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Effect of Steeping Regime on Barley Malt Quality and Its Impacts on Breeding Program Selection

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ABSTRACT
When making malt, the endosperm is hydrated during steeping to make stored starch available for extraction. Differences in steep regime impact malt quality. Differences in malt quality results between the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) and Montana State University (MSU) malt quality laboratories are primarily due to differences in steep regime and are reported herein. Evidence suggests that differences in steep regime in this study were primarily due to length of water immersion versus air rests, rather than other differences (e.g., temperature of steep, sorting of seed, or length of germination). The difference in steep regime caused a difference in the level of endosperm modification. To confirm this finding, three different steep regimes on seven different lines were tested and it was found that the impact on quality varied depending on the trait and in some cases on the genotype. The steep regime was found to have affected both moisture uptake and quality of hydration. Finally, the implications of these findings on malt quality analysis and breeding for malt quality are discussed.

KEYWORDS
Barley; breeding; malt; quality; steep regime

Introduction

Malting innovation combined with breeding of modern barley varieties has shaped major advances in the malting process over the last century, shortening processing times while increasing malt volumes. Although the direct impact of these practices has led to greater output, the details of how process changes impact malt quality are not fully understood. The evolution of the malting and brewing industry, with more varied quality requirements for malt barley, emphasizes the importance of understanding the impact of steeping practices on malt quality. The brewing industry has recently shifted with a marked increase of craft all-malt brewing, as opposed to the long-standing macro-breweries, which can make use of adjuncts in their production. Along with this shift has come a need for malts with different performance profiles. Craft brewers are looking for malts with lower protein and free amino nitrogen (FAN), more moderate soluble protein/total protein (S/T) and enzyme potential (α-amylase and Diastatic Power), and low β-glucan. Malt modification is a result of the interaction between malt regime and variety. Therefore, understanding the impact of steep regime on malt quality is an important goal, especially as the industry evolves.

To select for varieties with adequate malt quality, breeders submit grain for malt quality testing. United States Department of Agriculture-Agricultural Research Service (USDA-ARS) annually provides 78,000–85,000 data points to 16 breeding programs across the United States. Recently, Montana State University (MSU) developed a malt quality laboratory to provide data points to the MSU barley breeding program, and to support craft maltsters and brewers. Results from both laboratories are used to select varieties with the best quality. Unlike commercial malt houses, where malt process conditions are perfected for a given grain sample, laboratories running breeding samples must test a large number of grain samples under conditions that consistently discriminate the potential malt quality of each line. Malt quality is a complicated set of traits determined not only by growing conditions, genetics, and malt processing individually, but also by interactions between those conditions. Since variations in malt process will favor a specific genetic makeup, it is critical for the breeder to understand the interplay between genetics and malt process.

Hydration of the endosperm is of critical importance to malt quality. Hydration is the trigger for germination that causes a cascade of events, activating or promoting the production of enzymes, such as those responsible for protein and β-glucan degradation, and starch mobilization. While temperature controls enzyme synthesis and activity, the amount of free water enables mobility and action of the enzymes.[1] Some enzymes exist within the mature barley kernel (such as β-amylase) and are activated or increased during germination; while others (such as α-amylase, β-glucanase, endopeptidase) develop during germination.[2] Conversion of protein into soluble protein and FAN

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requires moisture, because necessary enzyme activities (transaminases and peptidases)[3] and degradation products[4] increase with water content. Peptidase activity is also increased by aeration during steeping.[5] The breakdown of β-glucan, a major component of cell walls in the barley endosperm,[6] is highly dependent on hydration, temperature, and length of germination.[3] High levels of unmodified β-glucan deter movement and subsequent action of enzymes such as α and β-amylases that need to diffuse and access their substrates.[4, 7] Additionally, hydration may be increased by the release of bound water when soluble β-glucans are degraded.[8] Degradation of β-glucan begins during steeping [9] and is first observed in the crushed cell layer between the scutellum and the endosperm.[10]

The evolution of malt processing specific to steeping practices are briefly described and moisture and hydration patterns within barley grains are reviewed to inform the current research, which dissects the impact of several malting regimes on malt quality.

The malting community has explored many malting methods with varying popularity in different regions and periods (reviewed in Briggs,[11] Briggs et al.,[12] and Brookes et al.[13]) Within these methodologies, variations in steep regimes primarily involve different timing of steep and air rests. For example, in the longstanding tradition of *immersion steeping*, grain is left continuously under water, potentially with periodic water replacement, until the desired percentage of moisture is reached.[13] Steeping in running water mimics the approach used by early Norwegian farmers, who immersed partly filled sacks of grain in streams, removing them at intervals to drain and rest.[11] On the other hand, *flush steeping* involves recurrent steeps and rests in a repeating time pattern (i.e., 4 hour steep/4 hour rest/4 hour steep, etc.).[13] *Spray steeping* has an initial immersion steep that brings the barley to 35% moisture, followed by an air rest and water sprayed intermittently on the barley until the desired percentage of moisture is reached.[14] Finally, *multi steeping* consists of varied steep and rest timing across roughly 48 h, bringing the grain to a final moisture of ~45%.[13] Driving forces behind the evolution of these methods include: 1) the practice of cleaning the barley via removal of effluent water, to ensure germination is not impeded by dust, bacteria, mold or dissolved materials from the grain's surface; 2) the realization that water sensitivity is overcome with an air rest after grain has reached 35–37% moisture, and that subsequent immersions could be used to obtain an ideal final moisture content of 41–46%;[12] and 3) the recognized benefits of air rests that dissipate surface water film and increase the speed of germination.[15]

The various steeping methods have advantages and disadvantages. For example, a *single steep* is less complicated and saves water; however, it is slower and provides less control over speed of hydration. Multiple studies have indicated that grain moisture increases more rapidly during air rests than under continual immersion. Briggs et al.[12] reported that the removal of surface moisture increased germination, similar to removal of the barley husk. Kirsop et al.[15] showed that removal of steep water by sample centrifugation consistently increased germination rates during malting. Holmberg et al.[16] successfully demonstrated a model for water uptake during steeping, where differences between inner and outer grain moisture levels impact the rate of water uptake, with water transfer rates increasing during air rests, as compared to wet steep phases. Ultimately widespread adoption of air rests has occurred and is a mark of many modern malting regimes.[11] Three immersions with intervening air rests are common in conical steep vessels,[17] but specific lengths of these phases can vary among varieties, seasons, and maltsters.[18]

Aided by a long history of research into cereal kernel structure, reviewed by O'Brien,[19] several studies have elucidated the relationship between kernel physical structure and hydration.([15, 20–28]) In *immersion* type regimes, three phases of water uptake have been proposed. In Phase 1, imbibition occurs roughly in the first 10 h and is marked by rapid water uptake. Factors impacting this initial hydration are seed morphology, permeability of the seed coat, and the availability of water.[29] This initial water absorption is chiefly a physical process that is focused through the micropyle into the endosperm. The directed pattern of water uptake is likely due to the grains' pericarp[22, 23, 30] and testa,[23, 31] which are layers that inhibit water penetration. Water entry is focused because the structure of the testa is modified in the region of the micropyle, and the pericarp is missing around the embryo. As early as 1896, Day[20] reported that the embryo of 24 h steeped grains had at least twice the moisture content compared to the endosperm, marking early recognition of the embryo's importance in water uptake. Multiple studies (Brown;[26] Buchinger;[31] Chapon;[30] Collins;[22] Dickson and Burkhart[25]) have indicated that the embryo itself is responsible for this preferential uptake. During Phase 1, the scutellum becomes hydrated and metabolically active,[32] while the endosperm absorbs water slowly and to a low degree. Phase 2, lasting from roughly 10 h through about 35 h of immersion steeping, is marked by a strong reduction in the rate of moisture uptake. During this phase, the embryo's metabolic activity increases, making use of available sugars and initiating the onset of growth via promotion by active compounds such as gibberellins.[13] The endosperm continues a slow but steady rate of hydration. In Phase 3, starting around 35 h, the visible onset of germination occurs and water is taken up at a steady linear rate, which correlates with metabolism.[33] As in Phase 2, water uptake only continues if there is a concurrent supply of nutrients from the endosperm, requiring an adequate supply of oxygen and appropriate temperature. Barley reaches optimal moisture after approximately 50 h of continuous steeping.[21]

Modern techniques, including physical dissection of seed tissues,[15] autoradiography,[27] and x-ray microanalysis[28] have further supported the three phases of water distribution in barley during malting. More recently, Yin et al.[34] and McEntyre et al.[8] utilized magnetic resonance to evaluate hydration patterns. McEntyre et al. simplified the moisture uptake curve to two phases: water uptake by the outer grain layers and internal redistribution. Although both processes

Table 1. Comparison summary of differences between the MSU and USDA malting regimes.

Parameter	MSU	USDA
Equipment	CLP Steep/germ and kiln[a]	Custom system by Standard Industries[b]
Tank capacity	15 samples, 1 control	36 samples
Plumpness	Over 5.5/64	Over 5.5/64
Steeping	48 h with multi-steep at 15 °C	Variable based on kernel weight, 24–48 h[c]
	10 h steep – 15 °C	4 h steep – 16 °C
	18 h rest – 15 °C	4 h rest – 18 °C
	6 h steep – 15 °C	4 h steep – 16 °C
	10 h rest – 15 °C	4 h rest – 18 °C
	4 h steep – 15 °C	4 h steep – 16 °C
		4 h rest – 18 °C
		4 h steep – 16 °C
		4 h rest – 18 °C
		4 h steep – 16 °C
		4 h rest – 18 °C
		4 h steep – 16 °C
		4 h rest – 18 °C
Steep out target moisture	45%	45%
Germination	96 h @ 15 °C	120 h @ 17 °C
Humidity	>98%	>98%
Agitation	5 min in every 30	3 min in every 30
Aeration	1 min in every 10	None
Kiln	24 h	24 h
	12 h @60 °C	10 h @ 49 °C
	6 h @65 °C	4 h @ 54 °C
	2 h @ 75 °C	3 h @ 60 °C
	4 h @85 °C	2 h @ 68 °C
		3 h @ 85 °C
		with 30 min temperature ramps
Total time	~7 days	~6–8 days, depending on kernel weight

MSU = Montana State University; USDA = United States Department of Agriculture.
[a]http://www.customlab.co.uk/products/sgk-combination-steep-germinator-and-kiln-vessel/.
[b]Standard Industries (Fargo, ND) patterned an apparatus described by Anderson in 1937[41] and Anderson and Mereclith in 1940[40].
[c]1000 corn weight is measured (in grams) for each variety and steep time (in h) is determined by subtracting 6 from the result.

occur concurrently, the internal redistribution of water is faster during air rests.[16] McEntyre et al.[8] also found that the crushed cells (a collection of cells between the embryo and endosperm and projecting into the endosperm) showed higher water concentration than surrounding endosperm tissues. The crushed cell tissues may have a role in water distribution into surrounding areas.[35]

As the metabolic processes occurring during water uptake are interrelated, the phases outlined during immersion steeping are not clearly distinct. The addition of air rests, as occurs in many modern steep protocols, has complicated effects on metabolic processes. For example, oxygen uptake by the seed is rapid, with most of the oxygen depleted from the steep liquid within 1 h of immersion of the grain;[36] however, both oxygen consumption and carbon dioxide emission rise in the liquid if it is continually aerated.[37] This rise in respiration is likely correlated with the rise in the rate of moisture uptake characterized by studies finding air rests increase speed of hydration (Briggs[11]; Kirsop et al.[15]; Holmberg et al.[16]). Additionally, the work of van Somere[38] showed that respiration rises to a maximum during the first 6 h after moistening and falls after 24–48 h of steeping. Steep regimes incorporating air rests invigorate the respiratory rate, allowing increased rates of metabolism and grain moisture uptake.

To summarize, the steeping method, including the amount of aeration, time of immersion, and temperature impacts the rate and quality of grain hydration and, therefore, will directly impact grain modification and final malt quality traits. Genetic makeup not only impacts malt quality, but also interacts with the malt process to change malt quality. Understanding the influence of different malting regimes used during the selection of varieties is critically important. Therefore, the impact of three steep regimes on final malt quality to better understand malt quality selection was examined.

Experimental

Barley material

Barley (*Hordeum vulgare* L.) varieties and lines were grown during 2016 and 2017 at the MSU Post Research Farm, Bozeman, MT, U.S.A. (latitude 45.41° N, longitude 111.00° W, elevation 1455 m) with plots consisting of 3 m rows seeded at 60 seeds m^{-1} for rain-fed trials and 90 seeds m^{-1} for irrigated trials in three replications with a randomized complete block design. Grain samples were pooled across replications for malting. Specific lines used in each experiment were as follows: 1) Initial comparison between USDA-ARS and MSU included Hockett, Odyssey, Metcalfe, Genesis, Synergy, Growler, Balster, Genie, Harrington, and Craft. 2) Comparison of within and between lab differences included Eslick, Lewis, Amsterdam, Hockett, Craft, MT124071, MT124148, MT124128, MT124601, MT090182, and MT090190. 3) Comparison of three malt regimes on a subset of seven lines included MT124601, Craft, MT124148, Hockett, Eslick, MT124071, and Odyssey.

Malting control

In 2017, MSU required a large volume of grain from a single source (environment) to determine consistency of malting across batches. Limagrain (Ft. Collins, CO, U.S.A.) kindly provided Odyssey barley to fill that need. With careful monitoring, Odyssey has a consistent malt profile making it a good choice for quality control. The USDA lab similarly uses the variety Conrad as a malting control.

Malting regimes

MSU

Malting was performed at MSU with the use of Custom Laboratory Products (Milton Keynes, U.K.) steep/germ tanks and a kiln (Table 1). Samples of barley (120 g), plumped over a 5.5/64" sieve, were loaded into round steeping cages (19.05 cm diam. x 12.7 cm tall), with four quadrants. Each steep tank accommodated four cages, allowing 16 samples to be malted simultaneously. Early in the development of the MSU Malt Quality Lab, steep regimes were tested to develop a method that would consistently bring the Odyssey control line, and on average other named varieties common to Montana, to 45% moisture at steep out. Included was a control line (Odyssey) in every steep run to ensure uniformity of steeping between runs. The developed steeping regime consisted of 48 h, in which grain was continually maintained at 15 °C and underwent a multi-steep program with a steep/rest pattern of 10 h steep, 18 h rest, 6 h steep, 10 h rest, and 4 h steep, with an average target moisture of 45%. Germination consisted of 96 h at a constant 15 °C. Throughout steeping and germination, humidity was maintained at >98% and agitation consisted of 5 min of cage turning at 0.61 RPM in every 30 min period. Aeration with moist air through the grain occurred for 1 out of every 10 min. After germination, samples were kilned via forced air in the CLP kiln over a 24 h period consisting of 12 h at 60 °C, 6 h at 65 °C, 2 h at 75 °C, and 4 h at 85 °C. Upon completion, samples contained on average 4.0% moisture and were manually de-culmed.

USDA Madison, WI, U.S.A. Laboratory

Samples malted at the USDA Madison, WI Laboratory were treated under the "Traditional Malting System"[39] (Table 1). Experimental malting equipment was custom fabricated by Standard Industries (Fargo, ND, U.S.A.), patterned on apparatuses described in.[40, 41] Cuboidal stainless-steel steeping cans had screen mesh bottoms and were 15.24 cm tall x 8.89 cm long x 8.89 cm wide. Cylindrical germination cans with tight-fitting lids and double rows of ten 0.32 cm diameter holes around the circumference were 14.61 cm diameter x 10.16 cm tall; and cylindrical kiln cans with mesh bottoms were 10.8 cm diameter x 16.5 cm tall. Samples were steeped for 24-48 h, dependent on kernel weight. Kernel weight was measured for each sample and a factor from 6 to 10 was subtracted from the result to determine steeping time for each sample, depending on barley origin. For example, a sample with a kernel weight of 46, from Montana, would be steeped for 40 h. The steeping regime included repeating 4 h immersions (16 °C) and 4 h air rests (18 °C) for the total steep time. Steep out target moisture was 45%. Samples were germinated for 120 h at 17 °C and >98% humidity, with turning 3 min of every 30 min. Moisture percentage was checked/adjusted to 45% once during germination. Kilning consisted of hot air blown through the samples over 24 h in a slow, controlled manner with a finished malt moisture of ~4.0%. The following stages were used: 49 °C for 10 h, 54 °C for 4 h, 60 °C for 3 h, 68 °C for 2 h, and 85 °C for 3 h, with 30 min temperature ramps between all but the first plateau.

Flush, multi-steep, and immersion regimes; MSU

To evaluate the differences in malt regime, barley samples were malted at MSU under three different steeping regimes. The first mimicked as closely as possible the USDA Madison, WI, U.S.A. program, a flush regime, utilizing 4-h intervals of immersions/rests and was designated as "Flush." This regime included: 1) sorting grain based on kernel weight and accordingly altering the steep length (subtracting a factor of 6 as previously described and in accordance to the USDA practices); 2) programming consecutive 4-h steeps and rests; 3) utilizing the respective 16 °C and 18 °C temperatures during steeping and 17 °C during germination; and 4) elongating the germination time to 120 h. The second regime utilized the standard MSU protocol which consists of multisteep phases and is designated as "Multi-Steep" and the third regime utilized a continual immersion steep, designated as "Immersion." The Immersion program consisted of a 48-h steep at 15 °C followed by 120-h germination at 15 °C, with all other settings matching the standard MSU regime.

Testing methods

Malt quality analysis

Testing by both the MSU and USDA laboratories followed the guidelines set forth by the American Society of Brewing Chemists (ASBC) with only minor modification to fit the work flow of each laboratory, as detailed in the following section.

MSU

Amylolytic Enzymes (diastatic power and α-amylase): Extracts were prepared from 12.5 g of grist in 250 mL of 0.5% NaCl. The samples were immersed in a 20 °C water bath for 30 min, with continual mixing followed by 30 min of filtration with return of the first 25 mL of filtrate. Validated analysis of diastatic power was performed in accordance with ASBC Malt-6 and the Gallery operating protocol "Diastatic Power," version 4–5/2016 and the Thermo Scientific D-Glucose reagent kit, product code: 984304. Validated analysis of α-amylase was performed in accordance with ASBC Malt-7 and the Gallery operating protocol "Alpha-Amylase in Malt, version 1–06/2016 (Thermo Fisher Scientific Vantaa, Finland). These results

were validated using the ASBC Lab Proficiency Collaborative.

Wort: Extraction was performed via ASBC Malt-4 with volumes reduced by 20% and 320 mL total of ultra-purified water was added to 40 g of grist for each sample. An initial addition of 160 mL pure water heated to 45 °C was combined with each 40 g sample, followed by 30 min of mashing, then samples were ramped to 70˚C at 1 °C/min. Upon reaching 70 °C, 80 mL of pre-heated pure water was added and 70 °C was maintained for 60 min. Mashing with continual stirring was completed in a 16-cup, benchtop mash bath (IEC, Sydney, Australia). Samples were cooled to room temperature, brought to a final volume of 360 g (40 g grist:320 mL water) and filtered to 160 mL into 500-mL Erlenmeyer flasks, with return of the first 80 mL of filtrate. Filtration time was recorded as the time from initial pour to accumulation of 160 mL. Densities of the extracts (° Plato) were determined on an Anton Paar DMA5000 densitometer. Samples of 1 mL were taken at the 1-h mark after filtration started and FAN, β-glucan, and soluble protein were measured with a Gallery analyzer. Validated FAN analysis was performed as described in D101313_08_Insert_Alpha-Amino_Nitrogen (Thermo Fisher Scientific) and described by.[42] Validated β-glucan analysis was performed as described in D10951_07_insert_Beta-Glucan_(High_MW) (Thermo Fisher Scientific, Waltham, MA, U.S.A.) and described by Kelly et al.[43] Validated soluble protein analysis was performed as described in D10415_02_Insert_Total_Protein_Biuret (Thermo Fisher Scientific). These results were validated using the ASBC Lab Proficiency Collaborative.

USDA, Madison Wisconsin Lab. Amylolytic Enzymes (diastatic power and α-amylase): Extracts were prepared from 10 g of grist in 200 mL of 0.5% NaCl. The samples were immersed in a 20 °C water bath for 2 h, with gentle swirling every 20 min. Next, they were filtered for 1 h, with return of the first 25 mL of filtrate. Diastatic power was determined via ASBC Method Malt-6C using the automated flow injection (Skalar San + Segmented Flow System) to measure reducing sugars (ferricyanide, 420 nm). The α-amylase was determined via ASBC Malt-7, using samples from the same extracts, analyzed in the same way as for diastatic power, after initial heating to 73 °C (ferricyanide, 420 nm). These results were validated using the ASBC Lab Proficiency Collaborative.

Wort: Extraction was performed via ASBC Malt-4, except that all amounts were halved and 100 mL of Reverse Osmosis-purified (R.O.) water was added to 25 g of grist, for each sample. Samples were extracted with a Congress Mash: 30 min at 45 °C, ramped to 70 °C at 1 °C/min, addition of 50 mL of R.O. water and maintenance of 70 °C for 60 min in custom fabricated water baths, with capacities of 12 steel beakers. Samples were cooled to room temperature and filtered into 250-mL Erlenmeyer flasks, with return of the first 50 mL of filtrate. Densities of the extracts (° Plato) were determined on an Anton Paar DMA5000 densitometer. The color, soluble protein, FAN, and β-glucan levels of each extract were determined using a Skalar San + System, employing the ASBC Methods, Beer-10, Wort-17, Wort-12, and Wort-18, respectively. These results were validated using the ASBC Lab Proficiency Collaborative.

Moisture Uptake

Initial percentage of grain moisture was determined for the Flush, Multi-Steep, and Immersion trials using a Near Infrared 1241 Grain Analyzer (FOSS Infratec, Hillerød, Denmark). Pre-malting grain weight was corrected for moisture using the following formula: grain weight – (grain weight * percentage of grain moisture). Grain (10 g) was loaded into 2 inch tea infuser balls (Winco STB-5, Lionsdeal.com), subjected to various malting regimes, and removed for evaluation at different time points. The grain was spread onto paper towels and patted dry between two paper towels to remove free moisture before determining hydrated grain weight. Percentage of moisture was calculated using the formula: (hydrated grain weight – pre-malting grain weight)/hydrated grain weight. Individual sets of seed were used for each time point evaluation.

SKCS kernel weight and hardness

The harvested whole barley samples were tested using a SKCS 4100 (Perten Instruments, Springfield, IL, U.S.A.). SKCS measurements were carried out on 300 grains of each sample and kernel weight (thousand kernel weight) and hardness index (HI) values were generated automatically by the instrument's software.

Chapon test and steep index calculation

Chapon tests[42, 43] were evaluated on grain after determination of grain moisture. Grain was re-loaded into its original 2 inch tea infuser ball and placed in boiling water for 1 min. Boiled grains were removed from the balls and 25 random seeds were split longitudinally. The boiling action gelatinized hydrated starches giving them a brown/translucent appearance, while un-hydrated starch remained in a crystalline form giving a white appearance. Visual scoring placed the seeds into one of five categories and points were assigned: 0 hydration = 0 points, less than half the seed hydrated = 1 point, between ½–¾ of the seed hydrated = 2 points, more than ¾ but less than fully hydrated = 3 points, and fully hydrated = 4 points. The steep index for each line was then calculated by adding the point value from each seed with a maximum score of 100 (25 seeds x 4 points). Individual seed sets were used for each time point evaluation.

Statistical methods

Within (r_{95}) and between (R_{95}) lab variation. Variation is inherent from sample to sample within and between laboratories. Limits of recommended allowable variation for each test have been established for both within laboratory (r_{95}) and between laboratories (R_{95}) when measuring a single sample and using the same protocol.[44] These values were determined by conducting inter-laboratory studies – such as

Table 2. Comparison of named variety malting results between USDA-ARS and MSU.

	Variety	Fine grind extract (%)	Soluble protein (%)	FAN (ppm)	β-glucan (ppm)	Soluble/ Total protein (%)	α-amylase (DU)	Diastatic power (ASBC)
MSU	Hockett	82.0	4.7	197	95	41.2	96	191
	Odyssey	79.3	5.0	241	54	37.3	99	196
	Metcalfe	81.4	5.6	252	58	47.1	106	166
	Genesis	80.7	4.8	206	133	45.0	78	152
	Synergy	79.3	5.8	259	52	43.3	104	210
	Growler	80.2	5.6	268	64	50.0	121	204
	Balster	82.6	5.2	242	63	55.0	120	152
	Genie	77.3	5.7	290	72	44.3	104	215
	Harrington	78.5	5.5	275	70	40.1	110	276
	Craft	77.5	4.9	208	91	36.0	90	221
USDA	Hockett	80.4	4.1	170	120	32.5	99	179
	Odyssey	78.8	3.7	124	156	26.2	54	135
	Metcalfe	80.4	4.6	214	61	34.7	107	167
	Genesis	78.6	4.0	140	321	31.1	71	158
	Synergy	80.9	4.4	186	31	37.3	100	136
	Growler	78.2	4.6	203	165	30.7	111	209
	Balster	83.9	4.6	197	105	35.2	111	159
	Genie	79.7	4.3	203	110	37.8	103	140
	Harrington	79.0	4.3	182	226	31.3	91	173
	Craft	78.0	3.9	128	314	28.8	66	158
MSU	Ave	79.9	5.3	244	75	43.9	103	198
USDA	Ave	79.8	4.2	175	161	32.5	91	161
	p value	0.86	**0.00**	**0.00**	**0.01**	**0.00**	**0.04**	**0.02**
	Spearman's R^2	0.25	0.42	0.39	0.47	0.20	0.59	0.00

MSU = Montana State University. USDA-ARS = United States Department of Agriculture-Agricultural Research Service. FAN = free amino nitrogen; Ave = average.
T test analysis performed as two-tailed, paired comparison.
Material: 2017 Intrastate Dryland.

performed by the ASBC Technical Committee. Statistical design for these tests make use of the Youden Block procedure where pairs of samples covering a meaningful range of values are sent to participating laboratories in order to generate a variety of random errors and allowing a robust estimate of the method's precision.[45] Here, the variation was compared with what was observed, with limits of recommended allowable variation.[44]

Pearson's correlation (R^2). Pearson's linear correlations were calculated (using the Excel formula = RSQ [known y's, known x's]) to reflect relatedness of magnitude among sample groups. This statistic is defined as the ratio of the covariance of two variables representing a set of numerical data, normalized to the standard deviation. A cutoff value of 0.70 was used, with $R^2 > 0.70$ indicating a relationship between two data sets.

Spearman's correlation (R^2). Spearman's linear correlations, rho, were calculated to reflect relatedness of rank-order among sample groups. This was accomplished by first assigning a rank order value to each data point with the Excel formula (=RANK.AVG [number, reference]), then calculating the correlation of the rank orders using the Excel formula (=RSQ [known y's, known x's]. A cutoff value of 0.70 was used, with $R^2 > 0.70$ indicating a relationship between two data sets.

ANOVA. Effects of steep regime and testing laboratory on malt quality measures were examined across three replicates of the control variety Odyssey. For each malt quality measure, steep regime and testing laboratory and their interaction were modeled as fixed effects, using ANOVA implemented in R (version 3.3.3).

Effects of steep regime and testing laboratory on malt quality measures were also examined across 11 varieties using a mixed-model ANOVA. For each malt quality measure, steep regime and testing laboratory and their interaction were modeled as fixed effects with variety modeled as a random effect using restricted maximum likelihood. The significances of the fixed effects were determined using Satterthwaite's approximation. The analysis was implemented in the R (version 3.3.3) using the package lmerTest.[46]

Results

USDA-ARS has long provided malt quality data to the breeding program at MSU. In 2016, MSU established a malt quality laboratory. The MSU laboratory now supplements the malt quality data provided by USDA-ARS, allowing for earlier-generation testing and testing in more environments. Both laboratories malted and analyzed grain from 10 varieties grown in Bozeman in 2017, each using their standard malting schedules previously outlined (Table 1). Most of the traits showed significant variation ($P < 0.04$ or less) for the means between the two laboratories, except for fine grind extract (Table 2). Even more problematic, the rank orders of the lines were considerably different when compared by Spearman's values of rank order correlation, with all values falling below 0.70, signifying a lack of relationship between the data sets. This is a matter of concern for a breeding program, as rank order difference could change selection decisions. The degree of difference between the two laboratories depended on the trait being measured. Additionally, all traits trend in the direction of lower modification for the samples malted by the USDA (i.e., lower soluble protein, FAN, ratio

Table 3. Consistency and repeatability of testing between the USDA and MSU laboratories.

Trait	USDA malted					MSU malted					Recommended limits of variation	
	USDA tested		MSU tested			USDA tested		MSU tested				
	Ave	Within lab variation	Ave	Within lab variation	Between lab variation	Ave	Within lab variation	Ave	Within lab variation	Between lab variation	Within lab r_{95}^{1}	Between lab R_{95}^{2}
Fine grind Extract (%)	**79.40**	0.20	**78.00**	1.46[a]	1.40	**80.00**	0.29	**78.90**	1.17[a]	1.10	0.40	1.40
Soluble protein (%)	**3.90**	0.05	**3.90**	0.11	0	**4.10**	0.04	**4.70**	0.10	0.60	0.20	0.64
Soluble/Total protein (%)	**31.90**	1.77[a]	**32.30**	1.20	0.40	**34.10**	1.82[a]	**39.00**	0.46	4.90	1.50	6.30
Diastatic Power (ASBC)	**119.00**	4.13	**123.00**	8.20	4.00	**144.00**	11.70[a]	**149.00**	3.29	5.00	10.00	30.00
α-amylase (DU)	**50.00**	10.20[a]	**58.00**	2.90	8.00	**60.00**	2.20	**85.00**	1.76	25.00[a]	5.00	15.00
β-glucan (ppm)	**153.00**	30.00[a]	**114.00**	2.70	39.00	**54.00**	7.00	**65.00**	22.80[a]	11.00	20.00	50.00
FAN (ppm)	**122.00**	8.30[a]	**127.00**	1.10	5.00	**170.00**	6.00	**191.00**	9.80[a]	21.00	7.00	40.00

[a]value larger than r95 or R95 MSU = Montana State University. FAN = free amino nitrogen; USDA = United States Department of Agriculture; Ave = average.
Three replicates of a single variety (Odyssey) were malted by both the MSU and USDA programs. Replicate malts from both programs were then tested by both laboratories. Averages of the replicates are presented here. Acceptable within-laboratory differences are reported as r_{95}^{1} and acceptable between-laboratory differences reported as R_{95}^{2}, established by Macleod in 2017 [45] for Fine Grind extract, Diastatic Power, α-amylase, β-glucan and FAN, and Lloyd in 1994 [44] for Soluble protein (%) and Soluble/Total protein (%). a Value larger than r_{95} or R_{95}.

of soluble to total protein, α-amylase, and diastatic power and higher β-glucan).

Due to the observed differences (Table 2), the USDA and MSU laboratories initiated a collaborative study to explore the causes of the variation. This collaboration consisted of a cross laboratory comparison study, where both laboratories malted a set of samples and, then, swapped a portion of each sample such that both laboratories could test both sets, allowing for comparison of testing results and malting regime.

To explore the differences in malting and analysis, MSU and USDA-ARS malted the control variety Odyssey in 3 replications and the means are reported in Table 3. To compare within-laboratory differences, the variation (range of difference between replications for each parameter within each laboratory's testing) is also reported. Comparing the variation of each trait with the recommended limits of within-laboratory variation (Table 3, Within-lab r_{95}), it was observed that both laboratories closely followed the limits, indicating that both laboratories produced consistent malt quality testing. Between-laboratory variation is also expected, with acceptable levels established as R_{95} (Table 3, Between-lab R_{95}).[44] Comparison of between laboratory variations fell within acceptable R_{95} for most traits, except for α-amylase in the MSU-malted sample, which was remarkable considering that the two laboratories were not using identical protocols. Thus far, differences in analysis were compared. However, when the malts made by the two labs were compared, more differences were observed with MSU malt tending to have higher extract, soluble protein, diastatic power, α-amylase and FAN, but lower β-glucan.

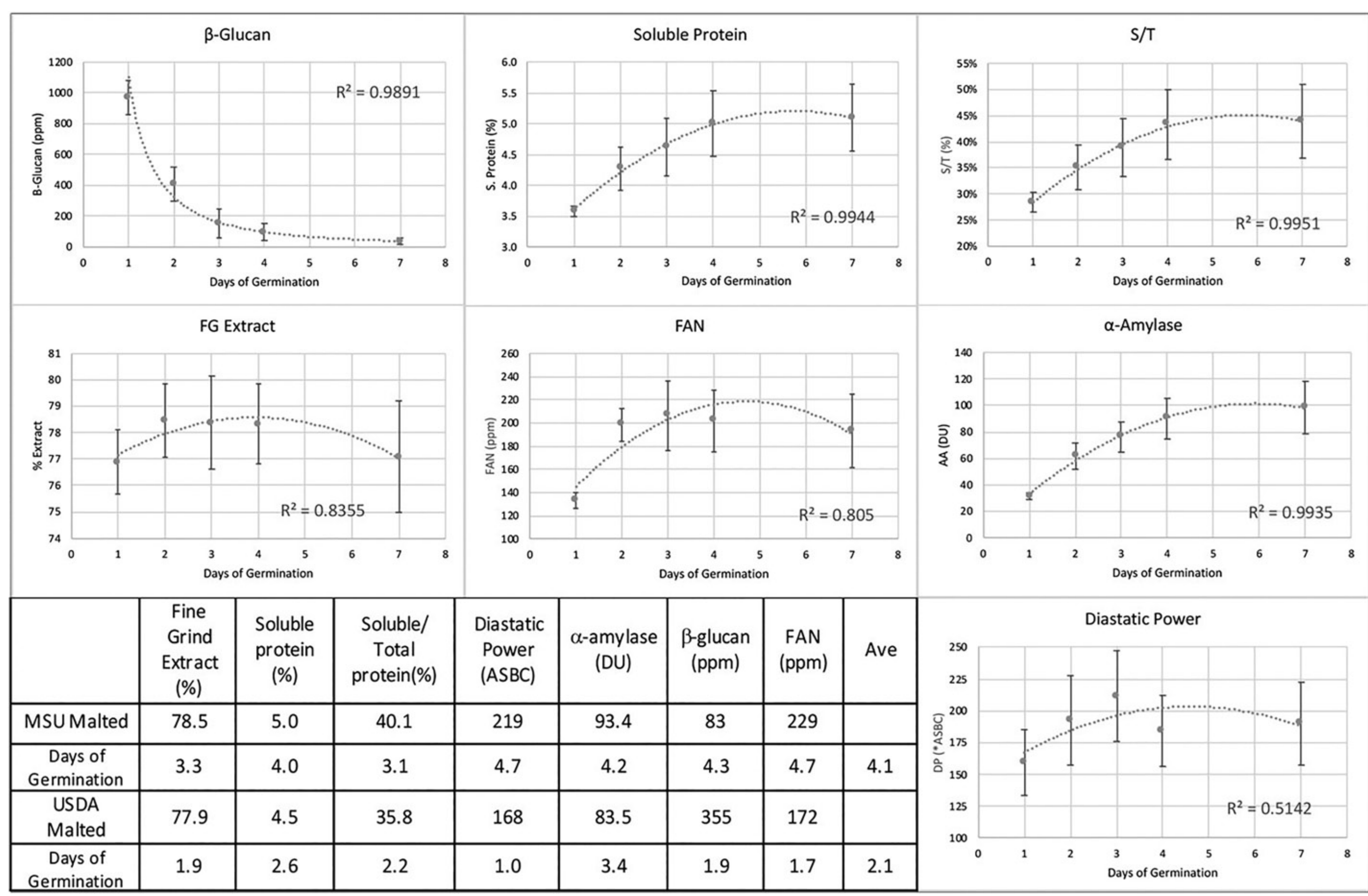
To determine if quality differences of the malts from the two laboratories were consistent across genotypes, both laboratories malted, exchanged, and tested 11 lines from the 2016 Bozeman field season (Table 4). At the bottom of Table 4, the *Difference between Testing* section displays the mean between laboratory variation for each trait (R_{95}) and shows that analysis for most traits was within recommended limits. Places with variations slightly outside the recommended R_{95} limits include the USDA-malted β-glucan: slightly over with a difference of 51.4 (limit = 50), and the MSU-malted ratio of soluble to total protein value: slightly over: 7.6% (limit = 6.3%), and the α-amylase was high: 25 DU (limit 15). However, the *Difference between Malts* section, which compares the analysis of malts from the two regimes, shows that most of the values were significantly different with most tests scoring at $P \leq 0.001$, indicating that regime significantly affects quality traits. These results combined suggest that the two laboratories provide consistent analyses and that the observed differences are primarily due to the malting regime. The malting regimes have the greatest impact on β-glucan ($P \leq 0.001$), diastatic power ($P \leq 0.001$), and FAN ($P \leq 0.001$). Furthermore, when comparing the mean values of all tests, a consistent trend exists with the MSU values representing greater modification than the USDA values (i.e., MSU malted-samples were found to have a higher ratio of soluble to total protein and FAN values, α-amylase, and lower β-glucan). These findings are

Table 4. Cross-comparison of program results looking at both malting regime and quality analysis.

	USDA Tested							MSU Tested						
	Fine Grind Extract %	Soluble Protein %	Soluble/ Total Protein %	Diastatic Power *ASBC	α-Amylase DU	β-Glucan ppm	FAN ppm	Fine Grind Extract %	Soluble Protein %	Soluble/ Total Protein %	Diastatic Power *ASBC	α-Amylase DU	β-Glucan ppm	FAN ppm
USDA Malted														
MT124071	80.2	4.58	37.4	162	81.3	197	175	79.0	5.0	43.9	161	84	147	200
Eslick	76.2	4.77	34.4	167	94.8	374	204	75.5	4.9	37.4	163	102	316	225
Lewis	77.4	4.81	33.5	241	99.5	450	194	74.8	4.8	35.9	221	96	348	208
MT124148	74.2	3.81	27.5	129	60.5	471	121	73.7	3.7	29.2	129	65	421	129
Hockett	79.0	4.36	34.8	193	90.8	337	161	78.7	4.6	38.3	178	92	261	172
Craft	77.7	4.27	31.9	166	66.4	430	135	77.4	4.3	33.0	166	74	385	142
MT124128	81.3	5.02	40.3	137	120.2	186	221	79.1	5.2	44.8	135	132	133	224
MT124601	79.4	3.97	33.4	156	68.8	531	130	78.4	4.1	35.4	153	65	476	134
MT090182	78.2	3.90	31.6	177	62.0	525	120	77.2	4.0	35.7	173	62	525	123
Amsterdam	79.2	4.58	32.5	207	63.5	405	176	77.9	4.8	38.8	193	64	316	202
MT090190	78.2	3.95	31.1	175	62.1	432	137	77.8	4.3	37.2	179	77	381	143
Mean	**78.4**	**4.38**	**33.7**	**170**	**81.2**	**381**	**166**	**77.4**	**4.6**	**37.8**	**166**	**85.7**	**329**	**177**
CV	**0.02**	**0.09**	**0.10**	**0.19**	**0.25**	**0.32**	**0.23**	**0.02**	**0.11**	**0.12**	**0.16**	**0.26**	**0.36**	**0.23**
MSU Malted														
MT124071	80.5	5.05	39.8	214	95.9	23	249	78.3	5.9	51.6	208	107	29	265
Eslick	76.5	5.19	36.7	236	96.6	34	269	75.0	5.9	44.8	235	121	41	313
Lewis	77.8	5.24	37.3	285	104.8	59	270	77.5	5.7	42.9	264	124	79	310
MT124148	75.9	4.21	31.7	169	69.6	82	182	75.1	4.6	37.9	177	90	70	196
Hockett	79.5	4.56	37.2	223	86.1	58	204	78.2	5.4	45.2	223	112	58	227
Craft	78.2	4.49	32.5	210	66.9	65	179	79.2	5.3	40.8	204	85	71	215
MT124128	82.0	5.16	42.0	162	98.1	66	262	81.6	5.8	48.2	168	130	70	278
MT124601	78.4	4.23	34.1	198	63.5	121	166	79.5	4.9	38.8	189	99	108	186
MT090182	78.2	4.13	33.8	246	65.2	115	162	76.3	4.7	41.2	245	99	78	180
Amsterdam	79.5	5.10	36.1	291	69.6	64	247	78.1	5.7	46.1	284	88	51	272
MT090190	78.7	4.38	37.1	240	69.8	82	189	78.6	4.9	44.4	230	89	79	207
Mean	**78.8**	**4.69**	**36.3**	**220**	**80.9**	**88**	**217**	**78.1**	**5.33**	**43.9**	**217**	**105.9**	**77**	**241**
CV	**0.02**	**0.09**	**0.08**	**0.20**	**0.19**	**0.78**	**0.19**	**0.02**	**0.09**	**0.09**	**0.17**	**0.15**	**0.51**	**0.19**
Difference Between Malts														
	USDA Tested							**MSU Tested**						
Difference	0.33	0.31	2.5	49.3	0.29	293	50.5	0.68	0.75	6.13	50.9	20.1	253	64.1
***P* value**	0.07	0.00	0.00	0.00	0.62	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00
Difference Between Testing														
	USDA Malted							**MSU Malted**						
Difference	0.7	0.22	4.1	4	4.8	51.4*	11	0.7	0.61	7.6*	3	25.0*	11	18
R_{95}	1.4	0.64	6.3	30	15	50	40	1.4	0.64	6.3	30	15	50	40

MSU = Montana State University. FAN = free amino nitrogen.

Barley samples from the 2016 Bozeman Fertility trial (Irr 150 N treatment) were malted by both programs and subsequently both programs tested both malts. R_{95} values are the recommended limits for between lab variation. *Signifies difference values that are greater than R_{95} for the comparison between results of the two laboratories. *P* values indicate results of a paired, two-tailed *t* test between malts.



	Fine Grind Extract (%)	Soluble protein (%)	Soluble/ Total protein(%)	Diastatic Power (ASBC)	α-amylase (DU)	β-glucan (ppm)	FAN (ppm)	Ave
MSU Malted	78.5	5.0	40.1	219	93.4	83	229	
Days of Germination	3.3	4.0	3.1	4.7	4.2	4.3	4.7	4.1
USDA Malted	77.9	4.5	35.8	168	83.5	355	172	
Days of Germination	1.9	2.6	2.2	1.0	3.4	1.9	1.7	2.1

Figure 1. Illustration of modification level impact on various malt traits in lines of interest.
*Points represent the means of quality traits at different points in the malting process, with error bars representing the standard deviation. Lines included are MT124601, Craft, MT124148, Hockett, Eslick, MT124071, and Odyssey from the 2017 field year. Samples were removed from the Montana State University (MSU) malting regime at different time points (days of germination beyond the steep phase). The best fit R^2 curve model for each data set was selected as follows: β-glucan uses a power curve; Fine Grind extract, free amino nitrogen (FAN), α-amylase, soluble protein, soluble/total protein, and Diastatic Power use a polynomial curve. The data table represents mean values (averaged across MSU and United States Department of Agriculture (USDA) results, Table 4, 2016 crop year) for the same lines malted in the standard MSU malting regime and standard USDA malting regime. The days of germination were calculated based on the curve model equations for each trait.

consistent with the initial observations seen in the named variety comparison of Table 2.

It was of interest to estimate the level of difference due to modification for each quality trait. Therefore, MSU malted 7 of the original 11 lines using the standard regime and tested samples at different modification levels (i.e., daily increments beyond the initial steep stage: 1 day, 2 days, 3 days, 4 days, and 7 days germination) (Figure 1). The curve for each parameter represents the mean change over time as modification progresses for the seven lines (2017 field year material). For direct comparison, Figure 1 (see inserted table) indicates the average value for each trait when the lines were malted in the standard USDA or MSU regime (from the cross-comparison study, Table 4, 2016 field year material). Most of the traits (fine grind extract, FAN, α-amylase, soluble protein, ratio of soluble to total protein, and diastatic power) evolve with a similar pattern as modification proceeds: increasing with time until about 4 days of germination, where there is either a plateau or decrease with further modification. The exceptional trait is β-glucan, which strongly decreases until about 4 days of germination and then plateaus, with well-modified values of below 100 ppm. Figure 1 (see table portion) displays equivalent days of modification for each trait, calculated using the curve model equations. The level of modification shows that on average the USDA samples were modified to 2.1 days of germination, while the MSU samples were modified to 4.1 days of germination, an average difference of two days less modified for the USDA samples. The three traits that were the most significantly affected in the cross-comparison study (β-glucan, FAN, and diastatic power [Table 4]), also show the largest differences here with modification of the USDA samples falling on average 2.5–3.5 days behind the MSU samples. The remaining tests were, on average, a day or more behind.

To identify the differences in malt regimes that had the greatest impact on malt quality, MSU malted the same seven lines simultaneously with three different steep regimes. The first regime reproduced the USDA procedure as closely as possible (Flush), while the other two regimes included the standard MSU regime (Multi-Steep), and an immersion regime (Immersion) that held grain under water in a 48 h continuous steep. The data generated by the Flush regime was highly comparable with the data generated by the USDA (Table 4), where quality results correlated on average at an R^2 of 0.63, while soluble protein, FAN, and diastatic

Table 5. Average malt quality data for seven lines from three different malting regimes.

Regime % Moisture, S.I.	Time to Malt		Malt Protein (%)	Fine Grind Extract (%)	Coarse Grind Extract (%)	Difference in Fine and Coarse Extract	Soluble Protein (%)	FAN (ppm)	β-Glucan (ppm)	α-Amylase (DU)	Diastatic Power (ASBC)	Soluble/ Total protein (%)
Immersion	8 days	Ave	12.1	78.2	77.8	1.5	4.4	184	66	83	193	37%
43.1%, 49		CV	0.06	0.02	0.03	0.85	0.14	0.14	0.42	0.20	0.17	0.19
Flush	8 days	Ave	12.2	76.9	76.5	2.1	4.3	162	126	75	170	36%
43.2%, 41		CV	0.07	0.04	0.03	0.91	0.09	0.15	0.78	0.27	0.24	0.16
Multi-Steep	7 days	Ave	11.6	78.3	77.1	1.3	5.0	202	93	90	175	43%
44.8%, 51		CV	0.05	0.02	0.02	0.38	0.11	0.13	0.61	0.17	0.16	0.16
P value	Flush x Imm		0.34	0.25	0.07	0.54	0.44	**0.01**	0.08	0.35	**0.01**	0.24
	Flush x Multi-Steep		**0.00**	0.25	0.28	0.32	**0.00**	**0.00**	0.12	**0.01**	0.68	**0.00**
	Imm x Multi-Steep		**0.00**	0.58	0.10	0.72	**0.00**	**0.00**	0.06	0.36	**0.02**	**0.00**
R^2	Flush x Imm		**0.86**	**0.86**	0.35	0.15	**0.73**	0.67	0.56	0.13	**0.93**	**0.93**
	Flush x Multi-Steep		**1.00**	0.51	0.67	0.02	0.62	**0.86**	**0.73**	0.51	0.56	**0.80**
	Imm x Multi-Steep		**0.86**	**0.80**	0.60	0.01	0.67	0.67	**0.93**	0.15	0.67	0.62

FAN = free amino nitrogen; Ave = average. Comparison of malt quality results of 7 lines malted with three malting regimes, one reproducing the USDA regime (Flush), an immersion consisting of a 48 h immersive steep (Immersion) and the Montana State University (MSU) standard recipe (Multi-Steep). The Flush and Immersion trials each had 5 days of germination, whereas the Multi-Steep regime had 4 days. Values below the steep regime name represent percentage of moisture and Steep Index at steep out (48 h). Typically, the goal percentage of moisture is 45% and the greater the Steep Index value, the better (fully hydrated kernels have a Steep Index value of 100).

P values calculated as two-tail, paired *t* tests; bolded values are significant at $p < 0.05$. R^2 values are Spearman's values of rank order correlation Bolded values are greater than 0.7, indicating that a relation in rank order exists.

power were best represented at R^2 values ranging from 0.73–0.76. Table 5 presents mean malt quality results for the seven lines under the three regimes. The Multi-Steep regime solubilized proteins more than the other two regimes, with the lowest malt protein, as well as the highest soluble protein, and the highest ratio of soluble to total protein. The Flush regime had the lowest extract of the three and the Immersion favored lower β-glucan and higher diastatic power. Flush was significantly different from Immersion for two traits, and significantly different from Multi-Steep for five traits. Immersion and Multi-Steep were significantly different for five traits. Overall, the traits involving protein had the largest differences between the three regimes. The three different steep regimes also impacted diastatic power, although the Flush and Multi-Steep were not significantly different here in contrast to the cross-comparison study, indicating that there may be inherent differences in the USDA malting program that were not captured in our reproduction.

From a breeding standpoint, significant mean differences, as indicated by the *p* values, are important when a cut off for acceptance is in place. However, rank order is also important, especially if a limited number of lines are advanced. The R^2 section of Table 5 represents the relationship of rank orders for the seven lines tested in each regime. Malt protein, soluble protein, the ratio of soluble to total protein, and FAN correlated well between the three regimes. Conversely, α-amylase, coarse grind extract and fine coarse grind extract difference had low correlations indicating that regime will have a greater impact on rank order of these traits.

To determine the interaction between steep regime and hydration, moisture and quality of hydration was tracked through steep and germination for all three regimes. Chapon tests[42, 43] determined the Steep Index to evaluate the quality of the endosperm's hydration. Results are displayed in Figure 2. Counting from initial wetting, measurements were taken at 24 h, 32 h, 40 h, 48 h (steep out), 72 h, and 144 h. The Immersion and Multi-Steep regimes had higher moistures and Steep Index values across all time points as compared to the Flush regime. This was consistent with the malt analysis from Table 5, which indicated greater modification for Immersion and Multi-Steep in the form of differences in fine and coarse grind extract, soluble protein, and β-glucan.

The MSU and USDA malting regimes also varied in that the USDA sorts seed by kernel weight and adjusts the steep time to reflect this value. Table 6a reports the kernel weight and hardness values for the lines included in the steep regime comparisons. To evaluate if sorting impacted this study, Pearson's correlations of magnitude were calculated comparing kernel weight and hardness with both moisture and Steep Index (shown in Table 6b). Kernel weight and hardness were highly correlated ($R^2 = 0.998$). However, for most time points, neither kernel weight nor hardness were correlated with moisture or Steep Index, except in the flush regime at 24 h. Kernel weight and/or hardness also showed a

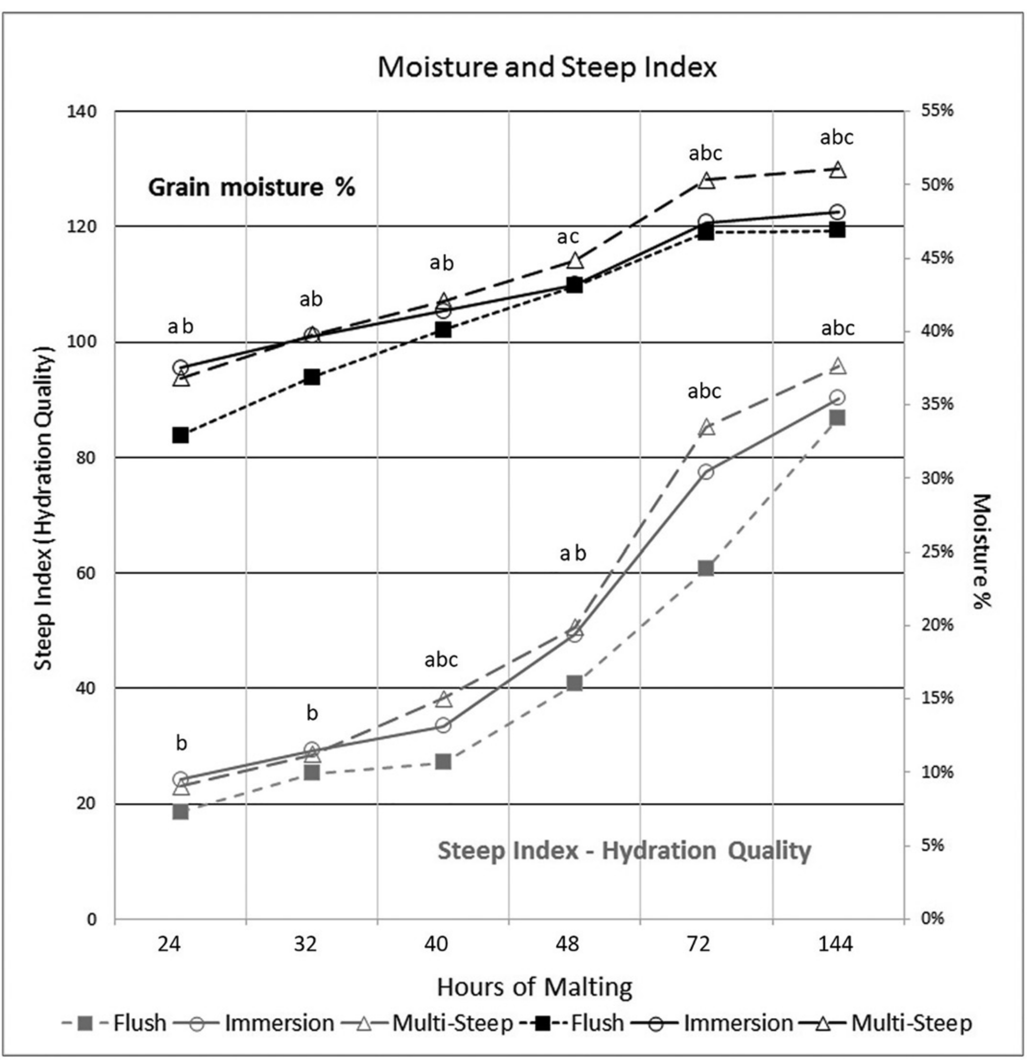


Figure 2. Mean grain moisture and Steep Index for seven lines and three different malting regimes across time.
Evolution of moisture (right y axis) and Steep Index (hydration quality – left y axis) in lines of interest from 2017 field material in three different malt regimes. Comparison is made between a malting regime built to mimic the United States Department of Agriculture's (USDA's) malting conditions (Flush), a 48 h immersion regime where grain was kept underwater for the initial 48 h steep (Immersion), and Montana State University (MSU's) standard multi-steep malting regime (Multi-Steep). Steep out was at 48 h for all regimes. The Flush and Immersion regime were completed after 168 h while the 3-Steep regime was completed at 144 h. *T* test results were calculated as two-tail, paired analysis. [a]indicates Flush and Multi-Steep are significantly different; [b]indicates Flush and Immersion are significantly different; [c]indicates Multi-Steep and Immersion are significantly different.

Table 6a and 6b. Kernel weight and hardness values correlated to moisture and hydration of the three malting regimes.

a	Line	SKCS Hardness (HI)	Kernel Weight (g)
	MT124601	720	41.7
	Craft	669	44.8
	MT124148	714	42.0
	Hockett	711	42.2
	Eslick	749	40.1
	MT124071	645	46.5
	Odyssey	648	46.3

b		Moisture R^2					Hydration (steep index) R^2				
Hours		24	32	40	48	144	24	32	40	48	144
Kernel Weight (g)	Flush	**0.81**	0.46	0.50	0.32	0.56	0.48	0.09	0.34	0.01	0.05
	Immersion	0.14	0.13	0.00	0.02	0.45	0.34	0.23	0.17	0.01	0.01
	Multi-Steep	0.37	0.35	0.37	0.61	0.00	0.05	0.22	0.47	0.33	0.31
Hardness (HI)	Flush	**0.82**	0.46	0.51	0.31	0.55	0.50	0.09	0.34	0.01	0.04
	Immersion	0.14	0.13	0.00	0.03	0.44	0.33	0.24	0.18	0.01	0.01
	Multi-Steep	0.36	0.34	0.38	0.63	0.00	0.06	0.22	0.49	0.35	0.32

Pearson's R^2 correlation of magnitude was calculated comparing both kernel weight and hardness to percentage of moisture and Steep Index for 7 lines of interest in the three steep regimes: Flush, Immersion, and 3-Steep. Bolded values indicate a relationship with R^2 greater than 0.70.

lack of relationship across all malt quality traits, except for fine grind extract (data not shown).

Discussion

Observed differences in malt quality when the same grain was malted and tested by the MSU and USDA laboratories prompted the current study. This work established consistency and repeatability of testing within and between the two programs, despite the laboratories having slightly varied protocols (see the Experimental section). The cross-comparison study indicated that the observed between-laboratory differences were primarily due to malting regime and not the post malt quality analyses. Major differences between malting regimes of the two laboratories include the practice of sorting by kernel weight to determine steep time,

the number and duration of steeps and rests, time held in germination, and temperature variations. Similarities between the two regimes were that grain was plumped over a 5.5/64" sieve before malting, a steep out target moisture of 45%, high level of humidity held within the malting chambers, and samples were dried at low temperatures in a slow fashion over a 24 h period, similar to base malt production. Bourne and Wheeler[3] demonstrated that increased temperature and prolonged germination time increased modification. Therefore, the fact that USDA malt was less modified was a surprise, since the USDA steeping was at higher temperatures (MSU = 15 °C throughout, USDA = 16 °C/18 C, and 17 °C), and germination time was longer (MSU = 96 h, USDA = 120 h). Consequently, differences in modification between USDA and MSU were not related to temperature or length of germination.

Seed-sorting by size and shape as a recommended and common practice was reported as early as 1933.[24] The purpose behind sorting is that seed size and/or shape can impact hydration and larger seeds are thought to take longer to hydrate. However, many factors can impact how a grain hydrates (including grain protein, starting moisture, hull characteristics), meaning that sorting by seed size alone does not account for all factors impacting rate of hydration. Additionally, as reviewed by Pollock,[24] grains with widths above 2–3 mm vary relatively little in their rates of water uptake. Also, during the first hours of steeping, water is absorbed most rapidly by large kernels, but percentage increase in weight is the same for variously sized kernels.[24] These studies suggest that separation of very small kernels (i.e. those smaller than 2–3 mm) improves homogeneity of the final malt, but further separation of larger kernels does not have identifiable impacts. Both the USDA and MSU malting regimes plump grain over a 5.5/64" (2.18 mm) sieve, removing thin kernels before malting, meaning that most kernels falling below the cutoff of 2–3 mm are removed. Table 6b displays the lack of relationship between seed size, moisture uptake, and grain hydration. In fact, the only observed correlation was found at the 24 h time point for the flush regime ($R^2 = 0.85/0.85$ for kernel weight and hardness), where seed was sorted and steeped varying lengths of time based on kernel weight, likely causing the correlation. This relationship was lost with time as the grains moved through the malting program and the moisture and hydration levels were impacted by other aspects of the program and/or genotype. The variation in kernel weight and hardness for the samples here was small, with range and CVs of 6 g/0.06 and 104 HI/0.06, respectively, for the two tests. Germplasm with more variation may better justify the need for sorting.

Steep regime (i.e., number and duration of steeps) has the largest impact on malt quality differences between the two programs. Table 5 directly evaluates the effect of three different steeping regimes on final malt quality. Changes in rank order of quality traits between the steep regimes indicate a genetic component impacting response to steep regime, and future research should genetically dissect response to steep. Variations and similarities between the three regimes can help us understand the impact of certain steep parameters on malt quality traits. For example, the Multi-Steep and Immersion regimes are similar in extended immersion times. Therefore, one could extrapolate traits with rank orders that are most similar between these two regimes (fine-coarse grind extract difference and β–glucan) are traits impacted by longer immersion times. Similarly, malt protein, FAN, and α-amylase rank orders were highly correlated in the Multi-Steep and Flush regimes, indicating that these traits were impacted by air rests. Finally, soluble protein, diastatic power, soluble/total protein rank orders were highly correlated in the Flush and Immersion regimes. One might expect that the malt quality from these two regimes would be very different, as there is the greatest difference in the length of immersion between the two. This could be an indication of the need for immersion times to be "just right" where the two extremes both negatively impact malt quality. The negative effect was not overcome by the extra 24 h of germination that both regimes had compared to the Multi-steep.

Granting that both MSU and USDA measure steep out moisture with a target of 45%, a typical measure to ensure malting uniformity, differences in percentage of moisture at steep-out were observed between the three malt regimes, with Multi-Steep samples on average at 44.8% moisture and the Flush and Immersion samples at ∼43% (Figure 2). Higher percentage of moisture resulting from the Multi-steep regime correlated with a higher Steep Index and greater protein modification (Table 5). While the Flush and the Immersion regimes have near identical mean percentage of moisture (43%), they have significantly different Steep Indices (40.7 and 49.3, respectively). The Immersion regime's higher Steep Index correlates with greater modification as measured by fine and coarse grind extract and β-glucan (Table 5), indicating a direct effect of hydration quality on final malt quality and suggesting that using percentage of moisture alone as a measure for malt uniformity is not ideal. A better estimation of grain hydration is a combination of percentage of moisture and Steep Index via the Chapon test.[42, 43] This argument is supported by findings of other researchers, including McEntyre et al.,[8] who reported that genotypic effects on water uptake and redistribution were independent; therefore, grain moisture contents determined after steeping might not reflect the true extent of hydration. Landau et al.[47] found that rapidly germinating barley showed extensive water uptake but diffusion through the endosperm was relatively slow. Kirsop et al.[15] also advised that moisture content of the grain may be a misleading indication that barley is adequately steeped. Studies in wheat have found that endosperm structure, initial grain moisture, and protein content all affect the rate of water distribution. Mealy endosperms are more easily penetrated by water than vitreous endosperms,[48] hydration increases as density of the endosperm decreases,[49] and thickness and composition of the kernel's outer layers cause hydration variation.[50] Due to similarities in kernel structure, each of these findings may relate to barley, although variations between the species (e.g., barley's multilayered aleurone) and hulls may also

affect hydration patterns. These factors point to the importance of directly evaluating the quality of a grain's hydration, as in a Steep Index, compared to using percentage of moisture alone to determine malt uniformity.

The Multi-Steep regime resulted in a higher Steep Index and higher moisture content at steep-out and malt with greater modification of protein than both the Flush and Immersion regimes (Figure 2, Table 5). However, the Multi-Steep did not result in malt with significantly higher extract or lower β-glucan (Table 5). This could be due to the longer time allowed for germination in the Immersion and Flush regimes (120 h) as compared to the Multi-Steep (96 h). Another possibility is that while air rests impact hydration, they may not have the same impact on all quality traits. Inconsistencies, such as a lack of significant difference for diastatic power between the Flush and Multi-Steep programs, could be explained by inherent variances that were not directly replicated in the attempt to match the USDA regime and which could impact final product (such as physical differences in the malting units, hydrostatic pressure, pH, and concentration of oxygen/carbon dioxide.[51]) Despite these discrepancies, there were measurable similarities between the Flush regime and the USDA's regime (R^2 average across traits = 0.63 when comparing Flush quality data to that of the USDA malted/tested, Table 4), and the trends observed in the three regimes (i.e. lower modification in the flush style) follow those observed in the initial comparison between MSU and USDA malt quality.

Although Multi-Steep regimes have become common [13,17], ideal immersion lengths can vary among varieties, seasons, and maltsters. A full understanding of how this practice impacts the quality of hydration is not yet in place. The initial hours of steeping are critical to the development of the components that determine malt quality. Kernel morphology, chemical composition, and environment, all affect the behavior of the kernel, during steeping. General regime recommendations such as offered by Schwarz and Li[18] state that highly vigorous barley may require only two phases of immersion, whereas short immersions with more frequent air rests (4–7 h) may be used to overcome water sensitivity. Brookes et al.[13] offer a comprehensive review of research performed on multiple and flush steepings and concluded that an initial steep bringing grain to 30–35% moisture is most effective; anything short of this landmark leads to insufficient water preventing the development of the embryo. Additional research performed by Bettner and Meredith[52] found that the effect of initial steeping time persists throughout the remainder of the steeping and germination phases, with an initial steeping time of 15 h wet producing the best-modified malt. Degradation of β-glucan occurs significantly during the initial steeping step.[9, 53] Runavot et al.[54] showed that barley malted under low hydration conditions resulted in delayed cell wall degradation and a two-day lag in β-glucan breakdown, similar to results reported in this study (Figure 1).

The initial short steep of flush regimes might stunt the development of the embryo as previously described. However, spray steeping suggests another explanation. Malting via spray steeping involves an initial steep, followed by subsequent sprinkling of water on the grain until the desired moisture is reached.[13] Several authors describe spray-steeped malts as being prone to low uniformity, due to inadequate hydration of the embryo, even though 45% moisture is reached.[4, 7] A poorly hydrated embryo will remove moisture from the endosperm to maintain embryonic growth rate, causing uneven rates of modification throughout the malt batch.[10] Short initial steep times may also cause low initial embryo hydration.

Understanding the impacts of steep regime on malt quality is key for both breeding programs and maltsters alike. Therefore, variations in steep regime were studied to better understand how malt recipe differences could impact selection decisions. Comparisons of results from the two laboratories consistently showed a pattern of reduced modification in the USDA samples as compared to MSU. Under modification could highlight important genotypic differences facilitating selections. For example, if considering β-glucan for a set of samples and using a cutoff point of 100 ppm, the USDA protocol provides stronger selection power, whereas the MSU program shows more lines in a favorable light. However, FAN, the ratio of soluble to total protein, and enzymes appear to be underestimated in the USDA regime. Traditionally, adjunct brewers have sought higher enzymes and soluble proteins, while all-malt brewers have sought lower enzyme activities. Selection in the MSU regime might be necessary to improve these traits. Of concern and potential interest is that the rank orders varied, at least to some extent, between the two systems, indicating that at least some varieties responded differently in each. Future work should genetically dissect the differences in response to the regimes.

Conclusions

Evidence is given that a critical factor impacting malt quality is length of immersion and air rests. Short steep intervals reduce grain hydration at steep out and overall lower grain modification in the final malt. Impacts of steep regime were observable in all malt quality traits and this study found that measurement of Steep Index at steep out was a better measure for malt uniformity than percentage of moisture alone. Although the Chapon test may be too labor intensive for regular breeding selections, it is an important tool for optimizing steeping regimes and could be implemented in genetic studies of endosperm hydration. As a general observation, the authors of this paper recognize that the Chapon test is an underutilized tool in the industry. Further research, using Steep Index, could establish it as a proxy for other quality traits. The impact of malt regime on malt quality not only has implications for maltsters, but also for barley breeders, because malt regime can impact selection decisions. The current research emphasizes the need for continued industry dialogue about the best steep practices to employ for malt quality improvement. The impact of selecting against lines that do not perform well under short immersions is currently not known. A variety that has stable

malt quality under most malting regimes would be preferred, however, that would also require more testing. A malt regime imposing the most stringent selection would be preferred if all traits of importance were impacted equally by the stringency imposed. A malting regime that best represents current malting practices could provide lines that perform best under those parameters. However, with all the variation in practices, agreeing on a single regime is difficult. Also, under best industry practices there may not be enough measurable difference between lines to impose selection. Another possibility would be to test under best industry practices, but to impose early termination of malting, such that more quality differences could be observed between lines. This study encourages future research to determine the genotypic effect on grain performance under different malt regimes.

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