D.E. Evans and J.M. Brauer

Review: “Are the days of Congress mashing over?”

The operating parameters chosen for the small-scale mash to determine malt quality are critical in defining malt quality. These key malt quality parameters include extract, attenuation, Kolbach Index (KI), wort FAN, viscosity, β-glucan, colour etc. The Congress mash (45°C – 30 min, 0.2 mm grist grind, 1:4 grist : water, ramp to 70 °C (1 °C/min), rest 25 – 60 min, lautering at 1:9 G:W ratio, RT) is currently the industry agreed small-scale mashing protocol for malt quality analysis. The Congress protocol appears to have its origins from a German Brewers Convention in Vienna in 1815, later formalized in 1907. As such, the Congress mash was developed to assess the malts of the time that were less modified and homogenous than before the innovations of malting engineers Nicholas Galland and Charles Saladin (c1870 – 1890) that ushered in modern pneumatic malting production. Prof Martina Gastl (TUM, Germany) concluded that the Congress mash favours cytolytic (and proteolytic) modification compared to modern mashing practice that mash in at 60 – 65 °C which favours amylolytic modification. Prof Barry Axcell (South African Breweries Ltd., RSA), among others, critiqued malt quality analysis for providing brewers with inferior practical information with respect to high gravity brewing, lautering, attenuation, foam, flavor and colloidal stability. Central to improving malt quality analysis was a complete revaluation of the small scale mashing protocol. This review sequentially considers what the likely optimum parameters are. The review concludes that potential starch hydrolysis into fermentable sugars is best determined by mashing in at 65 °C for 60 min (compromise between starch gelatinization and DP enzyme thermostability), a grist grind of 0.7 mm (possibly 0.4 mm), addition of 1.4 mM Ca^{2+} (56 ppm Ca^{2+}), a 1:3 grist:water ratio (higher gravity) and mash out temperature of 78 °C which is broadly in line with modern commercial brewing practice. For small-scale practical convenience and efficiency, ‘lautering’ at 1:9 G:W ratio and at RT, as with the Congress mash was preserved. Comparison of this style of mash shows broad agreement (correlation) with the Congress mash for extract, attenuation, wort FAN, KI, β-glucan and protein, however the ranking of samples is substantially different. This perspective is important for brewers, but critical for malting barley breeders whose selection indices between candidate breeding lines are largely based on agronomic and quality rank to provide improved resolution between high malting quality progeny.

Descriptors: Malt quality, small-scale mashing, malting barley breeding

1 Introduction

“Are the days of Congress mashing over?” was the prescient and provocative statement originally coined nearly 30 years ago by Stenholm et al. [102]. Stenholm and colleagues had recognized the inherent limitations of the small-scale Congress mashing protocol that did not match modern methods of mashing nor brewhouse equipment. This perspective was expanded upon by Axcell [8] who questioned commercially; “Why is it that malt analysis is still universally used if it gives such limited information?” At a similar time in Germany, Back and Narziss [11] were attempting to re-interpret and extend the conventional results yielded by Congress mashing to explain malt behavior within the context of modern brewing practice. Axcell et al. [9] had earlier considered the value of the conventional malt Certificate of Analysis (CoA) and concluded that; “Malts however, are often purchased with similar analyses that behave completely differently on brewing.” In particular, “It is well known that, within a particular brewing company, problems suddenly appear without warning and often disappear as quickly. This may be traced back to a particular delivery of malt. Examination of the malt analysis will probably reveal nothing.” Given the expense in producing a conventional CoA for each malt batch and subsequent, this was a damning assessment of the value of the conventional CoA and a strong recommendation for its modernization. In fact, many large brewing companies these days mandate specific variations or additions to the CoA to harmonize with their brewing practices and objectives. It would therefore appear that CoA’s are primarily valuable as a basis for commercial transaction between maltsters and brewers more than providing the brewer with a prediction of the performance of that malt in brewing.

Resolving the deficiencies in the CoA highlighted above by Stenholm, Axcell, Back and Narziss primarily, centers on the Congress mashing protocol. Since ~ 1990/2000, the EBC [111] and ASBC [1,

https://doi.org/10.23763/BrSc24-02evans

Authors

Evan Evans (ORCID ID: 0000-0001-8765-9227), The Tassie Beer Dr Consulting, Lindisfarne, Tasmania, Australia; John M. Brauer (ORCID ID: 0000-0002-8973-497X), EBC Executive Officer, The Brewers of Europe, Brussels, Belgium; corresponding author: tassiebeerdr@gmail.com

2] small-scale Congress mash protocols have been harmonized. The essential details of the Congress mash are summarized and compared with the putative commercial modern styles and as summarized by the mash parameters in table 1. As Axcell [9] observed: "few breweries operate a mashing sequence based on the Congress temperature profiles, little information is derived regarding brewhouse behavior of the malts." A more detailed drawing of the Congress temperature program, in comparison with several representations of basic commercial mash temperature programs is presented in figure 1. These temperature programs outline modern infusion style mashes and traditional decoction (single, double) style mashes. It is a general requirement in adjunct brewing that unmalted, non-Triticeae grains (e.g., corn, rice) be cooked (boiled) in a cereal cooker [12], resulting in essentially a single decoction mash program (Fig. 1C).

While having some similarities with the parameters of the Congress temperature program, there are also many substantive differences with modern commercial mashing practices (Table 1, Fig. 1). Modern mashes tend to be of higher gravity (G:W, grist to water ratio) these days to optimize the utilization of the brewhouse and fermenters (capital). Stewart and colleagues provides a useful overview of high gravity (wort 14 – 17 °P, 6 – 8 % beer ABV) or even very high gravity brewing (18 – 25 °P, beer >8 % ABV) in modern brewing practice [105, 106]. Returning to table 1, comparing the Congress mash parameters to those for commercial mashes identified that the mash in temperatures are substantially higher, the final mash out temperature is higher, the grist is coarser, the G:W is thicker, calcium is added to the liquor and the final wort gravity after mash separation (lautering) is more concentrated. Apart from adjunct brewing which by definition requires cooking to properly gelatinize the adjuncts starch, decoction style mashes are little used these days due to the added expense in terms of time, labor and energy [32]. In contrast to the mash tuns of 100 years ago, todays steam jacketed mash tuns readily and efficiently enable brewers to adjust the mash temperature as required [32]. In addition, a recent reassessment of the perceived benefits of decoction mashing (e.g., greater Maillard products) over infusion mashing have been found to be questionable [64, 65].

The Congress mash protocol is routinely used in the measure of malt quality parameters and is based on mashing methods and brewing practices from Europe in the late 19th century to emulate brewing practices of that period. The temperature program of

Table 1 Comparison putative commercial, Congress and small-scale mash protocol parameters

	Putative Commercial mashes		Small-scale					
Mashing parameters	**100% malt**	**Adjunct mash[1]**	**Congress[2]**	**IoB 65 °C HWE[3]**	**Isothermal 65 °C[4]**	**Tepral HG[5]**	**VTT[6]**	**MIM 65 °C[7,8]**
Mash in temp.	62 – 65 °C	50±5 °C[1]	45 °C (30 min)	65 °C (60 min)	65 °C (60 min)	50 °C[9] (15 min)	48 °C (30 min)	65 °C (60 min)
Adjunct temp/ramp (conversion temp)	NA	62 – 65 °C	(1 °C/min)	NA	NA	63°C[9] (15 min)	63°C (30 min)	NA
Saccharification rest	~70 °C	~70 °C	70 °C (25 – 60 min)	NA	NA	75 °C (15 min)	72°C (30 min)	NA
Final mash temp.	~76 – 78 °C	~76 – 78°C	70 °C	65 °C	65 °C	75 °C	80 °C	74/78 °C[6,7]
Calcium addition	0.3 – 0.5 mM (~40 – 70 ppm)	0.3 – 0.5 mM (~40 – 70 ppm)	No	No	No	No	2.56 mM pH 5.5	1.4 mM (56 ppm Ca^{2+})
Malt milling	"roller mill"	"roller mill"	Disc mill 0.2 mm	Disc mill 0.7 mm	Disc mill 0.2 mm	Disc mill 0.2 mm	Disc mill 0.7 mm	Disc mill 0.7 mm
G:W ratio	1:3 ± 0.5	1:3 ± 0.5	1:4 → 1:6	1:7.2	1:7.0	1:3.5 → 1:5.3	1:4	1:3 → 1:6
Final G:W ratio	1:3 ± 0.5	1:3 ± 0.5	1:9	1:9	1:9	1:9	1:5	1:9
Lautering	Hot lauter (sparging)	Hot lauter (sparging)	RT 'lauter'	RT 'lauter'	RT 'lauter'	Hot 'lauter'	Hot 'lauter'	RT 'lauter'
Wort boiling	Trub removed	Trub removed	NA	NA	NA	NA	NA	NA
Wort gravity	>10 °P	>10 °P	~ 8 – 9 °P	~8 – 9 °P	~8 – 9 °P	~8 – 9 °P	'high °P'	~8 – 9 °P
Total mash time	variable	variable	~85 min	~70 min	~70 min	~80 min	100 min	70 min
Residual gravity after fermentation	Av 2.11 °P (1.19 – 4.22 °P)	Av 2.11 °P (1.19 – 4.22 °P)	0.7 – 1.3 °P (24 h rapid)[10]	–	–	–	–	0.5 – 1.2 °P (24 h rapid)[10]

NA = not applicable, RT = Room temperature [1] Evans [32] [2] Congress mash [2, 31]

[3] EBC Analytica 4.6.1 (formally IoB recommended Method 4.2) IoB 65 °C = Institute of Brewing, hot water extract (65 °C) [60]

[4] Isothermal 65°C mash,[30, 79] MEBAK R-217.00.002 [2016-03] derived from IoB HWE (difference, 0.2mm grist grind MEBAK vs 0.7 grist grind IoB)

[5] Tepral high gravity [80-82] [6] VTT high gravity mash [102, 103] [7] MIM 65 °C = Modified Infusion Mash 65 °C, mash out 74 °C [37]

[8] MIM 65 °C, mash out 78 °C [40] [9] ramp 50-63 °C 13 min (1°C/min) and ramp 63-75 °C 12 min (1 °C/min)

[10] Rapid small-scale attenuation test, according to Evans and Hamet [35]

the Congress small-scale mash (Fig. 1A) emulates a temperature controlled, single stepped decoction mashing program. Historical small-scale mashing equipment and the received knowledge from the Weihenstephan Brewing School (Technical University Munich) indicates that the Congress mashing protocol had its origins at the German Brewers Convention of 1815 in Vienna (pers. comm. Ass Prof Jens Voigt, University of Applied Sciences, Trier, Germany). As such, the Congress mash protocol sought to mimic the decoction procedures commonly used in Central Europe at that time.[97] The modern incarnation of what we describe as the Congress mash evolved somewhere between an International Congress held in 1898 [115] and 1907 [4, 77]. The standardized protocol was approved by the EBC in 1975 [87].

The Congress mash protocol has a direct bearing on a number of important malt quality parameters listed in on the CoA as they are assessed on the wort produced by this mash protocol. The parameters assessed include extract, attenuation (AAL), soluble nitrogen (N), Kolbach Index (KI), wort viscosity, free amino nitrogen (FAN), wort β-glucan, wort colour, wort haze/turbidity and wort pH. Axcell [8] identifies that the Congress mash and derived parameters provides the commercial brewer with little to no information on high gravity brewing, lautering, potential haze formation, foam, flavour and colloidal stability.

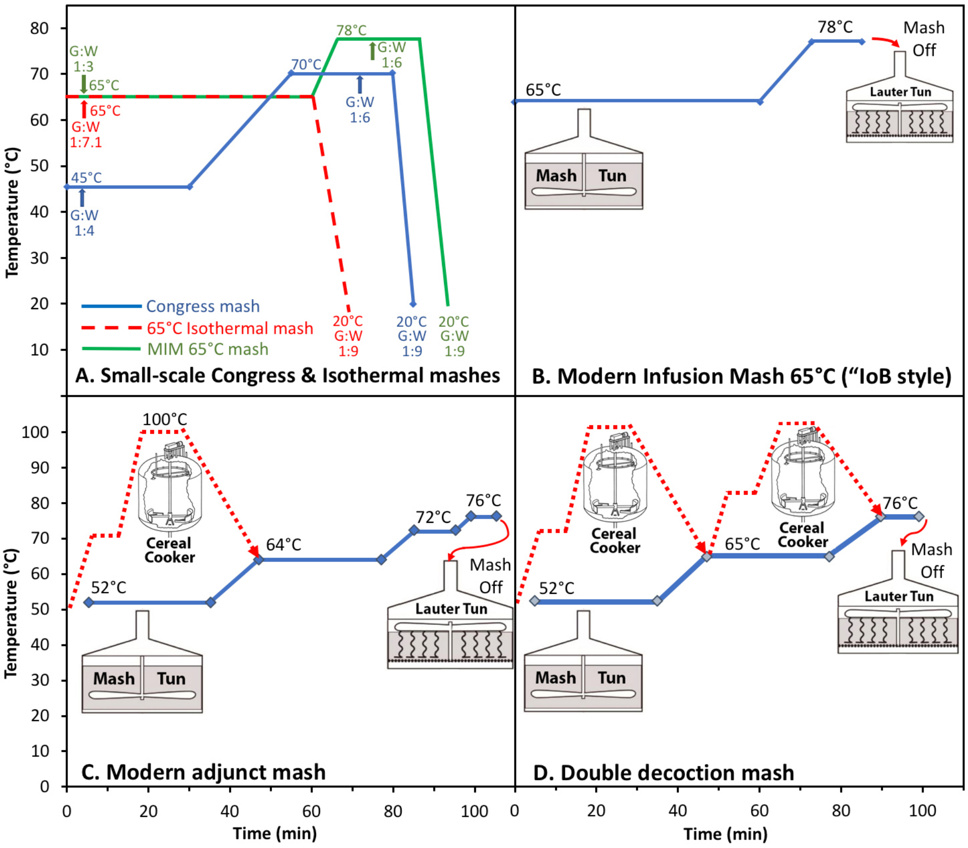

Fig. 1 **Typical basic mashing temperature programs: A. Congress, 65 °C infusion and MIM 65 °C mash programs used for small scale malt analysis (for comparison), B. Modern infusion style mashing program, C. Mash program for inclusion of cereal adjuncts, D. A double decoction mash program. Figure adapted, with permission by the ASBC and MBAA© from Evans [32]**

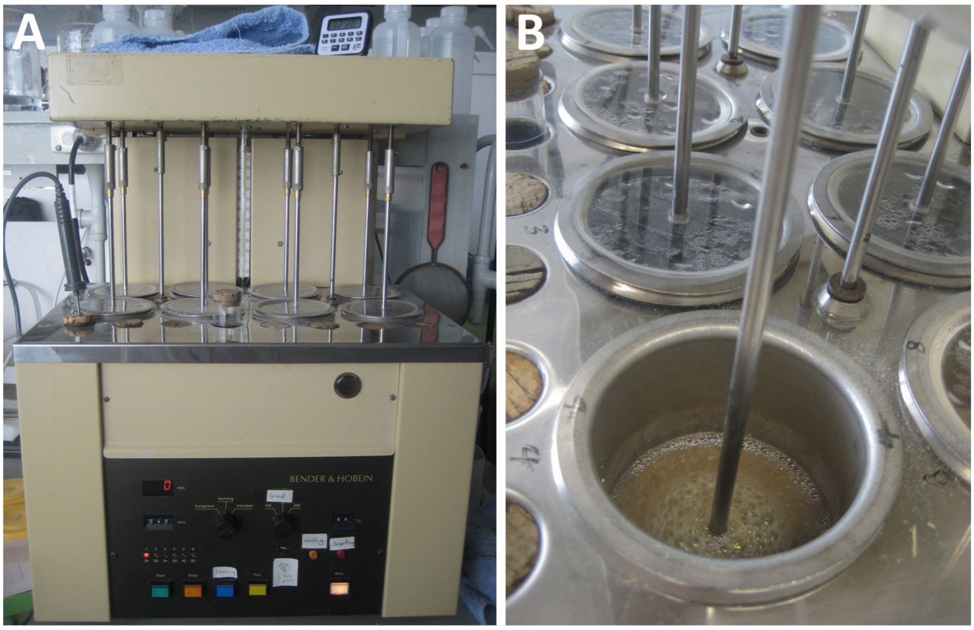

Fig. 2 **A. Traditional overhead stirring Congress mash bath (Bender and Holbein, Munich, Germany), and B. Close up view of mash in progress. Figure reproduced with permission from the ASBC and MBAA© from Evans [32]**

Practically the Congress mash is typically undertaken in mash baths as shown in figure 2. The mash bath consists of mash stirrers (overhead in this example) stirring the mash in stainless steel pots while sitting in a temperature-controlled water bath. More modern versions of mash baths that can be more easily temperature programmed and stir bar driven (bottom) are commercially available from Crisps (CampdenBRI bath, UK) or the IEC mash bath (Thornbury Australia). Care is required with bottom stirrer-bar mixing as the stirrer-bar acts as an additional grist grind creating fines which may interfere with assessments of lautering efficiency and possibly extract [49]. The grist for the Congress mash is prepared by using a disc mill (DLFU) with a gap setting of 0.2 mm (fine grind, Table 1). In terms of grist milling, it has been concluded that a coarse grind grist (0.7 mm, DLFU mill) was probably more akin to actual modern commercial practice [37, 102, 103]. The Congress G:W (1:4) has also been considered to be too ‘thin,’ compared to what many commercial brewers are currently using. Table 1 indicates that a G:W ratio of 1:3 ± 0.5 is most

Table 2 **Summary of the thermostability and pH optima of key malt enzymes. Table reproduced with permission by the ASBC$^{©}$ from Evans [32]**

Brewing actions	Primary action	Malt enzymes	Mash thermostability	pH optimum	Mash outcomes
Starch hydrolyzing enzymes	Mashing	α-Amylase	> 70 °C	5.2 – 5.5	Combined action produces yeast fermentable sugars (e.g. maltose, glucose, etc.)
		beta-Amylase	~ 65 °C	6.0 – 6.5	
		Limit dextrinase	~ 65 °C	5.0 – 6.5	
		α-Glucosidase	~ 50 °C	4.3 – 4.5	
Cell wall hydrolyzing	Malting	Endo-β-glucanase	<50 °C	3.7 – 5.0	Low Mwt. β-glucan
		Endo-xylanase	55 – 60 °C	4.5 – 7.0	Low Mwt. pentosans
		Cellulase	> 60 °C	4.0 – 6.0	Mash/wort filterability
Protein hydrolyzing & releasing	Malting	**Endo proteinases:**			Protein release - foam
		Cysteine	50 – 60 °C	3.8 – 4.5	Reduced haze protein
		Metallo	50 – 60 °C	5.3 – 8.5	
		Aspartic	50 – 60 °C	3.5 – 4.5	
		Serine	50 – 60 °C	5.5 – 6.5	
		Exo peptidases:			Release FAN (amino acids)
		Carboxypeptidases	55 °C	5.0 – 6.5	Release 75% amino acids
		Aminopeptidases	55 °C	7.0 – 8.5	
Lipid releasing/ oxidizing	Malting and Mashing?	Lipase	50 – 60 °C	6.8	Decreases wort turbidity
		Lipoxygenase: LOX 1	~ 45 °C	6.0 – 6.5	Staling – foam instability
		LOX 2	< 45 °C	6.0 – 6.5	

likely most aligned to commercial practice which is in agreement with conclusions of Piendl [90] and Evans et al. [37].

Concerted efforts have been made to modernize the Congress mash to better conform to parameters used modern by modern brewers. For a range of small-scale mashes table 1 outlines the differences in milling fineness, temperature program, G:W (thickness), liquor calcium addition, final mash out temperature, lautering temperature, mashing time and final wort gravity. Although a 0.2 mm disc mill grind (Congress) maximizes the malt extract number, it is generally considered not to be a good reflection of a coarse (0.7 mm) commercial grist (VTT, MIM 65 °C). Next to the thickness of the mash is limited to being greater than > 1:2, with G:W ratios > 3:1 being fully independent of the quantity of water present [22, 81, 83]. This indicates that the apparent 1:7.0 G:W of the 65 °C Infusion mash [47, 79] to be substantially thinner than the Congress mash and modern commercial practice (Table 1). The other operational component of a small-scale mash protocol is that it should be efficient (labor and time) to run in the malt quality laboratory.

A summary of the thermostability and pH of the key malt enzymes is provided in table 2. It is judged that starch hydrolysis primarily occurs during mashing while the hydrolysis of cell wall non-starch polysaccharides and proteins mainly but not exclusively during malting [32]. With respect to the Congress mash, the rest at 45 °C preserves protease and β-glucanase activity which is now generally considered no longer necessary with today's properly homogenously modified malts [32]. It was malting engineers Nicholas Galland and Charles Saladin who ushered in this era of pneumatic malting to more evenly modify malts (c1870 – 1890) [68, 115].

It is now understood that the optimal temperature for conversion – starch gelatinization and diastatic power (DP) enzyme survival in mashing is in the range 60 – 65 °C [34, 36, 38, 90, 102, 103]. Bamforth [12] contends that, "mash conversion temperatures of around 65 °C will comfortably ensure the gelatinization of malt starch." The need of a saccharification rest at 70 – 72 °C is questionable if the time for conversion is sufficiently long (~ 60 min) according to Evans et al. [37]. The final mash out temperature is typically 74 – 78 °C (Table 1) with a number of modernized small scale mashes adopting this consensus temperature (Tepral, VTT and MIM 65 °C). Mini mashes, 1 ml Eppendorf mashes, have been proposed by Kerr et al. [61] and Schmitt et al. [95] for assessment of breeding lines and for laboratory experiments. In practice, the quanties of malt involved (~ 200 mg) and the required finess of grind (e.g., Udy mini hammer mill) would suggest such micro mashes were unlikely to provide a sufficiently representative sample for malt quality analysis.

The key question to be asked is, do each of the key stakeholders in the malting barley chain, brewers (company purchasers), maltsters and barley breeders have differing requirements with regards the quality parameters derived from the small-scale mash? Axcell [8, 9] proposes that commercial brewers require consistent quality in line with agreed specifications and will want any potential malt problems effectively 'red flagged.' In addition, information on malt performance with respect to high gravity brewing, lautering, foam, flavor and colloidal stability would also be valuable. Maltsters, on the other hand, are interested in barley varieties that are fast modifying to efficiently produce malt that conforms to the brewers malt quality specifications [115]. Historically, the dissatisfaction with malt analysis has led to increasing numbers of parameters being added to the malt specs. Interestingly, very few malt analysis parameters ever get removed. Around 1910, a typical malt analysis would consist of about 10 parameters (extract, F/C difference, CWE, diastatic power, moisture, colour, arsenic and screenings). Basically, maltsters want to efficiently run (cost and labour) as few universally accepted tests (e.g., small-scale mash styles) as possible.

Malting barley breeders, like maltsters, also have efficiency constraints in their programs which require large numbers of samples to be efficiently analysed. Two perspectives on breeders objectives with regards quality can be gained from Pitz [91] and Nagamine and Kato [86]. Barley breeder's primary objective is yield (e.g., agronomy, N utilization efficiency, disease resistance etc), for without competitive yield even high malting quality varieties are not viable because the malting premium is not high enough and only a proportion of the crop is graded malting (e.g., 30 – 50 % depending on region). Modern barley breeders have embraced marker assisted selection (MAS) and genomic selection (basket of malt quality related alleles at multiple genomic locations) to expedite genetic progress in their programs, it follows that traits being selected can ideally be traced back to a small number of major gene loci (multiple alleles) or QTL's (quantitative trait loci) [70, 89]. Of course, the small-scale mash is an important conduit to identifying desirable genes or gene blocks. Although each of these stakeholders in the malting brewing chain are important, at the end of the day, the amount and quality beer brewed, and the efficiency of the process is the end game and the quality that the small-scale mash should identify.

It is thus not surprising that the European brewing industry is currently re-assessing the small-scale mash protocol, the most recent meeting being 26th October 2023 Joint Euromalt-EBC Workship in Brussels [29]. Therefore, the aim of this review is to examine each of the parameters selected for small scale mashing (grist grind, mash temperature program, G:W ratio, mash out temperature) for the determination of the CoA of malt quality. It is anticipated that judicious selection of the small scale mash parameters will assist in striving for the malt analysis goals set by Axcell [8, 9].

2 Systematic Assessment of Small-Scale Mash Parameters for a Modernized Protocol

The following discussion will map out a systematic and step wise discussion of the key parameters for a small-scale mash using the Congress mash as a starting point (Table 1).

A. Selection of the mash temperature program

The primary enzymatic task during mashing is the hydrolysis of starch which is a compromise between the temperature of required for starch gelatinization and that for sufficient preservation of the DP enzymes, particularly beta-amylase and limit dextrinase (LD) [12, 36]. Table 3 shows the typical T_p for barley, malt and a range of potential adjuncts. T_p is a measure of peak gelatinization temperature, with some degree of gelatinization occurring at temperatures lower and higher than the T_p. In general, most barley malt starch gelatinizes between 61 and 65 °C which is consistent with the general perception of most brewers that, "mash conversion temperatures of around 65 °C will comfortably ensure the gelatinization of starch" [12]. There are a number of factors which can practically increase the T_p of malt starch. These include:

- different proportions of A (large) and the generally higher T_p small (B granules) [5, 69];
- Differing starch granule proportions of amylopectin to amylose where a higher proportion of amylose typically increases starch T_p [12, 14];
- Increased levels of storage protein [57, 100].

Table 3 Reported starch gelatinization temperatures primarily determined by differential scanning calorimetry (DSC) [12, 18, 50, 71, 118]. Table reproduced with permission by the ASBC© from Evans [32]

	Gelatinization temperature	
Starch		**T_p (°C)**
Barley normal	Lowest	52
	Highest	60 – 68
	Majority	59 – 63
Barley malt	Majority	61 – 65
Maize (Corn)[1]	range	62 – 77
Maize (waxy)[1]	range	62 – 80
Oats	range	52 – 64
Rice[1]	range	61 – 82
Rice, short grain[1]	range	65 – 68
Rice, long grain[1]	range	71 – 74
Rye	range	49 – 61
Sorghum[1]	range	69 – 75
Wheat	range	52 – 64

[1] Adjuncts of these materials should always be cooked (heated to 100 °C) before brewing

The growing environment of the barley crop, [42] in particular higher temperatures during grain fill, and the resulting dryness of soil, have been observed to lead to higher starch gelatinization temperatures [85, 110]. It is then perhaps not surprising that when starch gelatinization temperatures exceed 64 – 65 °C, sub-optimal levels of wort fermentability begin to be noticed by brewers [15, 102, 103].

The principle mash starch degrading DP enzymes are α-amylase, beta-amylase and LD [12, 36, 74, 101]. To date the evidence from mashing indicates that α-glucosidase has a relatively minor role in starch hydrolysis during mashing [12, 36, 74, 101]. Of the two α-amylase isoform families α-amylase 1, the dominant α-amylase 2 comprises 80 – 90 % of the total α-amylase activity in malt [75, 73]. Bertolft [16] also observed that α-amylase 2 was the most sensitive isoform to Ca^{2+} thermostabilization. It is widely understood that α-amylase is thermostable at mashing temperatures above 65 – 70 °C in the presence of Ca^{2+}[12, 34, 41], thus is the most thermostable of the DP enzymes. For many years, based on pure enzyme thermostability studies it was thought that the thermostability of both beta-amylase and LD was below 60 °C. It has since been demonstrated that under practical mashing conditions the practical thermostability of both beta-amylase and LD was greater than 60 °C, and effectively 65 °C [34, 98, 101]. Of the DP enzymes only beta-amylase has been found to have a practically significant thermostable variant, Sd2H [28] which conferred an wort attenuation of ~ 2 % point advantage in some circumstances [38, 26].

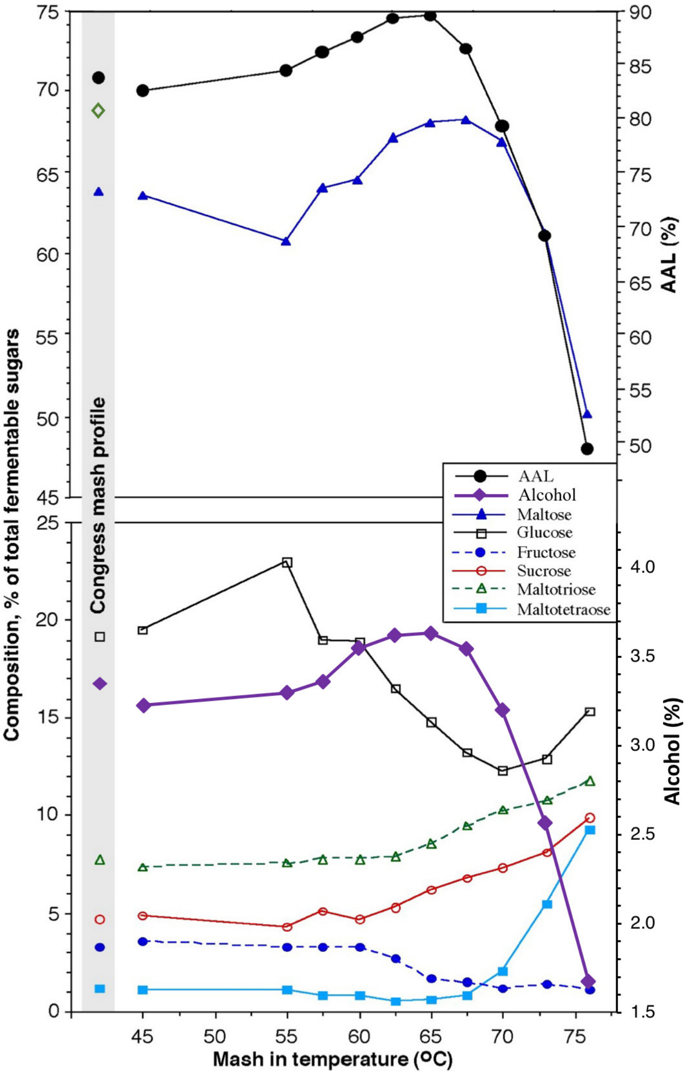

Fig. 3 **Fermentable sugar profiles derived from small-scale mashes, grist grind 0.2 mm, G:W (1:4) mashed in at 10 different temperatures, based on the Congress mash protocol and compared with the Congress mash, alcohol and apparent attenuation limit (AAL) summary from eight different varieties of commercial malts. Figure adapted, with permission from the ASBC© from Evans [36] after Palmer [88]**

The optimum mash temperature for the hydrolysis of starch into yeast fermentable sugars was identified from a series of mashes with increasing mash in temperatures based on the Congress mash (Fig. 3). Notably, as the mash in temperature rises from 45 °C to 76 °C, the level of AAL and alcohol reach a maximum at ~ 65 °C and maltose slightly later at 67.5 °C, after which all three measures rapidly deteriorate. In terms of extract (Fig. 4) when compared to the Congress mash (80.8 %), mashing in temperature reduces extract slightly between mash in temperatures of 45 – 55 °C and 70 – 76 °C. However, the extract recovered when mashed in at 65 °C (80.6 %) was almost the same as for the Congress mash. Interestingly, compared to the Congress, FAN (Fig. 4) increased by 6.6 % points for the 55°C mash (45°C mash), after which it continually decreased to the 76 °C mash in. Mashing in at 65 °C resulted in a 15.4 % point FAN decrease compared to the 55 °C mash. This demonstrated that FAN producing enzymes are still somewhat active at 65°C.

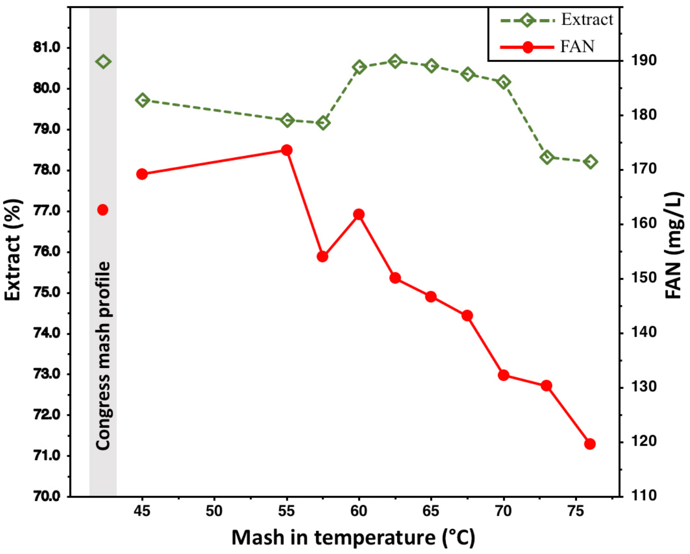

Fig. 4 **Extract and FAN small-scale mash profiles, grist grind 0.2 mm, G:W (1:4) mashed in at 10 different temperatures, based on the Congress mash protocol and compared with the Congress mash, summary from eight different varieties of commercial malts. Figure adapted, with permission from the ASBC© from Evans [36]**

Figure 3 also shows that AAL and alcohol were useful proxies for the level of maltose and other fermentable sugars. Compared to the EBC mash, the AAL was 5.7 % higher and alcohol was 0.25 % higher at 65°C. Interestingly, the mash in temperature has a substantial impact on fermentable sugar composition, particularly maltose:glucose. Such changes in the sugar spectrum, particularly the proportion of glucose and fructose to maltose, have been shown to influence yeast metabolism and the subsequent ester content and flavor of the beer [112, 116]. Intriguingly, after a mashing in temperature of 55 °C, the level of glucose dramatically decreases (Fig. 3). This is coincident with the thermostability of α-glucosidase being inactivated at temperature > 50 °C[84], suggesting that α-glucosidase may play a more important role in mashing than previously understood [12, 36]. Combined, the compromise temperature between starch gelatinization and starch hydrolysis into fermentable sugars was 65 °C, which was in accordance with the 60 – 65 °C range previously identified [90, 101].

Conventionally brewers, when mashing in, use mash liquor with a somewhat higher temperature to warm up the grist which is normally at room temperature to attain the desired mash temperature [32]. This “strike temperature” could conceivably inactivate some of the more thermolabile malt enzymes in mashing. Evans et al. [37] tested the impact of a 71 °C strike temperature (mash: 65 °C for 50 min, 0.2 mm grist grind, 2.2 mM $CaSO_4$ (88 ppm Ca^{2+}), 1:4 G:W, mash out 70 °C, lautering at 1:9 G:W ratio, RT) and observed that extract was not significantly different, while AAL and alcohol content were slightly but not consistently different. For convenience, Evans et al. [37] concluded that attemperating the dry grist is the mash pot (cylinders) for 10 min attemperated to 65 °C, thus avoiding the need for use of a strike temperature was most efficient.

Brewers normally increase the mash temperature of the mash to between 76 – 78 °C for mash out [32]. The higher temperature aids lautering efficiency and may aid the final solubilization of some final malt components. The mash out temperature for the Congress mash is 70 °C while for the small scale mashes outlined in Table 1 it is 65 – 80 °C. Evans et al. [37] assessed the difference between mashing out at 70 °C and 74 °C (mash: 65 °C for 50 min, 0.2 mm grist grind, 2.2 mM $CaSO_4$ (88 ppm Ca^{2+}), 1:4 G:W, lautering at 1:9 G:W ratio, RT). Again, this difference in mash out temperature did not significantly affect extract, while resulting in small but not necessarily significant reductions in AAL and alcohol content. It was concluded that mashing out temperature had a minor effect on the metrics measured. Later, Evans et al. [40] adopted a mash out temperature of 78 °C for small scale mashes which was more consistent with commercial brewing practice.

B. Inclusion of Ca and adjustment of mash pH

A combination of salts typically including calcium (e.g., $CaSO_{4.}2H_2O$) are used to condition mashing liquor [32]. Typically, the concentration of Ca^{2+} is in the order of 1.0 – 1.75 mM (40 – 70 ppm Ca^{2+}). Calcium promotes the thermostability of α-amylase 2, reduces pH, precipitates oxalate (as beer stone) and assists in the flocculation of yeast at the completion of fermentation. Evans et al. [37] compared the effect of mashing with or without 2.2 mM $CaSO_4$ (88 ppm Ca^{2+}, mash: 65 °C for 50 min, 0.2 mm grist grind, 1:4 G:W, mash out 70 °C, lautering at 1:9 G:W ratio, RT). Overall, there was no significant difference for the addition of Ca for extract and minor and variable differences for AAL and alcohol content. It was noted that the pH of mashes to which 2.2 mM $CaSO_{4.}2H_2O$ (88 ppm Ca^{2+}) had been added were on average 0.11 pH units lower. However, Evans and Fox [34] did observe that with one of three varieties used for Congress mashes in that study that α-amylase thermostability, was observed to undergo a substantial loss of activity at 70 °C when additional Ca was not present. Possibly this was a result of the terroir that this barley was grown on, as substantive variation for Ca and other micronutrients contents in malt has been observed [39]. Consequently the decision to include 2.9 mM Ca^{2+} (116 ppm Ca^{2+}) was most likely prudent.

Stenholm et al. [102] has followed typical brewery practice and adjusted their mashes to pH 5.5 with 0.5 M H_2SO_4. However, in practice there is substantial buffering capacity inherent in malt in terms of its contingent of proteins and other components [13, 76, 109], such that the final wort pH was on average 5.83 with a range of 5.69 – 5.96 (0.3 mM Ca^{2+}, 12 ppm Ca^{2+}), quite a narrow range [40]. Given the time-consuming nature of adjusting pH, such a procedure was not considered for laboratory scale mashing testing. Perhaps it would be beneficial to consider including the capacity of the mash to buffer pH as well as the wort pH. The buffering would perhaps be an indicator of modification.

C. Grist Coarseness

Brewers conventionally use a range of malt mills from hammer mills, to wet mills and two, four or six dry roller mills [32]. Hammer mills are typically matched with mash filters, while some steep conditioning (wet) mills by their nature are limited to maximal operational temperatures of 63 – 65 °C due to increasing issues with starch gelatinization (pers. comm. Daniel Carey, New Glarus Brewing). Briggs et al. [18] states that “The objective of milling is to break up the malt and adjuncts to such an extent that the greatest yield of extract is produced in the shortest time in the mashing equipment in use.” While extract efficiency and yield are promoted by finer grist, it follows with traditional lauter tun systems, that there is also an imperative to keep the husk as intact as possible to enable efficient wort flow and separation. In practice, the majority of small to large breweries use a six (or four) dry roller mill. The roller dimensions (diameter 250 – 350 mm) are required to achieve the correct angles for grabbing, crushing and shearing of the grain [32]. It is immediately obvious that the conventional mill of choice for malt quality testing, a disc mill (e.g., DFLU Bühler Miag, Germany) has a somewhat different mode of action. However, operationally the disc mill is a reasonable compromise with respect to laboratory efficiency. This raises the question, what is the optimal mill setting for malt analysis and what are the consequences of that choice.

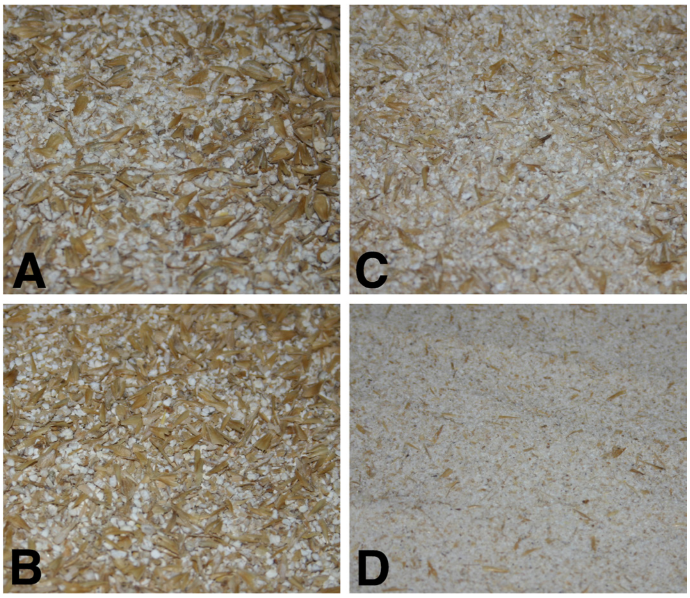

Fig. 5 **Pictures of grist milled by different means. A. Commercial 6 roller mill; B. 1.0 mm disc mill; C. 0.7 mm disc mill; D. 0.2 mm disc mill. Figure reproduced with permission by the ASBC© from Evans [37]**

Table 4 **Sieve proportions of disc milled malt (essentially an EBC sieve set) compared with a commercially milled malt (6-roller mill). Table reproduced with permission by the ASBC© from Evans [37]**

	6-roller mill	**Disc mill with setting:**		
	Commercial grist	0.2 mm grind	0.7 mm grind	1.0 mm grind
Sieve	**Fraction proportions (%)**			
1.25 mm	46.4	2.1	9.8	27.2
1.0 mm	8.5	1.8	14.6	20.2
0.50 mm	16.4	9.1	41.5	24.1
0.25 mm	9.5	37.9	13.0	9.7
0.125 mm	6.0	17.1	6.5	5.2
Pan	13.3	32.1	14.6	13.7

Evans et al. [37] directly compared samples of malt from the same malt batch milled by a disc mill at setting 0.2 mm Congress fine grind grist, setting 0.7 mm Congress coarse grind grist and setting 1.0 mm, with that of malt milled by a commercial six roller mill (Fig. 5). In comparison with the 6-roller milled grist the appearance of the grist is too fine at 0.2 mm, to coarse at 1.0 mm, and perhaps somewhat too fine at 0.7 mm, with the husk integrity notably de-

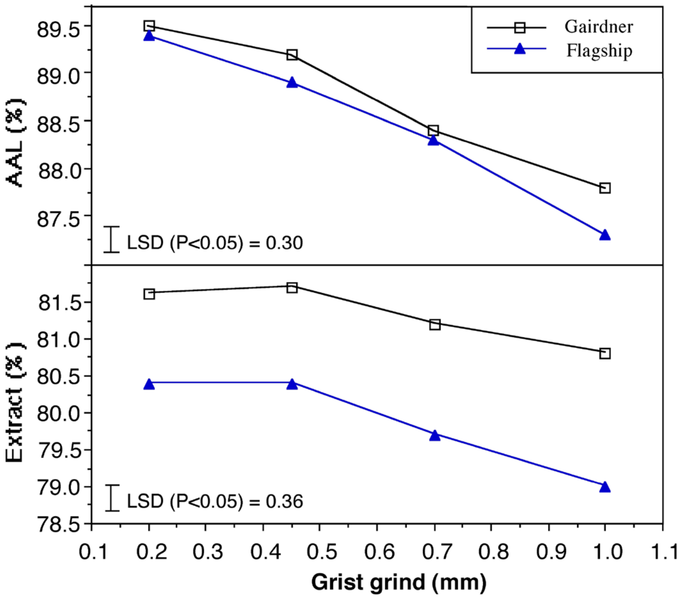

Fig. 6 **Effect of grist grind fineness. Mashing includes 2.2 mM $CaSO_4.2H_2O$ – 88 ppm Ca^{2+}, addition on a 65 °C for 50 min duration, 1:4 G:W ratio, finish temperature at 74 °C for mash out (lautering at 1:9 G:W ratio, RT). Figure reproduced with permission by the ASBC© from Evans [37]**

graded. The sieve classification proportions in table 4 essentially quantitate these observations. The performance of the various disc mill grinds in small-scale mashing are shown in figure 6. Extract increases as disc gap decreases from 1.0 mm to 0.4 mm (mash: 65 °C for 50 min, 1:4 G:W, 2.9 mM $CaSO_4$ – 116 ppm Ca^{2+}, mash out 74 °C, lautering at 1:9 G:W ratio, RT), where after extract level was essentially constant. AAL on the other hand increased consistently from 1.0 mm to 0.2 mm (~ 2 % points). The rationale for the AAL increase will be discussed, along with grist to water ratio, in the next section. Both Evans et al. [37] and Stenholm et al. [102, 103] both settled on the 0.7 mm grist grind as optimal because discussions with commercial brewers indicated that they regarded this grind as being the closest match to their experience. For potential of lautering efficiency, a G:W ratio of < 1:4 would be in keeping with commercial practice [102], however most small-scale mashes separate at 1:9 G:W which ensures laboratory efficiency and avoids the requirement of sparging (Table 1).

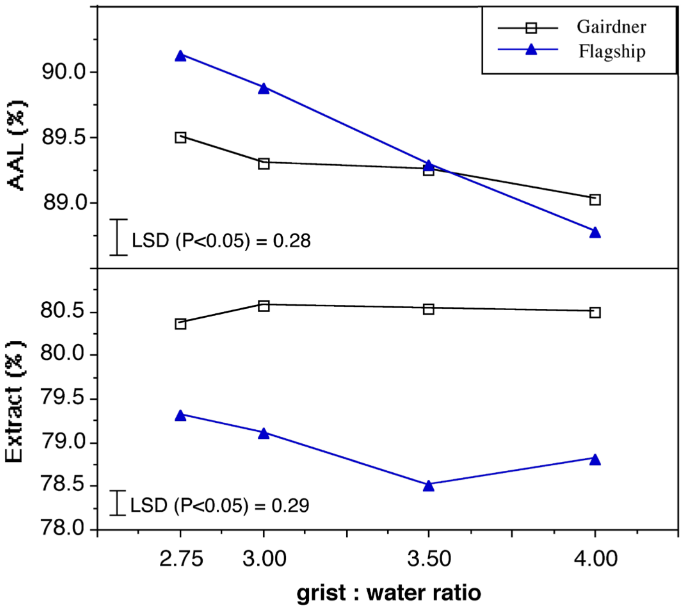

Fig. 7 **Effect of G:W. Mashing includes 2.2 – 3.0 mM $CaSO_4.2H_2O$ – 88 – 116 ppm Ca^{2+}, addition on a 65 °C for 50 min, 0.7 mm grist grind, finish temperature at 74 °C for mash out (lautering at 1:9 G:W ratio, RT). Figure reproduced with permission by the ASBC© from Evans [37]**

D. Grist to Water ratio – gravity

The G:W for the fine grind (0.2 mm) Congress mash is 1:4 [2, 111]. Axcell [8] identified the gravity (high gravity brewing) of the small-scale mash as one of the primary deficiencies of the Congress mash protocol. Figure 7 outlines the effect of G:W (1:4 to 1:2.75) on the extract and AAL parameters (mash: 65 °C for 50 min, 0.7 mm grist grind, 1:4 G:W, 2.2 – 2.9 mM $CaSO_4$ – 88 – 116 ppm Ca^{2+}, mash out 74 °C, lautering at 1:9 G:W ratio, RT). For the two malt samples used, extract appears to be only slightly affected by G:W ratio within the range observed. In contrast, AAL increases as the mash becomes thicker, especially for the Flagship (Sd2H) sample. The positive influence of mash thickness on AAL is perhaps not surprising as relatively thermolabile enzymes such as beta-amylase and LD can be thermally stabilized in the presence of other proteins or solutes. Such a conclusion is in line with the basic biochemical principles [6, 10]. An extreme example is the commonly used Betamyl assay (Megazyme) that relies on BSA (bovine serum albumin) to stabilize dilute malt beta-amylase solutions for measurement [78]. Not surprisingly, it has been observed that increasing modification/KI (soluble N) [36, 90] and Henson and Duke [51, 53] found increased malt solutes/osmolytes were indicators of malt quality and AAL in particular. Thus, it is also reasonable to propose that finer milled grists would potentially have greater and earlier (during mash) solute solubilization, which explains the rising AAL in figure 6.

There are however, limits to how a low G:W or high a gravity of mash can be practically performed. Muller [83], observed that mashing performance (grist 0.2 mm, disc milled) was very poor at a GW of 1:2, acceptable at 1:3 and somewhat variable for 1:3 – 1:2.5. Moll and Flayeux [81] concluded from trials with a 0.2 mm grist (disc milled) that 1:3.5 G:W were acceptable. These authors noted the extract performance of thicker mashes were variable, with different malt varieties/samples displaying differing performance. One of the primary factors in determining mashing performance with respect of G:W ratio is the influence of water availability for starch gelatinization [23]. de Schepper and Courtin [23] observed that thicker – higher gravity mashes (0.2 mM grist, disc milled), that mash sugar yield decreased, particularly at G:W ratios less than 1:4. Although a 0.7 mm grist grind was used for figure 7, de Schepper and Courtin [23] observations do not appear to be in accord with those of figure 7 and figure 3. These authors used a 45-62°C ramped mash compared to mashing in at 65°C. It would appear that at lower mash temperatures (e.g., 62°C) that higher proportions of water are required to achieve 'rapid' starch gelatinization and hydrolysis. Such an understanding may be valuable option for brewers seeking to produce beers with increased body or low alcohol beers? From a practical perspective, there is as yet no published reports from brewers practicing high gravity (wort 14 – 17 °P, 6 – 8 % beer ABV) [32] or even very high gravity brewing (18 – 25 °P, beer > 8 % ABV) would have noticed and complained

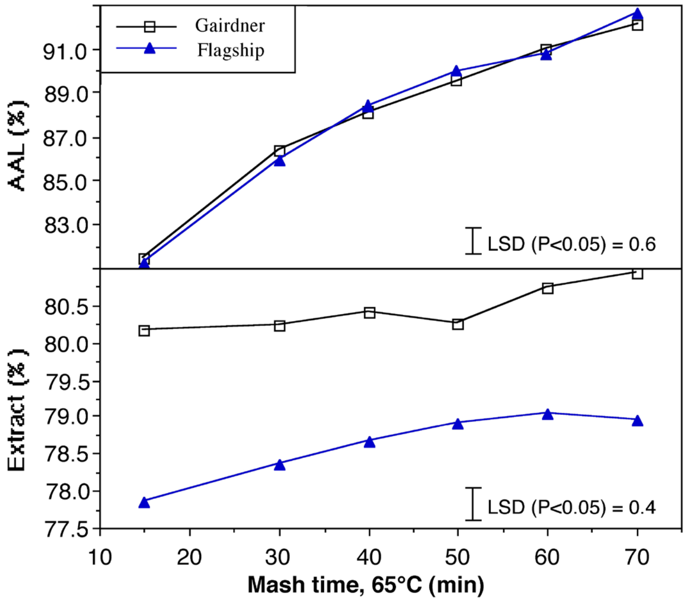

Fig. 8 **Effect of mashing time at 65 °C. Mashing includes 2.9 mM $CaSO_{4.}2H_2O$ – 116 ppm Ca^{2+}, addition on a 65 °C for 0.7 mm grist grind, 1:3 G:W, finish temperature at 74 °C for mash out (lautering at 1:9 G:W ratio, RT). Figure reproduced with permission by the ASBC© from Evans [37]**

about such an issue with their brewing model [105, 106]?

Maltose is another factor with respect to potential enzyme thermostability and inhibition, particularly in the mid to later stages of mashing when maltose level is elevated. It has been concluded that maltose can both stabilize beta-amylase activity [52, 90], while it has been reported that maltose is a potential inhibitor of beta-amylase [24]. Investigators need to be wary as maltose can competitively inhibit low dp substrates for beta-amylase with a Ki of 20 – 30 mM [72], that can impact the use of conventional beta-amylase substrates in mash time or thermostability studies.

In conclusion, considering estimations of practical brewing parameters G:W (Table 1), the mash performance observed in figure 7, with the discussion about, it was concluded that the 1:3 G:W with 0.7 mm grist was a satisfactory compromise, as chosen by Evans et al. [37]. However, further study is warranted of a ~ 0.4 mm grist grind to maximize mash extract as modern commercial lauter tuns recover ~ 98 % and mash filters ~ 101 % of laboratory extract [3, 113]. Despite the 1:7 G:W of the Isothermal 65 °C small scale mash protocol (Table 1) saves one step in the malt quality laboratory, it may be inappropriate, despite its fine 0.2 mm grist grind because the enzyme protecting solutes would at a much lower concentration that 1:3 G:W small scale mash and conventional modern commercial brewing practice (Table 1).

E. Mash time

The final major mash parameter that requires consideration is the duration of the mash. A useful starting point would be to propose that the mash time should be sufficient to ensure that extract solubilization and starch hydrolysis should be near the end point but not so long to slow operations within a malt quality testing facility. Figure 8 assessed the influence of mash duration on extract recovery and AAL performance (mash: 65 °C for 15 – 70 min, 0.7 mm grist grind, 1:3 G:W, 2.9 mM $CaSO_{4.}2H_2O$ – 116 ppm Ca^{2+}, mash out 74 °C, lautering at 1:9 G:W ratio, RT). Extract recovery increased gradually between 15 min to ~ 60 min where the Flagship sample extract increased 1.1 % and the Gairdner sample 0.5 %. In contrast, the AAL observed for both varieties assessed increased 10.5 % for both Flagship (Sd2H beta-amylase variety) and Gairdner (Sd1 beta-amylase variety). Interestingly, the AAL for both variety samples was 81.3/81.4 % respectively, after just 15 min of mashing suggesting that the majority of starch hydrolysis occurred relatively rapidly during this time. A mash time of 60 min with the above mash parameters would be expected to provide brewers with an indication of the maximal level of fermentable sugars that a malt can produce/performance. Depending on the beer style to be brewed the brewer could select brewing parameters and times to modulate the production of fermentable sugars to the style of beer desired, be that light/lite, full bodied or low alcohol beer.

Evans and Fox [34], observed with the MIM 65 °C mash that after 60 min of mashing, that there had been little loss of α-amylase activity while approximately 50 % of beta-amylase and LD activity could be recovered at this time in the mash. With a thinner 1:6 G:W mash, de Schepper et al. [24, 25] observed that after 60 min of mashing α-amylase was partially inactivated and approximately 10 – 20 % of beta-amylase activity remained. It remains to be determined if the increases in AAL after 15 or 30 min of mashing are more the result of combined DP enzyme action or increasingly α-amylase action with regards to freshly gelatinized starch [71]. If so, this period could be considered as that of extended saccharification.

F. Wort boiling and trub removal

A final minor query to resolve with reference to commercial brewing practice, is to ascertain if wort boiling and trub removal impact small-scale mashing determination of extract and AAL. Evans et al. [37] conducted an experiment using four commercial malt varieties that were small-scale mashed (65 °C for 60 min, 0.7 mm grist grind, 1:3 G:W, 2.9 mM $CaSO_4$ – 116 ppm Ca^{2+}, mash out 74 °C, lautering at 1:9 G:W ratio, RT), with the separated wort boiled for 60 min with a carrageenan fining agent that promotes trub formation (Whirlfloc T P424, Deltagen) being added 15 min into the boil. After boiling the wort was cool to RT and centrifuged to remove the trub formed. No significant changes were observed in either the resultant extract or AAL small-scale measures. Wort boiling and trub removal could, however, have impacts in commercial brewing such as reducing FAN and FAN assimilation across fermentation. The work of Kuhbeck et al. [66, 67] showed trub can promote conventional fermentation. However, the rapid attenuation test uses a very high yeast pitching rate, aeration and constant shaking over 24 hrs at 21 °C [35] which would nullify any putative benefits that would be contributed by trub. No change to the small-scale mashing protocol from the section E. Mash time above was made.

3 Comparison of Small-Scale Mash Outcomes

It was Axcell's contention that modernization of the CoA for malt analysis would better predict malt performance during brewing, now has a firm basis for discussion. Axcell [7-9] Central to that discussion are the parameters selected for the small-scale mash

Table 5 **Comparison of small scale mash malt quality outcomes. Congress, Isothermal 65 °C and MIM 65 °C protocols (Table 1). The same set of 29 commercial Australian malts (set A) was assessed by both the MIM 65 °C and Congress mashes [37], while 64 European malts (set B) were assessed with both the Congress and Isothermal 65 °C and protocols [47]**

Malt parameter	Mash program	Average	Minimum	Maximum
Extract (% dwt)	MIM 65 °C	79.0	75.8	81.7
	Congress set A	80.1	77.3	82.6
	Congress set B	83.2	81.4	85.6
	Isothermal 65 °C	83.0	80.4	?
AAL (%)	MIM 65 °C	88.9	83.9	93.9
	Congress set A	82.4	77.8	87.9
	Congress set B	84.6	77.5	88.9
	Isothermal 65 °C	87.9	81.3	92.9
Wort FAN (mg/L)	MIM 65 °C	150	118	198
	Congress set A	165	134	203
	Congress set B	165	115	226
	Isothermal 65 °C	144	108	196
Soluble N (mg/L)	Congress set A	669	558	870
	Isothermal 65 °C	611	510	775
Bradford N (mg/L)	MIM 65 °C	327	235	448
	Congress set A	367	266	588
KI (%)	Congress set B	45.6	36.3	53.7
	Isothermal 65 °C	41.8	33.9	49.6
Wort β-glucan (mg/L)	MIM 65 °C	114	47	362
	Congress set A	113	43	272
	Congress set B	101	15	313
	Isothermal 65 °C	195	14	612
Wort viscosity (mPa.s)	MIM 65 °C[1]	1.472	1.416	1.654
	Congress set B	1.443	1.400	1.492
	Isothermal 65 °C	1.478	1.425	1.579

[1] Data extracted from Evans et al.,[40] n = 92 commercially malted Australian and Canadian barley

that are pivotal in defining most of the key malt parameters such as extract, KI, wort β-glucan, viscosity etc. Gastl et al. [43] concludes that the small scale Congress, by way of the 45 °C mash rest step, provides too great a concession towards the modification action of cell wall (and protein) hydolyzing enzymes whose mash thermostability is < 60 °C (Table 2). Such mashing conditions are well suited to the less homogenous and well modified (higher β-glucan) malts of century ago. Given that modern malts are superior and more homogenous, today's brewers can largely overlook the low temperature mash stand (45 °C) which also minimizes the foam and flavor stability damaging action of lipoxygenases (Table 3) [32, 33]. As a consequence, modern brewers undertaking full malt mashes (Table 1) tend to mash in for the first rest at 62 – 65 °C, although unmalted adjunct brews are typically mashed in at ~ 50 °C to accommodate the inclusion of the 'cooked' (~ 90 – 100 °C) as a form of 'decoction' mashing regime.

It follows that a mash in temperature of 65 °C is an appropriate compromise between malt starch gelatinization temperature and the persistence of beta-amylase and LD if maximizing fermentable sugar yield is the brewer's objective (Table 3, 4, Fig. 3, 4). Both the small-scale Isothermal 65 °C and MIM 65 °C protocols (Table 1) mash in at 65 °C to provide brewers, maltsters and barley breeders a measure of the maximum for amylolytic modification [43]. Gastl and her colleague's motivation was to provide a superior assessment of malt quality for modern malting barley breeders lines and putative varieties to more accurately selected improved modern brewing performance for the selection of new malting barley varieties from the German "Berliner Programm" (formerly the "Frankfurter Programm").

A comparison of selected malt quality parameters derived by the Congress, Isothermal 65 °C and MIM 65 °C small-scale mash methods is provided in table 5. These key malt quality parameters are assessed in turn as follows.

Extract: Comparing a fine grind Congress/Isothermal 65 °C (0.2 mm), with the coarse grind (0.7 mm) MIM 65 °C mash which was on average 1.1 % lower. This is broadly in line with what would be expected for a Fine-Coarse difference. Comparing the Congress and Isothermal 65 °C mashes, that use a 0.2 mm grind, it was observed that the Isothermal 65 °C is ~ 0.2 % lower on average which may be attributable to the thin 1:7 G:W which is probably not as protective of thermolabile enzymes. In practice, modern breweries mashing followed by mash separation by lauter tun yields ~ 98% of laboratory extract, while mash filters achieve ~ 101 % of laboratory extract [3, 113]. Although the 0.7 mm grind grist appears closer to brewer reality (Table 4, Fig. 5), the 0.2 mm grind is perhaps more accurate for estimating the critical extract parameter. Figure 5 suggests an 0.4 mm (or slightly coarser) option, between the 0.2 and 0.7 mm grinds which yields the same level of extract and almost the same level of AAL.

Attenuation (AAL): The MIM 65 °C mash protocol was optimized to maximize fermentable sugar release. Accordingly, it provides a 6.5 % increase over the Congress mash (Table 5) due to optimization of parameters for a 1:3 G:W ratio (DP enzyme osmolyte protection), mashing in at 65 °C (Fig. 3) and a 60 min at 65 °C (Fig. 8). A shift to a 0.2 mm grind may result in a slight increase in AAL (Fig. 6,7). Not surprisingly, the 1:7 G:W for the Isothermal 65 °C mashed malts only increased AAL by 3.3 % compared to the comparative Congress mashes (1:4 G:W). Such an outcome is almost certainly the result of the reduced solute/osmolyte mash environment for the DP enzymes in the Isothermal 65 °C mash thus contravenes the stipulation of that the small scale mash should be conducted at 'high gravity' [8]. To commercial brewers average AAL levels approaching 90 % would on first appearance appear unrealistically high. However, the rapid attenuation test uses a very high yeast pitching rate, aeration and constant shaking over 24 h at 21 °C [35] ensuring minimal yeast growth and near complete fermentable sure utilisation. Brewers have traditionally calculated a "real degree of fermentation," [20, 21, 58] which uses a factor (~ 0.81) to correct AAL to that which is more realistic in practical brewing. The factor contains allowances for extract used for yeast growth and metabolism and other factors.

Wort FAN, Soluble N, Bradford protein and KI: In terms of wort FAN, the Congress mash, as a function of the mild initial 45 °C rest (protease) mash conditions (Table 2). Table 5 shows that higher FAN levels were generated by the Congress mash, than the MIM 65 °C mash which was commensurately higher than the Isothermal 65 °C mash. The differential between the MIM 65 °C (1:3 G:W) and Isothermal 65 °C (1:7 G:W) mashes can potentially be explained by the more dilute nature of the Isothermal 65 °C mash. Figure 4 shows that FAN production during mashing peaks at ~ 55 °C and reduces consistently as mashing temperature is increased. Still at 65 °C there is significant FAN generation in a 65°C mash and this would be expected be assisted by the 1:3 G:W mash conditions of the MIM 65 °C mash (thermal protection of enzymes). These results demonstrated that despite the recorded thermolability of the FAN generating protease enzymes (Table 2), they still have significant activity to generate FAN at mash temperatures of 65 °C and above. The related results comparing the Congress and Isothermal 65 °C mashes, essentially conform to the discussion for FAN. Finally wort protein measured by the Bradford (Coomassie blue binding assay) is considered to be an indication of foam positive protein in beer and presumably wort [33, 37]. It is also noted that the MIM 65 °C mash extracts slightly less Bradford protein than the Congress mash.

Wort β-glucan and viscosity: The cell wall hydrolyzing enzymes have similar levels of mash thermostability as the proteolytic enzymes (Table 2). Similar to FAN, the levels of wort β-glucan and viscosity are slightly lower when comparing the Congress and MIM 65 °C mashes with the Isothermal 65 °C mash being slightly higher (Table 5). These observations further underline the greater putative persistence of the cell wall hydrolyzing enzymes. The averages for wort viscosity suggest that all three mash protocols produce wort quite similar viscosities. Given that the levels of wort β-glucan appear adequately controlled by good malting and brewing practice, it appears to reduce wort viscosity and subsequent inefficiencies in mash separation and beer filtration that other culprits such as arabinoxylan/xylanase further assessment [19, 27, 40, 94, 96].

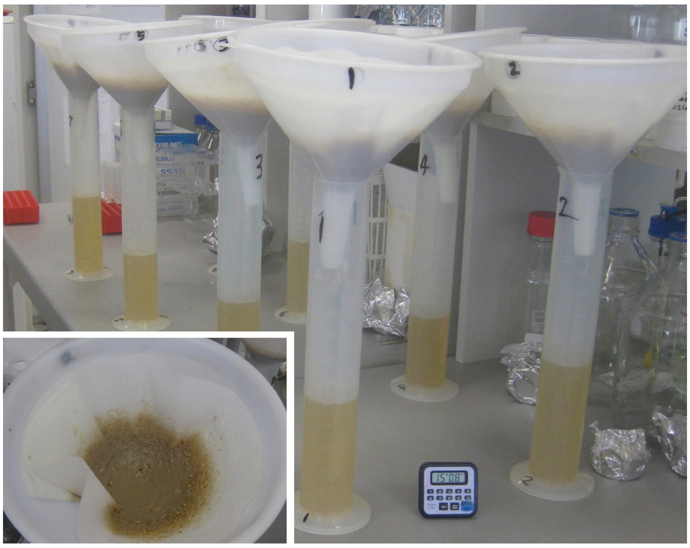

Fig. 9 A small-scale, inexpensive, efficient and functional lautering efficiency test that can be added on to the mash protocol [37]. Figure reproduced with permission by the ASBC and MBAA© from Evans [32]

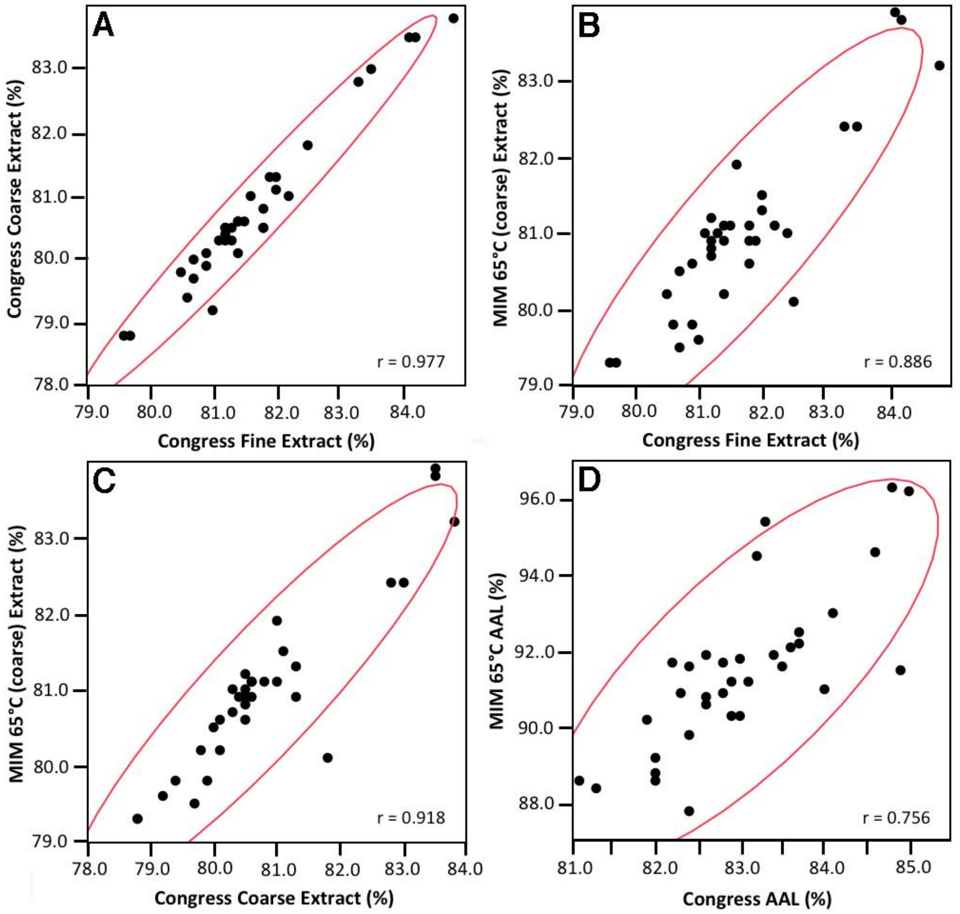

Fig. 10 Scatterplots showing the relationships between A. Congress extracts, B. MIM 65 °C coarse and Congress fine extract, C. MIM 65 °C coarse and Congress coarse extract and D. Congress and MIM 65 °C AAL. Reproduced from Cornaggia et al. [19], with permission from the Journal of the Institute of Brewing

Over the years there have been a number of concerted attempts

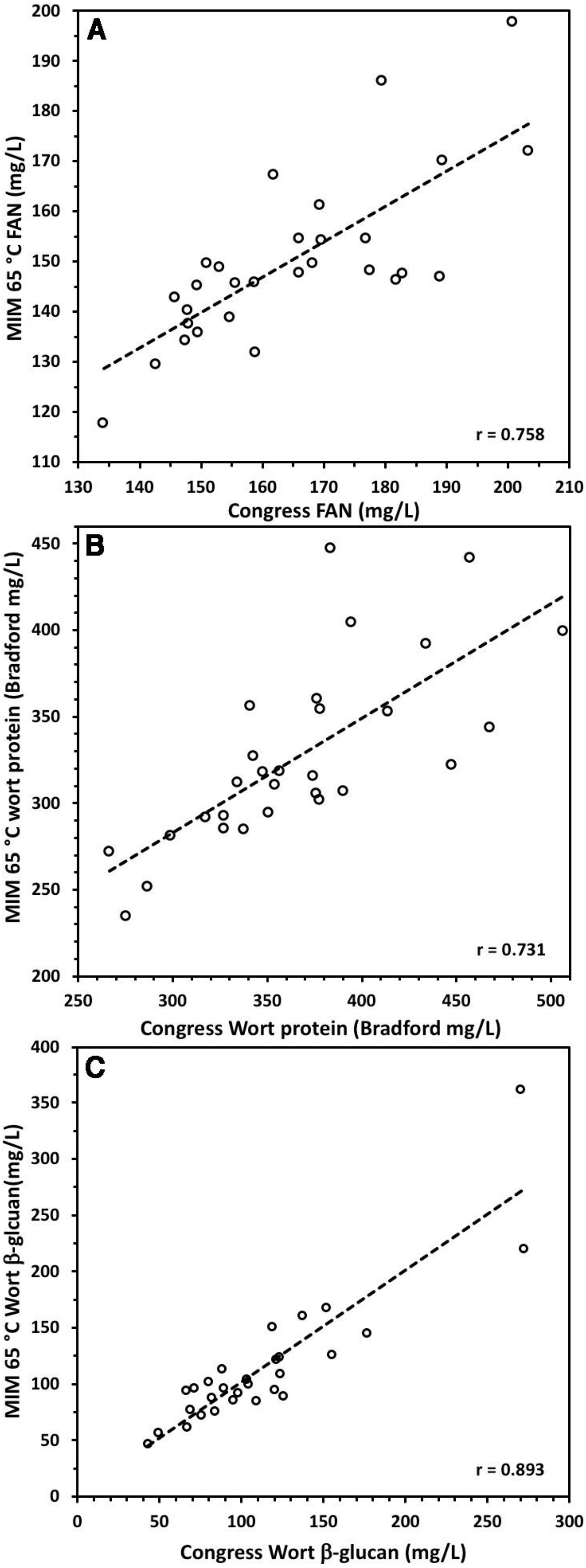

Fig. 11 **Scatterplots showing the relationships between A. Congress and MIM 65 °C wort FAN; B. Congress and MIM 65 °C wort protein (Bradford); and C. Congress and MIM 65 °C wort β-glucan (From unpublished data from Evans et al [37])**

to develop a small-scale lautering test. Moll and Flayeux [80, 81] developed an elegant system of lautering analysis that was conducted at practical brewing temperatures (80 °C) relying on a manual time course of lautering performance that was very time consuming to run in the laboratory. Of course, commercial mash separation is conducted with further hot water additions or sparges. Stenholm et al. [102] also measured the time course of lautering in an attemperated system at 80 °C with a thick mash (1:5 G:W), but this too was very time consuming to operate. Hot lautering also introduces problems with respect to evaporation for malt quality parameters based on wort concentration (e.g., extract, KI, etc.). Evans et al. [37] applied the "KISS principle" ('Keep it simple stupid' thought to have been first coined by Lockheed aircraft engineer, Kelly Johnson c1960 SR-71 Blackbird). In itself, it is a derivation of Occam's Razor attributed to 14th Century philosopher, William of Occam) famously adapted for Bill Clintons 1997 presidential election campaign. Figure 9 demonstrates the 'end-point' (25 min) measurement of mash separation at RT (1:9 G:W) collected into 500 ml measuring cylinders through Machery-Nagel MN-722 filters. In addition, the traditional practice of returning the first ~ 50ml of the 'lautered wort' to be refiltered was deemed unnecessary, as the MN-722 filters are sufficiently fine. Also, the mash filter papers were not 'fluted' as this action was observed to make little difference and potentially added experimental variation.

This small-scale lautering protocol adds little extra laboratory work but surprisingly, the results were reasonably reproduceable and could coarsely discriminate between different malt. Importantly, inclusion of exogenous glucanase and/or cellulase enzymes substantially increased the amount of wort measured [37]. This lautering test appears to provide discrimination between malts despite the thinner G:W ratio despite the absence of sparging, both of which would increase the time taken and require substantial extra laboratory labour. Interestingly, Evans et al. [40] observed that over a period of approximately a decade that the lautering efficiency measure by this test of commercial malt samples improved substantially. Whether this is a case of superior malting, malting varieties or just an unexplained seasonal seasonal quirk Axcell et al.[9] is yet to be determined. The wort collected can also be easily used for assessing wort/beer filtration by the SWIFT test,[104] which has been found to be correlated with wort viscosity, xylanse, KI and wort β-glucan [19, 40].

A major consideration with the Berliner Programm's objective to modernise the Congress mash protocol was in relation to selection rankings for malt quality parameters. In breeding programs, the setting of selection indices (e.g., percentage of breeder's lines discarded ~ 33 %) is intrinsically linked to the ranking a particular line achieves over a series of quality and agronomic parameters. Gastl et al. [44, 46, 48] noted that the correlations between Congress and Iso 65 °C mashes was r < 1.0 for parameters including extract, attenuation, soluble N, FAN, β-glucan and viscosity. Figure 10 provides a snapshot of those correlations between parameters derived from the Congress compared with the MIM 65 °C mash. As would be expected Congress fine (0.2 mm) and coarse extract (0.7 mm, Fig. 10A) were highly correlated (r = 0.977). In contrast the correlation between MIM 65 °C extract (0.7 mm) and Congress fine (Fig 10B) and coarse (Fig. 10C) were reduced, r = 0.866 and r = 0.918 respectively. For the comparison between MIM 65 °C and Congress

AAL (Fig. 10D) was substantially reduced (r = 0.756). Presumably, the modernisation of the Congress procedure for the MIM 65 °C mash in terms of the 1:3 G:W, temperature program (65 °C), mash time at 65 °C for 60 min result in these difference that would also substantially change the malt sample ranking. Further, Figure 11 compares the Congress mash for the amount of wort FAN (Fig. 11A), Bradford protein (Fig. 11B) and β-glucan (Fig. 11C) with equivalent analyses. Broadly these correlations are broadly consistent with Gastl et al. [44-46, 48] in that the comparative measures were well correlated but provided substantially different rankings between the Congress and 65 °C mashing method.

4 Conclusions

This review commenced with the provocative question, "Are the days of Congress mashing over?" [102] We contend that Stenholm was correct and that the 100 – 200 year old Congress small-scale mash should have been superseded long ago. With respect to Axcell's [8, 9] plea to malt analysis to more effectively reflect modern brewing practice with respect to high gravity brewing, lautering, potential haze formation, foam, and flavor stability, this review provides a number of positive options for approaching these goals.

The review systematically assesses, based on a Congress mash protocol starting point, ways in which a new protocol could be instituted to more closely emulate modern commercial brewing practice. To begin with, it was demonstrated that a 65 °C mash in temperature was the optimal compromise between starch gelatinization and preservation of the thermolabile starch degrading enzymes, primarily beta-amylase and LD. A grist grind of 0.7 mm using a disc mill was identified as perhaps the closest to that produced by a commercial six roller dry mill. However, a 0.2 mm grist grind produced ~ 1 % higher extract which needs to be considered because modern lauter tun and mash filters approach or exceed 100 % of Congress extract in practice. Possibly the compromise of a ~ 0.4 mm grind could be explored. The higher 1:3 G:W (higher gravity) was deemed optimal compared to the Congress mash (1:4 G:W). The higher G:W results in improved enzyme stability due to increased mash solute/osmolyte concentrations. Although the wort AAL was approximately 82 % 15 min into a mash, the optimum time for maximal starch hydrolysis into fermentable sugars was observed to be 60 min for a 65 °C mash. Such a mash as outlined above (65 °C for 60 min, 0.7 mm grist grind, 1:3 G:W, mash out 78 °C, lautering at 1:9 G:W ratio, RT) was found amenable to assess the lautering performance of the malt sample simply with minimal new equipment of time requirements for staff. Given that brewers typically add Ca^{2+} at 40 – 70 ppm (1.0 – 1.75 mM) to the mash liquor (Daniel Carey, Per. Com.) it was deemed prudent to add 1.4 mM Ca^{2+}- 56 ppm Ca^{2+}, (e.g., $CaSO_{4.}2H_2O$ or $CaCl_2$) to the mash liquor. Overall, it was found that different protocols could result in different analytical rankings (e.g., AAL, FAN, KI etc.) which is particularly important to malting barley programs seeking to expedite progress towards developing new and superior malting varieties.

Testing for potential foam, haze formation and flavor stability is problematic as our understanding of the important beer traits are either poorly understood as yet or are heavily dependent on brewer's choices during the brewing process. Possibly the simple measure of Bradford protein in wort may be of assistance as this measure generally biased in favour of foam positive proteins. A more modern determination of KI (protease action) could also be of assistance for foam stability A further option would be select lipoxygenase null varieties [55, 56, 99, 108, 117] which reduce the production of the very foam damaging lipid hydroperoxides [62, 63, 114]. A valuable benefit is lipoxygenase null malt will also produce less beer staling compounds. With respect to beer flavour, recent studies have suggested shown differences between malt samples, particularly with reference to the level of malt protein and KI [17, 54, 107]. Finally, with respect to beer colloidal stability, a number of proteins have been identified to promote the development of beer haze including a barley trypsin inhibitor CMe (BTI-CMe) and other barley trypsin and amylase inhibitors [59, 92, 93].

Acknowledgements

We would like to thank for critical contributions and comments Prof Barry Axcell (former Chief Brewer SABMiller, RSA), Dan Carey (New Glarus Brewing, WI, USA), Prof Martina Gastl (TUM, Germany), Prof Patrick Hayes (Oregon State University, OR, USA), and Dr Xiang Yin (Boortmalt, MN, USA),. Participants at the joint Euromalt-EBC workshop on the isothermal-65 vs. Congress mash procedures held on 26 October 2023, at The Brewers of Europe House at Rue Caroly 23 – 25, 1050 Brussels, are equally acknowledged for their thought-provoking and detailed discussions during that workshop.

5 References

1. American-Society-of-Brewing-Chemists, Malt-4 (Extract) and Wort-18 (β-glucan in congress wort by fluorescence), Methods of Analysis, 9th Edition, The ASBC: St. Paul, MN, 2009.
2. American-Society-of-Brewing-Chemists Methods of Analysis, St. Paul, MN.: American Society of Brewing Chemists, Online.
3. Andrews, J.: A review of progress in mash separation technology, Master Brewers Association of the Americas Technical Quarterly, **41** (2004), no. 1, pp. 45-49.
4. ANON Malt analysis: Industry-wide change in analysis approach for barley malt 5 EBC, BRAUWELT International, 2020.
5. Ao, Z. and Jane, J.-L.: Characterisation and modelling of the A- and B-granule starches of wheat, triticale and barley, Carbohydrate Polymers, **67** (2007), no. 1, pp. 46-55, DOI: 10.1016/j.carbpol.2006.04.013.
6. Arakawa, T. and Timasheff, S.N.: Stabilization of protein structure by sugars, Biochemistry, **21** (1982), no. 25, pp. 6536-6544, DOI: 10.1021/bi00268a033.
7. Axcell, B.C.: Don't let sleeping dogmas lie – a personal journey in brewing, Brewer and Distiller International, **14** (2018), pp. 28-33.
8. Axcell, B.C.: Malt analysis – prediction or predicament, Master Brewers Association of the Americas Technical Quarterly, **35** (1998), no. 1, pp. 28-30.
9. Axcell, B.C., Morrall, P., Tulej, R. and Murray, J.: Malt quality specifications – A safeguard or restriction on quality, Master Brewers Association of the Americas Technical Quarterly, **21** (1984), no. 3, pp. 101-106.
10. Back, J.F., Oakenfull, D. and Smith, M.B.: Increased themal stability of proteins in the presence of sugars and polyols, Biochemistry, **18** (1979), no. 23, pp. 5191-5196, DOI: 10.1021/bi00590a025.

11. Back, W., and Narziss, L.: Malt parameters and beer quality, BRAUWELT International, **15** (1997), pp. 29-37.
12. Bamforth, C.W.: Barley and malt starch in brewing: a general review, Master Brewers Association of the Americas Technical Quarterly, **40** (2003), no. 2, pp. 89-97.
13. Bamforth, C.W.: pH in brewing: an overview, Master Brewers Association of the Americas Technical Quarterly, **38** (2001), no. 1, pp. 1-9.
14. Bamforth, C.W. and Fox, G.P.: Critical aspects of starch in brewing, BrewingScience, **73** (2020), no. 9/10, pp. 126-139, DOI: 10.23763/BrSc20-16bamforth.
15. Bekkers, A., Vissenaekens, J. and Baks, T.: Attenuation limit of malt as a function of gelatinisation characteristics of starch. European Brewery Convention Congress, Proceedings. Venice, p. presentation #19, 2007.
16. Bertoft, B., Andtfolk, C. and Kulp, S.-E.: Effect of pH, temperature, and calcium ions on barley malt α-amylase isoenzymes, Journal of the Institute of Brewing, **90** (1984), no. 5, pp. 298-302.
17. Bettenhausen, H.M., Barr L., Broeckling, C.D., Chaparro, J.M., Holbrook, C., Sedin, D. and Heuberger, A.L.: Influence of malt source on beer chemistry, flavor, and flavor stability, Food Research International, **113** (2018), pp. 487-504, DOI: 10.1016/j.foodres.2018.07.024.
18. Briggs, D.E., Boulton, C.A., Brookes, P.A. and Stevens, R.: Brewing: science and practice. New York: CRC Press, 2004.
19. Cornaggia, C., Evans, D.E., Draga, A, Mangan, D. and McCleary, B.V.: Prediction of potential malt extract and beer filterability from conventional and novel malt quality assessment parameters, Journal of the Institute of Brewing, **125** (2019), no. 3, pp. 294-309, DOI: 10.1002/jib.567.
20. Cutaia, A.J. and Munroe, J.H.: A method for the consistent estimation of real degree of fermentation, Journal American Society of Brewing Chemists, **37** (1979), no. 4, pp. 188-189.
21. Cutaia, A.J., Reid, A.-J. and Speers, R.A.: Examination of relationships between original, real and apparent extracts, and alcohol in pilot plant and commercially produced beers, Journal of the Institute of Brewing, **115** (2009), no. 4, pp. 318-327.
22. De Rouck, G., Jaskula, B., De Causmaecker, B., Malfliet, S., Van Opstaele, F., De Clippeleer, J., De Brabanter, J., De Cooman, L. and Aerts, G.: The influence of very thick and fast mashing conditions on wort composition, Journal American Society of the Brewing Chemists, **71** (2013), no. 1, pp. 1-14.
23. De Schepper, C.F. and Courtin, C.M.: High mashing thickness negatively infuences gelatinisation of small and large starch granules and starch conversion effciency during barley malt brewing, Food Hydrocolloids, **131** (2022), p. 107745, DOI: 10.1016/j.foodhyd.2022.107745.
24. De Schepper, C.F., Buv́ e, C., Van Loey, A.M. and Courtin, C.M.: A kinetic study on the thermal inactivation of barley malt α-amylase and β-amylase during the mashing process, Food Research International, **157** (2022), p. 111201, DOI: 10.1016/j.foodres.2022.111201.
25. De Schepper, C.F., Michiels, P., Buv́ e, C., Van Loey, A.M. and Courtin, C.M.: Starch hydrolysis during mashing: A study of the activity and thermal inactivation kinetics of barley malt α-amylase and β-amylase, Carbohydrate Polymers, **255** (2021), p. 117494, DOI: 10.1016/j.carbpol.2020.117494.
26. Edney, M.J., Eglinton, J.K., Collins, H.M., Barr, A.R., Legg, W.G. and Rossnagel, B.G.: Effects of the high heat stable beta-amylase allele on fermentability of malts with low vs high diastatic power, European Brewery Convention Congress, Proceedings. Venice, p. paper #50, 2007.
27. Egi, A., Speers, R.A. and Schwarz, P.B.: Arabinoxylans and their behaviour during malting and brewing, Master Brewers Association of the Americas Technical Quarterly, **41** (2004), no. 3, pp. 248-267.
28. Eglinton, J.K., Langridge, P. and Evans, D.E.: Thermostability variation in alleles of barley beta-amylase, Journal of Cereal Science, **28** (1998), no. 3, pp. 301-309, DOI: 10.1016/S0733-5210(98)90010-8.
29. Euromalt-EBC Isothermal-65 °C & Congress Mash Procedures Workshop, 26 October 2023 at The Brewers of Europe, Brussels – protocol and recommendations (unpublished). 2023.
30. European-Brewery-Convention, 4.6 Hot water extraction of malt: constant temperature mash, Analytica-EBC, E.A. Committee, Editor, Verlag Hans Carl.: Nurnberg, Germany. p. 1-3, 2020.
31. European-Brewery-Convention Analytica-EBC, ed. E.A. Committee. Vol. 7th Edition. Nuremberg, Germany: Verlag Hans Carl., 2008.
32. Evans, D.E.: Mashing, Pilot Knob, Minneapolis, MN: American Society of Brewing Chemists and Master Brewers of the Americas, 2021.
33. Evans, D.E. and Bamforth, C.W.: Beer foam: Achieving a suitable head, Handbook of alcoholic beverages: Beer, a quality perspective, C.W. Bamforth, Russell, I., and Stewart, G.G., Editor, Elsevier: Burlington MA. pp. 1-60, 2009. DOI: 10.1016/B978-0-12-669201-3.00001-4.
34. Evans, D.E. and Fox, G.P.: The comparison of DP enzyme release and persistence with the production of yeast fermentable sugars during modified IoB 65 °C and Congress programmed mashes, Journal American Society of Brewing Chemists, **75** (2017), no. 4, pp. 302-311, DOI: 10.1094/ASBCJ-2017-4707-01.
35. Evans, D.E. and Hamet, M.A.G.: The selection of a dried yeast strain for use in the apparent attenuation limit malt analysis (AAL) procedure, Journal of the Institute of Brewing, **111** (2005), no. 2, pp. 209-214, DOI: 10.1002/j.2050-0416.2005.tb00668.x.
36. Evans, D.E., Collins, H.M., Eglinton, J.K. and Wilhelmson, A.: Assessing the impact of the level of diastatic power enzymes and their thermostability on the hydrolysis of starch during wort production to predict malt fermentability, Journal of the American Society of Brewing Chemists, **63** (2005), no. 4, pp. 185-198, DOI: 10.1094/ASBCJ-63-0185.
37. Evans, D.E., Goldsmith, M., Dambergs, R. and Nischwitz, R.: A comprehensive reevaluation of the small-scale Congress mash protocol parameters for determination of extract and fermentability. Journal of the American Society of Brewing Chemists, **69** (2011), no. 1, pp. 13-27, DOI: 10.1094/ASBCJ-2011-0111-01.
38. Evans, D.E., Goldsmith, M., Redd, K.S., Nischwitz, R. and Lentini, A.: Impact of mashing conditions on extract and its fermentability, and the level of wort free amino nitrogen (FAN), β-glucan and lipids, Journal of the American Society of Brewing Chemists, **70** (2012), no. 1, pp. 39-49, DOI: 10.1094/ASBCJ-2012-0103-01.
39. Evans, D.E., Paynter, B.H., Izydorczyk, M.S. and Li, C.: The impact of terroir on barley and malt quality – a critical review, Journal of the Institute of Brewing, **129** (2023), no. 4, pp. 211-258, DOI: 10.58430/jib.v129i4.38.
40. Evans, D.E., Stewart, S.L., Stewart, D.C., Han, Z., Han, P. and Able, J.A.: Profiling malt enzymes related to impact on malt fermentability, lautering and beer filtration performance of 94 commercially produced malt batches, Journal American Society of Brewing Chemists, **80** (2022), no. 4, pp. 413-426, DOI: 10.1080/03610470.2021.1979891.

41. Evans, D.E., van Wegen, B., Ma, Y. and Eglinton, J.K.: The impact of the thermostability of α-amylase, β-amylase and limit dextrinase on potential wort fermentability, Journal of the American Society of Brewing Chemists, **61** (2003), no. 4, pp. 210-218.
42. Fox, G.P., Staunton, M., Agnew, E. and D'Arcy, B.: Effect of varying starch properties and mashing conditions on wort sugar profiles, Journal of the Institute of Brewing, **125** (2019), no. 4, pp. 412-421, DOI: 10.1002/jib.585.
43. Gastl, M.: High quality malting barley – advances in breeding, BRAUWELT International, **30** (2012), no. 1, pp. 22-25.
44. Gastl, M., Becker, T. and Ritenauer, M.: Kongressmaische – isotherme 65 °C-Maische: ein Brückenschlag, BRAUWELT, **156** (2016), no. 45-46, pp. 1360-1363.
45. Gastl, M., Geißinger, C., Kupetz, M., Whitehead, I. and Becker, T. Kongressmaische – isothermes 65°C-Maischverfahren: Auswertung der Frühvermälzung 2018, BRAUWELT, **158** (2018), no. 49, pp. 1470-1474.
46. Gastl, M., Geißinger, C., Whitehead, I. and Becker, T.: Kongressmaische – isothermes 65 °C-Maischverfahren: Auswertung der Frühvermälzung 2017, BRAUWELT, **157** (2017), no. 49, pp. 1462-1466.
47. Gastl, M., Knofel, T. and Becker, T.: Umstellung auf die isotherme 65 °C-Maische – Neubewertung der Qualitätsmerkmale, BRAUWELT, **154** (2014), no. 10, pp. 274-276.
48. Geißinger, C., Whitehead, I., Hofer, K., Hess, M., Habler, K., Becker, T., and Gastl, M.: Influence of Fusarium avenaceum infections on barley malt: Monitoring changes in the albumin fraction of barley during the malting process, International Journal of Food Microbiology, **293** (2019), pp. 7-16, DOI: doi.org/10.1016/j.ijfoodmicro.2018.12.026.
49. Hadaway, T., Evans, D.E., Hodson, B., Ford, C.M. and Logue, S.: Modification of an IEC mash bath to optimize the recovery of brewers extract, Proceedings of the 10th Australian Barley Technical Symposium. Canberra, ACT, 16th-20th September 2001, p. T4, 2001. DOI: http://www.regional.org.au/au/abts/2001/t4/hadaway.htm.
50. Haywood, R.: Brewing adjuncts, The Brewer, **82** (1996), pp. 309-311.
51. Henson, C.A. and Duke, S.H.: A comparison of standard and nonstandard measures of malt quality, Journal of the American Society of Brewing Chemists, **66** (2008), no. 1, pp. 11-19, DOI: 10.1094/ASBCJ-2007-1210-01.
52. Henson, C.A. and Duke, S.H.: Maltose effects on barley malt diastatic power enzyme activity and thermostability at high isothermal mashing temperatures: I. β-amylase, Journal of the American Society of Brewing Chemists, **74** (2016), no. 2, pp. 100-112, DOI: 10.1094/ASBCJ-2016-2734-01.
53. Henson, C.A. and Duke, S.H.: Osmolyte concentrations as an indicator of malt quality, Journal of the American Society of Brewing Chemists, **65** (2007), no. 1, pp. 59-62.
54. Herb, D., Filichikin, T., Fisk, S., Helgerson, L., Hayes, P., Benson, A., Vega, V., Carey, D., Theil, R., Cistue, Jennnings, R., Monsour, R., Tynan, S., Vinkemeir, K., Li, Y., Nguygen, A., Onio, A., Meints, B., Moscou, M., Romagosa, I. and Thomas, W.: Malt modification and its effects on the contribution of barley genotype to beer flavor, Journal American Society of Brewing Chemists, **75** (2017), no. 4, pp. 354-362, DOI: 10.1094/ASBCJ-2017-4976-01.
55. Hirota, N., Kuroda, H., Takoi, K., Kaneko, T., Kaneda, H., Yoshida, I., Takashio, M., Ito, K. and Takeda, K.: Brewing performance of malted lipoxygenase-1 null barley and effect on the flavor stability of beer, Cereal Chemistry, **83** (2006b), no. 3, pp. 250-254.
56. Hirota, N., Kuroda, H., Takoi, T., Kaneko, T., Kaneda, H., Yoshida, I., Takashio, M., T., Ito, K. and Takeda, K.: Development of a novel barley with improved beer foam and flavour stability – the impact of lipoxygenase-1-less barley in the brewing industry, Master Brewers Association of the Americas Technical Quarterly, **43** (2006), pp. 131-135.
57. Hu, S., Dong, J., Fan, W., Yu, J., Yin, H., Huang, S., Liu, J., Huang, S. and Zhang, Y.: The influence of proteolytic and cyctolytic enzymes on starch degradation during mashing, Journal of the Institute of Brewing, **120** (2014), no. 4, pp. 379-384.
58. Huerta-Zurita, R., Horsley, R.D. and Schwarz, P.B.: Is the apparent degree of fermentation a reliable estimator of fermentability, Journal American Society of Brewing Chemists, **77** (2019), no. 1, pp. 1-9, DOI: https://doi.org/10.1080/03610470.2018.1553459.
59. Iimure, T., Nankaku, N., Watanabe-Sugimoto, M., Hirota, N., Tiansu, Z., Kihara, M., Hayashi, K., Ito, K. and Sato, K.: Identification of novel haze-active beer proteins by proteome analysis, Journal of Cereal Science, **49** (2009), no. 1, pp. 141-147.
60. Institute Of Brewing: Methods of analysis. London: Institute of Brewing, 1997.
61. Kerr, E.D., Caboche, C.H., Josh, P. and Schulz, B.L.: Benchtop micromashing: high-throughput, robust, experimental beer brewing, Scientific Reports, **11** (2021), no. 1, p. 1480, DOI: 10.1038/s41598-020-80442-7.
62. Kobayashi, N., Kaneda, H., Kuroda, H., Watari, J., Kurihara, T. and Shinotsuka, K.: Behaviour of mono-, di-, and trihydroxyactadecanoic acids during mashing and methods for controlling their production, Journal of Bioscience and Bioengineering, **90** (2000), no. 1, pp. 69-73.
63. Kobayashi, N., Segawa, S., Umemoto, S., Kuroda, H., Mitani, Y., Watari, J. and Takashio, M.: A new method for evaluating foam-damaging effect by free fatty acids, Journal of the American Society of Brewing Chemists, **60** (2002), no. 1, pp. 37-41.
64. Kohler, P., Krottenthaler, M., Herrmann, M., Kessler, M. and Kühbeck, F.: Tests on infusion and decoction method, BRAUWELT International, **24** (2006), no. 1, pp. 33-37.
65. Kraus-Weyermann, T. and Dornbusch, H.: Are decoction mashes still necessary?, BRAUWELT International, **39** (2021), no. 1, pp. 24-27.
66. Kühbeck, F., Back, W. and Krottenthaler, M.: Influence of lauter turbidity on composition, fermentation performance and beer quality – a review. Journal of the Institute of Brewing, **112** (2006), no. 3, pp. 215-221, DOI: 10.1002/j.2050-0416.2006.tb00716.x.
67. Kühbeck, F., Back, W. and Krottenthaler, M.: Influence of lauter turbidity on wort composition, fermentation, performance and beer quality in large-scale trials, Journal of the Institute of Brewing, **112** (2006), no. 112, pp. 222-231, DOI: 10.1002/j.2050-0416.2006.tb00717.x.
68. Kunze, W.: Technology of brewing and malting, T. Wainwright T., Editor, VLB: Berlin, 1999.
69. Langenaeken, N.A., De Schepper, C.F., De Schutter, D.P. and Courtin, C.M.: Different gelatinization characteristics of small and large barley starch granules impact their enzymatic hydrolysis and sugar production during mashing, Food Chemistry, **295** (2019), no. 15, pp. 138-146, DOI: 10.1016/j.foodchem.2019.05.045.
70. Looseley, M.E., Ramsay, L., Bull, H., Swanston, J.S., Shaw, P.D., Macaulay, M., Booth, A., Russell, J.R., Waugh, R. and Thomas, W.T.: Association mapping of malting quality traits in UK spring and winter

barley cultivar collections, Theoretical and Applied Genetics, **133** (2020), no. 9, pp. 2567-2582, DOI: 10.1007/s00122-020-03618-9.

71. Lund, D.: Influence of time, temperature, moisture, ingredients, and processing conditions on starch gelatinization, Critical Reviews in Food Science and Nutrition, **20** (1984), no. 4, pp. 249-273.
72. Ma, Y., Stewart, D.C., Eglinton, J.K., Logue, S.J., Langridge, P. and Evans, D.E.: Comparitive enzyme kinetics of two allelic forms of barley (*Hordeum vulgare* L.) β-amylase, Journal of Cereal Science, **31** (2000), no. 3, pp. 335-344, DOI: doi:10.1006/jcrs.2000.0303.
73. MacGregor, A.W., Bazin, S.L. and Izydorczyk, M.S.: Gelatinization characteristics and enzyme susceptibility of different types of barley starch in the temperature range 48 – 75 °C, Journal of the Institute of Brewing, **108** (2002), no. 1, pp. 43-47.
74. MacGregor, A.W., Bazin, S.L., Macri, L.J. and Babb, J.C.: Modelling the contribution of alpha-amylase, beta-amylase and limit dextrinase to starch degradation during mashing, Journal of Cereal Science, **29** (1999), no. 2, pp. 161-169, DOI: 10.1006/jcrs.1998.0233.
75. MacGregor, A.W., Marchylo, B.A., and Kruger, J.E.: Multiple α-amylase components in germinated cereal grains determined by isoelectric focusing and chromatofocusing, Cereal Chemistry, **65** (1988), no. 4, pp. 326-333.
76. MacWilliam, I.C.: pH in malting and brewing – a review, Journal of the Institute of Brewing, **81** (1975), no. 1, pp. 65-70.
77. Mallett, J.: Malt: A practical guide from field to brewhouse, Boulder, Colorado, USA: Brewers Publications, 2014.
78. McCleary, B.V. and Codd, R.: Measurement of β-amylase in cereal flours and commercial enzyme preparations, Journal of Cereal Science, **9** (1989), no. 1, pp. 17-33.
79. MEBAK,R-207.00.002 (2016-03), Isothermal 65 °C mash procedure, MEBAK – wort, beer and beer based beverages, F.-J. Methner, Editor. Mebak: Weihenstephan, Germany. p. 205-206, 2020.
80. Moll, M. and Flayeux, R.: Comparison of different malt analyses, Journal of the Institute of Brewing, **92** (1986), no. 6, pp. 572-583.
81. Moll, M. and Flayeux, R.: New mashing system for determination of laboratory extract, Journal of the Institute of Brewing, **87** (1981), no. 6, pp. 345-348.
82. Moll, M., Flayeux, R. and Jeandemange, J.: The analysis of industrial malts by means of the Tepral Method, Monatsschrift für Brauerei, **80** (1980), pp. 170-174.
83. Muller, R.: The influence of grain components on infusion mash performance, Journal of the Institute of Brewing, **97** (1991), no. 2, pp. 93-100.
84. Muslin, E.H., Karpelenia, C.B. and Henson, C.A.: The impact of thermostable α-glucosidase on the production of fermentable sugars during mashing, Journal of the American Society of Brewing Chemists, **61** (2003), no. 3, pp. 142-145.
85. Myllarinen, P., Schulman, A.A., Salovaara, H. and Poutanen, K.: The effect of growth temperature on gelatinization properties of barley starch, Acta Agricultural Scandinavia, Section B, Soil and Plant Science, **48** (1998), no. 2, pp. 85-90, DOI: 10.1080/09064719809362484.
86. Nagamine, T. and Kato, T.: Recent advances and problems in malting barley breeding in Japan, Japan Agricultural Rresearch Quarterly, **42** (2008), no. 4, pp. 237-243.
87. Oliver, G.: The Oxford Companion to Beer, Oxford: Oxford University Press Inc, 2011.
88. Palmer, J.J.: How the mash works, How to brew: Everything you need to know to brew beer right the first time, Brewers Association: Boulder, Co. pp. 141-152, 2006.
89. Pauli, D., Brown-Guedira, G. and Blake, T.K.: Identification of malting quality QTLs in advanced generation breeding germplasm, Journal of the American Society of Brewing Chemists, **73** (2015), no. 1, pp. 29-40, DOI: 10.1094/ASBCJ-2015-0129-01.
90. Piendl, A.: Malt modification and mashing conditions as influencing factors on the carbohydrates of wort, The Brewers' Digest, **48** (1973), pp. 58-84.
91. Pitz, W.J.: An analysis of malting research, Journal of the American Society of Brewing Chemists, **48** (1990), no. 1, pp.33-44.
92. Robinson, L.H., Healy, P., Gibson, C.E., Eglinton, J.K. and Evans, D.E.: The identification of a barley haze active protein that can influence beer haze stability. Part I: The genetic basis of a barley malt haze active protein. Journal of Cereal Science, **45** (2007a), no. 3, pp. 335-342.
93. Robinson, L.H., Juttner, J., Milligan, A., Eglinton, J.K. and Evans, D.E.: The identification of a barley haze active protein that can influence beer haze stability. Part II: Characterisation of the barley SE protein as a Barley Trypsin Inhibitor of the chloroform/methanol type, Journal of Cereal Science, **45** (2007b), no. 3, pp. 343-352.
94. Sadosky, P., Schwarz, P.B. and Horsley, R.D.: Effect of arabinoxylans, β-glucans and dextrins on the viscosity and membrane filterability of a beer model system, Journal of the American Society of Brewing Chemists, **60** (2002), no. 4, pp. 153-162, DOI: 10.1094/ASBCJ-60-0153.
95. Schmitt, M.R. and Budde, A.D.: Malting extremely small quantities of barley, Journal American Society of Brewing Chemists, **69** (2011), no. 4, pp. 191-199, DOI: 10.1094/ASBCJ-2011-0728-01.
96. Schwarz, P.B. and Han J.Y.: Arabinoxylan content of commercial beers, Journal of the American Society of Brewing Chemists, **53** (1995), no. 4, pp. 157-159.
97. Schwarz, P.B., Li, Y., Barr, J., and Horsley, R.D: Effect of operational paramenters on the determination of laboratory extract and associated wort quality factors, Journal of the American Society of Brewing Chemists, **65** (2007), no. 4, pp. 219-228.
98. Sissons, M.J., Taylor, M. and Proudlove, M.: Barley malt limit dextrinase: its extraction, heat stability and activity during malting and mashing, Journal of the American Society of Brewing Chemists, **53** (1995), no. 3, pp. 105-110.
99. Skadhauge, B., Lok, F., Breddam, K., Olsen, O., Bech, L.M. and Knudson, S.: Barley with reduced lipoxygenase activity, W.I.P. Organization, Editor, Carlsberg Breweries and Heineken Supply Chain BV: Denmark, 2010, 120.
100. Slack, P.T., Baxter, E.D. and Wainwright, T.: Inhibition by hordein of starch degradation, Journal of the Institute of Brewing, **85** (1979), no. 2, pp. 112-114, DOI: 10.1002/j.2050-0416.1979.tb06837.x.
101. Stenholm, K. and Home, S.: A new approach to limit dextrinase and its role in mashing, Journal of the Institute of Brewing, **105** (1999), no. 4, pp. 205-210.
102. Stenholm, K., Home, S., Pietila, K., Jaakkola, N. and Leino, E.: Are the days of congress mashing over?, Proc. Barley Malt Wort Symp., Institute of Brewing (Central and South African Section), Zimbabwe, pp. 149-163, 1996.
103. Stenholm, K., Home, S., Pietila, K., Macri, L.J. and MacGregor, A.W.: Starch hydrolysis in mashing, European Brewery Convention Congress, Proceedings. Maastricht, pp. 142-145, 1997.
104. Stewart, D.C., Freeman, G. and Evans, D.E.: Development and assessment of a small-scale wort filtration test for the prediction of beer filtration efficiency, Journal of the Institute of Brewing, **106** (2000), no. 6, pp. 361-366, DOI: 10.1002/j.2050-0416.2000.tb00526.x.

105. Stewart, G.G.: High-gravity brewing and distilling – past experiences and future prospects, Journal American Society of Brewing Chemists, **68** (2010), no. 1, pp. 1-9, DOI: 10.1094/ASBCJ-2009-1214-01.
106. Stewart, G.G. and Murray, J.: A selective history of high-gravity and high-alcohol beers, Master Brewers Association of the Americas Technical Quarterly, **47** (2010), no. 2, pp. 1-6, DOI: 10.1094/TQ-47-2-0416-01.
107. Stewart, S., Sanders, R., Ivanova, N., Wilkinson, K.L., Stewart, D.C., Dong, J., Hu, S., Evans, D.E. and Able, J.A.: The influence of malt variety and origin on wort flavor, Journal American Society of Brewing Chemists, **81** (2023), no. 2, pp. 282-298, DOI: 10.1080/03610470.2022.2041156.
108. Takoi, K., Kuroda, K., Hirota, H., Takeda, N., Kaneko, K., Kaneda, T. and Hirotaka, T.: Barley lipoxygenase 1 gene, method of selecting barley variety, material of malt alcoholic drinks and process for producing malt alcholic drink, W.I.P. Organization, Editor. Sapporo Breweries Limited: Japan. 2004. 83.
109. Taylor, D.G.: The importance of pH control during brewing, Master Brewers Association of the Americas Technical Quarterly, **27** (1990), no. 4, pp. 131-136.
110. Tester, R.F., South, J.B., Morrison, W.R. and Ellis, R.P.: The effects of ambient temperature during the grain filling period on composition and properties of starch from four barley genotypes, Journal of Cereal Science, **13** (1991), no. 2, pp. 113-127, DOI: 10.1016/S0733-5210(09)80029-5.
111. The Brewers of Europe/EBC Analytica-EBC (online via BrewUp.eu). https://brewup.eu/ebc-analytica/search.
112. Verstrepen, K.J., Derdelinckx, G., Winderickx, J., Thevelein, J.M., Pretorius, I.S. and Delvaux, F.R.: Flavor-active esters: adding fruitiness to beer, Journal of Bioscience and Bioengineering, **96** (2003), no. 2, pp. 110-118, DOI: 10.1016/S1389-1723(03)90112-5.
113. Wackerbauer, K.: Lauter Tun vs mash Filter, BRAUWELT International, **14** (1996), pp. 164-165.
114. Yabuuchi, S. and Yamashita, H.: Gas chromatographic determination of trihdroxyoctadecenoic acids in beer, Journal of the Institute of Brewing, **85** (1979), pp. 216-218.
115. Yin, X.S.: Practical brewing science: malt. St Paul, Minnesota: American Society of Brewing Scientists, 2021.
116. Younis, O.S. and Stewart, G.G.: Sugar uptake and sequent ester and higher alcohol production by *Saccharomyces cerevisiae*, Journal of the Institute of Brewing, **104** (1998), no. 5, pp. 255-264.
117. Yu, J., Huang, S., Dong, J., Fan, W., Huang, S., Liu, J., Chang, Z., Tian, Y., Hao, J. and Hu, S.: The influence of LOX-less barley malt on the flavour stability of wort and beer, Journal of the Institute of Brewing, **120** (2014), no. 2, pp. 93-98.
118. Zhu, F.: Barley starch: composition, structure, properties, and modifications, Comprehensive Reviews in Food Science and Food Safety, **16** (2017), no. 4, pp. 558-579, DOI: 10.1111/1541-4337.12265.

Received 4 April 2024, accepted 6 May 2024