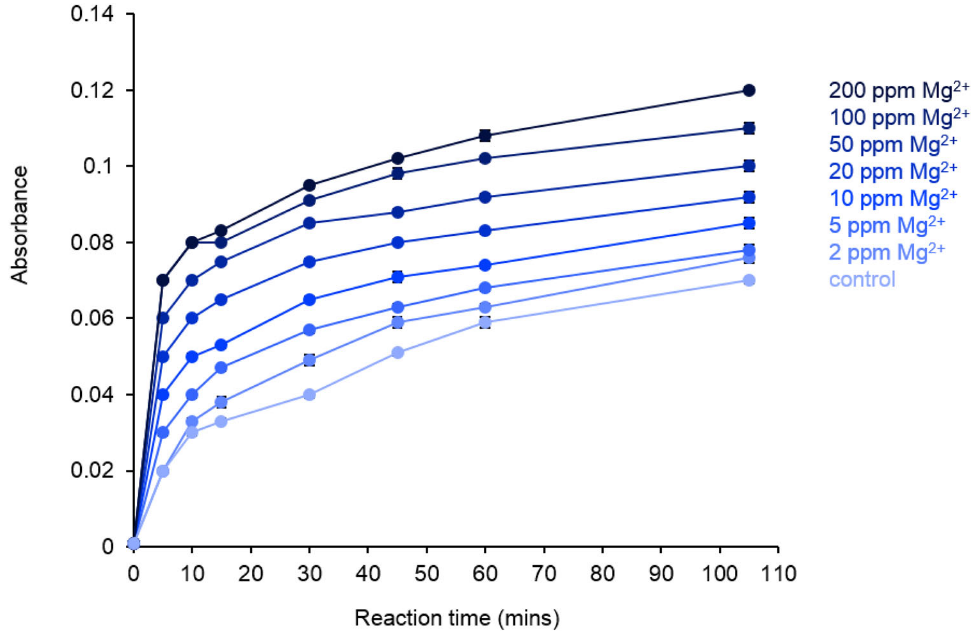

Figure 3. Influence of magnesium on absorbance (430 nm) of a maltose-proline model system.

products, ranging from 4.8 ppm in organically grown pale malt to 99.6 ppm in Dark Munich malt (Table 2), could be attributed to variability in potash fertilizer, as implied by previous correlation analysis,[14] variability in growing conditions, or release of magnesium during heat processing of specialty malts. Interestingly, one of two beers in our testing panel brewed with organic barley malt produced a high magnesium measurement at 144.7 ppm. One potential source of variability among samples is the application of magnesium salts in pre-isomerization of hop extracts and pellets; however, this would represent a small contribution and for the purposes of this study are likely negligible.

The broader discrepancy between all-malt and adjunct beers may be partly explained by greater application of specialty (generally, toasted or roasted) malts in all-malt beers, given the higher magnesium found in specialty malts. It is equally likely that among the beers brewed with adjunct grains, those showing the lowest magnesium contained the highest contributions of rice. Indeed, two of the three lowest magnesium concentrations measured contain rice, at 62.6 and 68.4 ppm, well below the adjunct beer average of 87.9 ppm (Table 1). Given these findings, an experimental evaluation of the impact of magnesium in simulated brewing conditions was then pursued.

Maillard chemistry

UV-Vis spectroscopy was employed to measure appearance of Maillard reaction products at various concentrations using model systems and brewhouse wort, with increasing absorbance at 430 nm indicating the formation of colored Maillard reaction products. All model systems produced a visible yellow color during the reaction, indicating Maillard product formation. Correspondingly, absorbance values increased with reaction time (Figures 3 and 4) The presence of magnesium in the Maillard reaction increased the absorbance values with reference to the control (0 ppm Mg^{2+}), and when magnesium concentration was increased from 2 ppm through 200 ppm, absorbance values increased correspondingly. The trend shown matched results obtained for the maltose-leucine system (Supplemental Online Figure S2). The maltose-phenylalanine system (Figure 4) shows slightly higher absorbance values than the maltose-proline (Figure 3) and maltose-leucine systems (Supplemental Online Figure S2) after 10 min, possibly due to differences in the reactivity of the amino acids as established by Kwak et al.,[42] where phenylalanine was found to be more reactive than proline and leucine (phenylalanine > proline ~ leucine).[42]

In the maltose-proline-phenylalanine-leucine model system (Figure 5), regardless of the presence of magnesium, absorbance values were higher for all reaction times than those shown in single amino acid systems (Figures 3 and 4; Supplemental Online Figure S2). This likely reflects increased complex pigment formation from a greater diversity of Maillard reaction products.

To test these model system findings against simulated brewing conditions, the effect of magnesium concentration on Maillard chemistry at different concentrations in boiling wort was investigated. The wort used had an amber color prior to the reaction, and unlike in model systems, it showed no visible color change in the course of reaction. However, similar to the results for the model systems (Figures 3–5), absorbance values increased across the reaction period for all treatments (Figure 6). This suggests a central position of magnesium relative to other metals in catalyzing Maillard reactions, given that in this system the spiked magnesium was supplementary to the existing metal content of the wort (Supplemental Online Table S1). The higher starting absorbance of wort (0.240) than that measured in the model systems is due to the contribution of

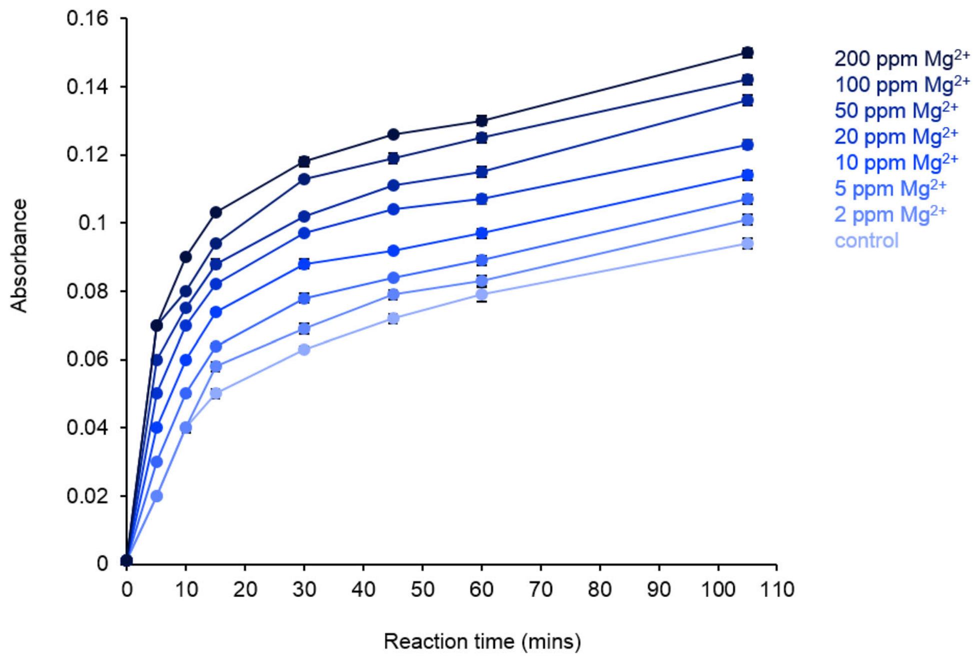

Figure 4. Influence of magnesium on absorbance (430 nm) of a maltose-phenylalanine model system.

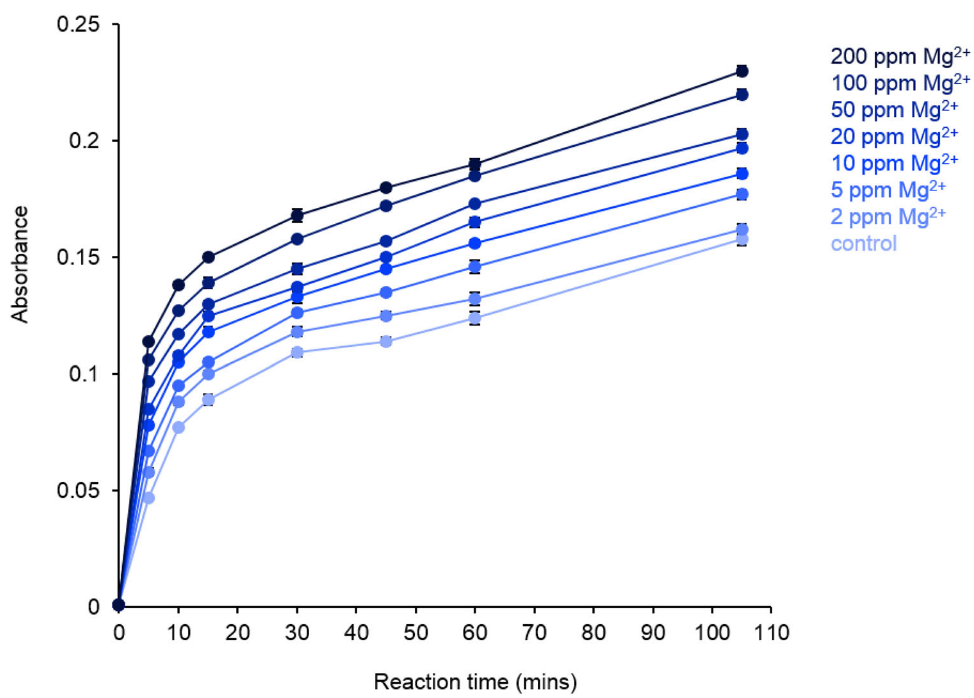

Figure 5. Influence of magnesium on absorbance (430 nm) of a maltose-proline-phenylalanine-leucine model system.

Maillard reaction products from specialty kilned and toasted malts in the brew recipe. The complex mixture of unreacted maltose, other reducing sugars and amino acids in the wort[36,43] is likely responsible for the additional Maillard reactions observed during the boil.

Change in absorbance at 430 nm serves as a proxy for change in concentration of the Maillard reaction products over time, given the direct relationship between absorbance and concentration.[44,45] This suggests that the increased absorbance values in the presence of magnesium is related to an increase in concentration of Maillard reaction products. The influence of magnesium could be explained by the findings of Matiacevich et al., whereby magnesium chloride was employed to decrease water mobility and increase Maillard reaction rates of a model system.[20] In the context of their findings, it is likely that herein as the magnesium concentration increased from 2 ppm through 200 ppm, the water mobility decreased correspondingly, and accelerated the Maillard reactions in all systems. However, for the wort system, other salts present could have possibly contributed

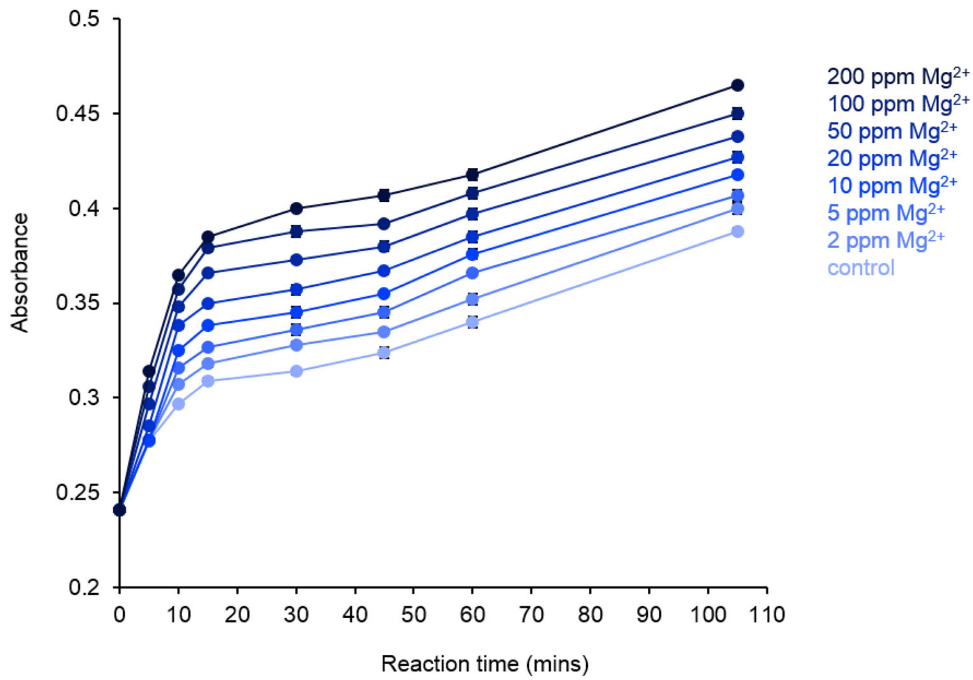

Figure 6. Representation of the influence of Mg^{2+} on the change of absorbance over time of wort.

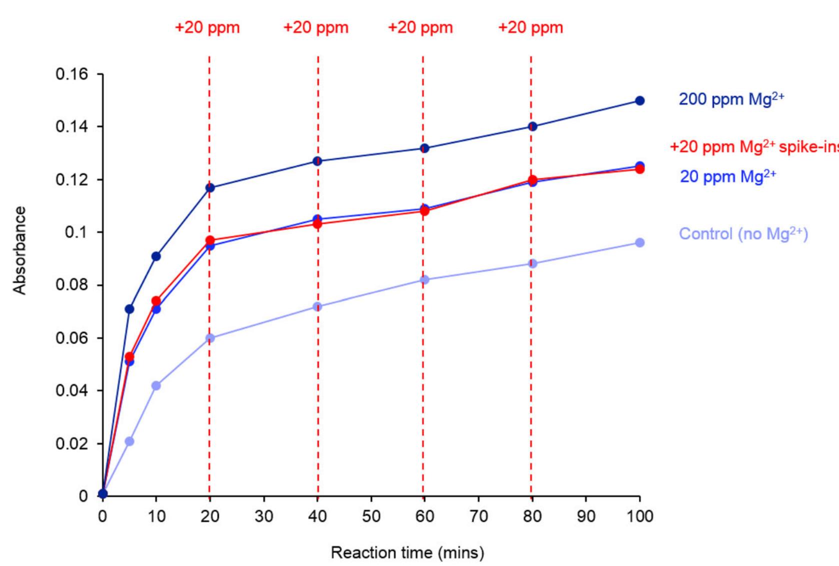

Figure 7. Representation of the influence of Mg^{2+} on the change of absorbance over time on the reaction between maltose and phenylalanine. Four experiments are represented here: no added Mg^{2+}, 20 ppm added Mg^{2+}, 200 ppm added Mg^{2+}, and 20 ppm Mg^{2+} repeatedly spiked at 0, 20, 40, 60, and 80 min.

to the decrease in water mobility (diffusion of water molecules).[5] In addition, the presence of magnesium ions in the Maillard reaction could facilitate nucleophilic addition reactions between the carbonyl groups and amino groups after Lewis acid activation of the carbonyl group,[46] given that Maillard chemistry occurs between carbonyl groups of reducing sugars and amino groups of amino acids, peptides or proteins.[43,47–49]

In all reactions studied—model systems and wort alike—the highest rate occurred early, followed by a change in slope to a value that was essentially uniform for all magnesium concentrations. Nonetheless, the reaction rate at early stages of the reaction is substantial, giving the Maillard reaction a substantial kickstart. One possible explanation is that magnesium ions are effectively sequestered by chelating agents generated through Maillard reactions or already present in the mixture,[50–52] and the magnesium ions lose efficacy as rate accelerators. This was modelled by adding 20 ppm magnesium at the start of the reaction and adding repeat aliquots of 20 ppm magnesium at 20, 40, 60, and 80 min (Figure 7). Additions at 20 min and onward had no effect on absorbance measurements, suggesting that deactivating agents are present in sufficient quantity to render additional magnesium ineffective. Another possibility is that irreversible, magnesium-catalyzed reactions occur quickly and consume one or more key species, limiting the system to reactions that occur at magnesium-independent rates.

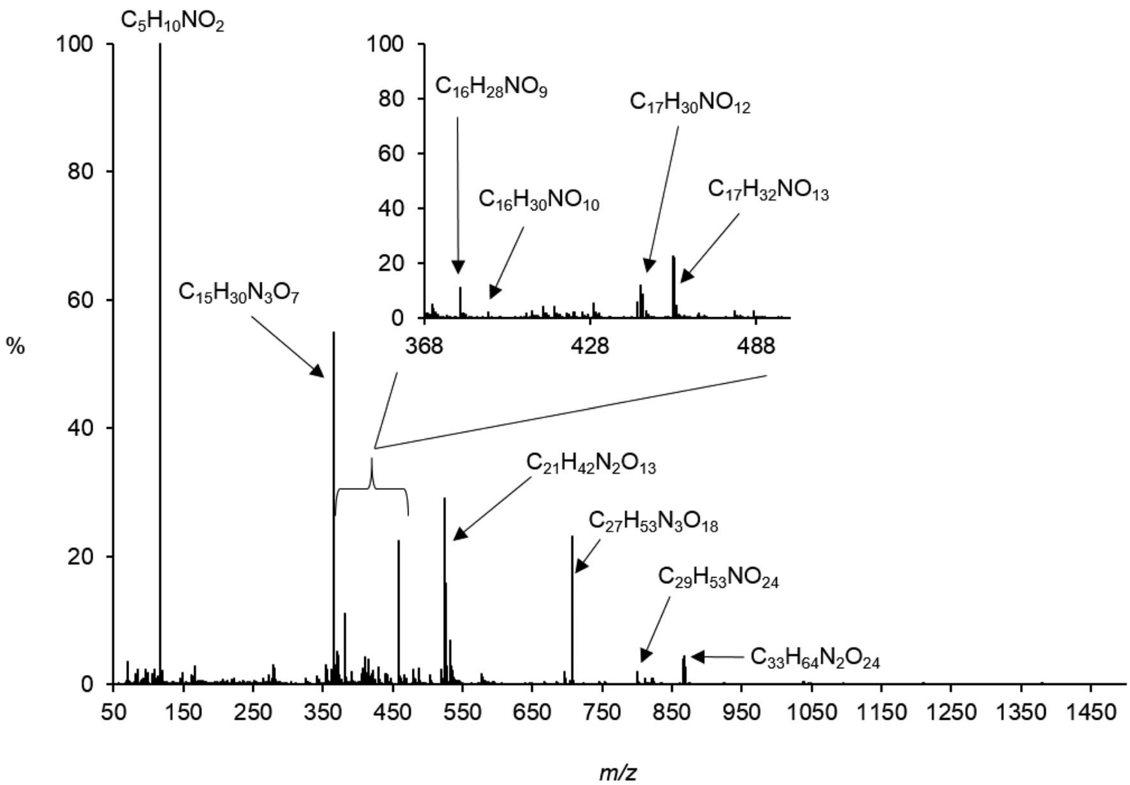

Figure 8. Positive ion mode ESI-MS of the Maillard reaction species of a maltose-proline system after reflux at 130 °C for 1 h. Inset: expansion of the *m/z* 368–488 range.

Characterization of reaction products

Investigation by ESI-MS revealed Maillard reaction species in the positive ion mode in a Mal/Pro system (Figure 8). The reaction products were identified as low molecular weight species. The base peak in Figure 8 represents a protonated proline species at *m/z* 116.1167 ($C_5H_{10}NO_2$). A reaction scheme leading to the formation of the observed ions is shown in Figure 9. The other model systems followed a similar reaction scheme (Supplemental Online Figures S4 and S5).

The Maillard reaction proceeded through a nucleophilic addition between the carbonyl group of the reducing sugar and the amino group of the amino acid to produce a Schiff base, which rearranged to form an Amadori product,[47,53–56] seen at *m/z* 440.0258 ($C_{17}H_{30}NO_{12}$) (Figure 8). The reaction continued via decarboxylation and dehydration to yield products at *m/z* 396.0127 ($C_{16}H_{30}NO_{10}$) and *m/z* 378.0646 ($C_{16}H_{28}NO_9$), respectively. The Amadori product further reacted with maltose to produce the species at *m/z* 799.5289 ($C_{29}H_{53}NO_{24}$). The species at *m/z* 364.6972 ($C_{15}H_{30}N_3O_7$) was identified as an aggregate of proline and water; this aggregate ion formed a cluster with maltose, observed at *m/z* 706.9716 ($C_{27}H_{53}N_3O_{18}$). An aggregate ion comprising the Amadori product and water was seen at *m/z* 458.0182 ($C_{17}H_{32}NO_{13}$). A cluster of the decarboxylated product at *m/z* 396.0127 ($C_{16}H_{30}NO_{10}$) and water was identified at *m/z* 414.0393 ($C_{16}H_{33}NO_{11}$). This species also formed a cluster with proline, and the aggregate ion was observed at *m/z* 529.6497 ($C_{21}H_{42}N_2O_{13}$). In addition, the observed ion at *m/z* 870.4815 ($C_{33}H_{64}N_2O_{24}$) represented an aggregate ion of maltose and the species at *m/z* 529.6497 ($C_{21}H_{42}N_2O_{13}$). To obtain further information on the reaction products, product ion scan experiments (ESI-MS/MS) were carried out. For example, the Amadori product at *m/z* 440.0258 ($C_{17}H_{30}NO_{12}$) produced a product ion at *m/z* 422.0425 ($C_{17}H_{28}NO_{11}$) by losing 18 Da (H_2O) (Supplemental Online Figure S6). This implied that the Amadori product readily undergoes dehydration.

Furthermore, no difference was observed in mass spectra for the various reaction times employed at reflux (5 min through 105 min); meanwhile, varying the magnesium concentration did not change the distribution of species in the mass spectra of all systems (Supplemental Online Figure S10 and S19). It is likely that any high molecular weight species formed were less surface active, thereby exhibiting poor ESI response. Previous research by the authors of this study reported that such behavior can occur depending on the environment of the analytes under study.[57] Also, a significant difference in the mass spectra could have been achieved for longer reaction times (10 h or more) as reported by Hemmler et al.[58] However, not more than 105 min was employed in order to simulate real world effects, whereby wort is typically boiled for 45–90 min before yeast fermentation.[59]

Conclusions

Addition of magnesium at levels typically found in barley facilitates Maillard reactions between sugars and amino acids by acting as a Lewis acid catalyst. Adjunct grains, in particular rice, contribute less magnesium than barley to participate in these color and flavor imparting reactions, highlighting a key distinction between 100% barley (or “all-malt”) and

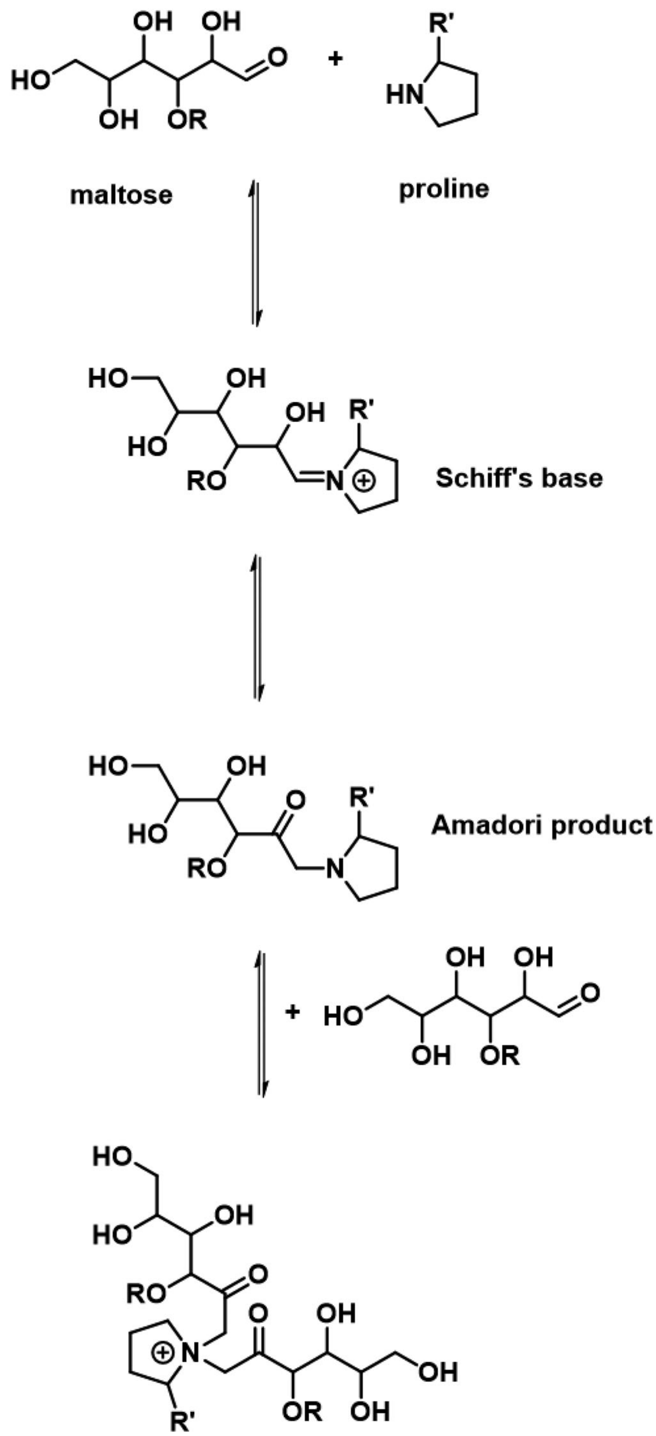

Figure 9. Maillard reaction scheme of a maltose-proline system.

adjunct brewing. Low molecular weight Maillard reaction species were characterized by ESI-MS. Examination of the reaction by UV-Vis spectroscopy showed that the catalytic effect of magnesium is significant but short-lived, persisting for about 20 min, at which point all reactions proceeded at the same rate regardless of magnesium supplementation. Further additions of magnesium at later stages in the reaction also had no accelerating effect on the rate of reaction. The results suggest that a contributing factor to the color and flavor of beer is the concentration of magnesium present at the start of the wort boil and that monitoring magnesium offers improved control over Maillard product formation in adjunct beers for flavor and color development. Future work will characterize in greater detail the loss of magnesium catalyst efficacy as the reaction proceeds and to investigate other metal-driven impacts of grain recipe variations in brewing that contribute to differences in sensory outcomes.

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