Isothermal and short mashing with or without pH adjustment and use of exogenous enzymes compared to infusion mashing

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ABSTRACT

In 16 case studies the traditional infusion mashing method was compared with isothermal mashing at 60-65°C combined with short mashing at half of the normal mashing time. Also, the effect of mash pH adjustment (5.4-5.6) and application of exogenous enzymes (proteases, glucanases, amylases, and amyloglucosidases) was studied. The results indicated that isothermal short mashing is a potential economic mashing process. Adjustment of the mash pH had a positive effect on the mashing performance. The use of exogenous enzymes in infusion mashings gave an increase in the amino acid concentration but this is not essential for the later fermentation process since basic wort free amino nitrogen (FAN) levels are already high enough.

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INTRODUCTION

The most important process in beer production is the fermentation of the sugars contained in the wort by the brewers' yeast. Mashing is the process for wort production to give as much soluble extract as possible from the initially insoluble components in the malt. Traditional mashing involves increasing the temperature of the mash to the optimum temperature for the enzymes that have to be activated, and maintaining a rest at that temperature. The rests occur at the temperature optima of the enzymes: proteinases and β-glucanases (45-50°C), β-amylases (62-65°C), and α-amylases (70-75°C). There are two types of traditional mashing processes: the infusion method and the decoction method (Briggs *et al.*, 2004; Kunze, 2004). The total duration of the traditional mashing is 2.0-2.7 h.

MATERIALS AND METHODS

In 16 case studies in 60 L mash tuns, the mashing process was investigated to see if it could be performed at one temperature (60-65°C) and shortened to half of the normal mashing time. Also, the effect of mash pH adjustment (5.4-5.6) and application of exogenous enzymes (proteases, glucanases, amylases, and amyloglucosidases) was studied.

The mashing processes were monitored extensively. Analytica EBC methods were used for the determination of the extract, pH, colour, β-glucan content, total nitrogen content, and free amino nitrogen (FAN) content. The extract was determined by density measurements with an Anton Paar DMA 4500 density meter. The fermentable sugars (fructose, glucose, sucrose, maltose and maltotriose) and maltotetraose were determined by High Performance Anion Exchange Chromatography (HPAEC) coupled to Pulsed Amperometric Detection (PAD). The CarboPac PA100 of Dionex was used and elution was according to Application Note 46 of Dionex (Anonymous, 1997). HPAEC-PAD was also used for comparison of the higher saccharides profiles of the samples. Oligosaccharides up to a polymerisation degree of 13 can be quantitatively analysed with HPAEC-PAD (Bruggink *et al.*, 2005; Van der Meulen *et al.*, 2004). Glucose concentrations were also determined with a glucose biosensor (Sire, Chemel). All data are the average of at least three determinations.

RESULTS AND DISCUSSIONS

The results and conclusions are gathered in Tables 1 and 2. The results of typical case studies are shown in Figures 1 and 2.

Worts of short- and long-duration isothermal mashes and of traditional mashes contained comparable amounts of extract and fermentable sugars and the brew house yield was in the same range. The FAN of wort of isothermal mashes was slightly lower than the FAN of wort of traditional mashes but still high enough (> 210 mg/L) for the fermentation process (Lekkas *et al.*, 2007; Kreisz, 2009). De Rouck *et al.* (2007) showed that a low FAN content has no effect on the yeast fermentation, the final attenuation, and the flavour stability of the beer. Wort of isothermal mashes adjusted to a mash pH of 5.4-5.6 had a final FAN that equalled the FAN of wort of traditional mashes. According to Kuhbeck *et al.* (2005) the FAN release is predetermined by malt modification. Finer grist causes higher initial FAN values at mashing-in which,

however, aligns towards the end of mashing even when short mashing (total mashing time of 85 min) is applied. The colour of the wort of isothermal mashes was lighter, especially for the short-duration isothermal mashes. The colour of the worts of the pH adjusted mashes of the case studies in Figure 1 is 7.03-7.75 EBC. These of the mashes of which the pH is not adjusted are 7.90-7.91 EBC. This correlates with less Maillard reaction at lower pH.

The β-glucan content is slightly higher in isothermal mashed wort than in infusion method mashed wort for the 60 L lab scale mashing and filter filtration of the mash. The β-glucan lab scale data can not be compared with the values of the industrial filtrate as paper filter filtration is used for the lab scale mash.

Mashing-in at a temperature of 63°C or higher and adjusting the pH of the mash to a pH lower than 5.6 has also a positive effect on the flavour quality and stability of the beer (Malfliet *et al.*, 2009). Under these mashing conditions the residual membrane bound lipoxygenase (LOX) of the malt is effectively inhibited with formation of less trihydroxy fatty acids. The concentration of trihydroxy fatty acids can be three to four times lower than the concentration of trihydroxy fatty acids of pitching worts of mashing-in conditions at lower temperature and pH >5.6 (Malfliet *et al.*, 2009).

Addition of exogenous enzymes to the mash did not result in an obvious higher extract yield but gave a FAN of about 50% higher in infusion mashings.

Table 1: Characteristics of the wort at the end of the mashing and of the industrial infusion method filtrate of the case studies of Figures 1 and 2. The content of glucose, maltose (M2), maltotriose (M3), maltotetraose (M4) and fermentable sugars (sum glucose, maltose and maltotriose; without fructose and sucrose content) are expressed in % of the extract. The brew house yield is calculated without taken in account the volume increase due to the addition of the grist.

	Case studies of Figure 1				**Case studies of Figure 2**				
Process conditions	165 min 60°C	165 min 60°C + Enzym mix	83 min 60°C		110 min 63°C pH not adjusted	119 min 50,63,72°C pH not adjusted	110 min 63°C pH adjusted	119 min 50,63,72°C pH adjusted	
Code Figure	Mash A	Mash AE	Mash B	filtrate	Mash A	Mash AT	Mash AP	Mash ATP	filtrate
pH start	6.22	6.16	6.08	-	5.75	6.00	5.78	5.73	-
pH end	5.94	5.93	5.93	5.95	5.70	5.85	5.48	5.68	5.36
EBC colour	6.01	5.68	4.25	6.04	7.91	7.90	7.03	7.75	7.30
FAN start (mg/L)	153	161	163	-	190	179	185	216	-
FAN end (mg/L)	240	243	210	328	244	258	289	290	296
Total N (mg/kg)	-	-	-	-	1320	1310	1370	1500	1460
Brew house yield (%)	84	83	81	-	68	66	69	68	-
% glucose	8.9	8.4	7.5	9.5	6.8	9.9	6.4	10.4	9.2
% M2	49.2	53.7	49.2	42.0	55.1	47.6	52.8	49.8	41.2
% M3	10.3	11.6	10.6	12.4	8.2	8.8	8.5	9.1	8.9
% M4	1.8	1.4	1.7	2.7	0.5	0.8	0.6	0.8	0.8
Fermentable sugars (%)	68.4	73.8	67.3	63.9	70.1	66.3	67.8	69.2	59.3
β-glucan (mg/L)	-	-	-	-	117.3	102.0	138.3	103.0	46.9

Table 2: Isothermal mashing and infusion mashing compared for some typical features: formation of glucose, extract, fermentable sugars and maltotetraose, brew house yield, β-glucan content, FAN, colour and effect of short isothermal mashing, pH adjustment, and use of exogenous enzymes.

Feature	**Isothermal mashing**	**Infusion mashing**
Glucose	Directly ~ same amount released	
	Increase to ~ two times the starting concentration	Increase to maximum three times the starting concentration
Maltose	Directly > 2/3 of the final concentration	Low formation at 50°C Fast and big increase at 62-63°C
	About the same final concentration	
Extract	Directly > 2/3 of the final extract	Directly 1/4 of the final extract Fast and big increase at 62-63°C
	About the same final extract	
Fermentable sugars	Comparable	
Maltotetraose	Formation merely at 70-78°C	
Brew house yield	Comparable	
β-glucan	Slightly higher in isothermal mashed wort than in infusion method mashed wort (lab scale)	
FAN	Direct about the same FAN (> 2/3 of final FAN) Infusion method mashed worts have a slighter higher FAN	
Colour	Isothermal mashed worts have a lighter colour than infusion method worts (the colour is even lighter for short isothermal mashed worts)	
Short isothermal mashing	~ Same results as long isothermal mashing	
pH adjustment	Higher FAN and higher total nitrogen Isothermal and infusion method mashed worts about the same final FAN Lighter colour	
Exogenous enzymes	No obvious extract increase	
	FAN the same	FAN > 50% higher

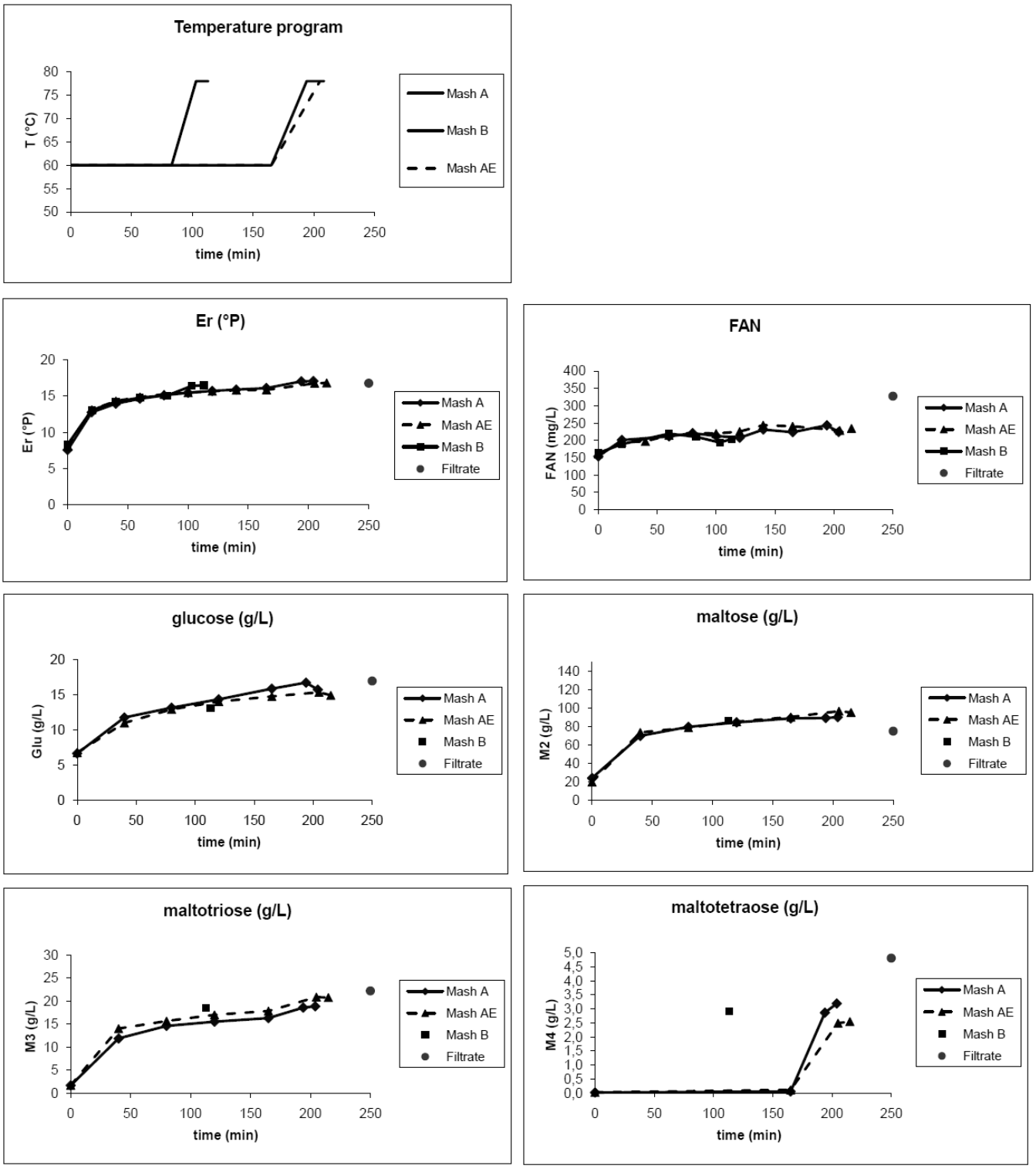

Figure 1: Results (extract expressed as Er in °P, FAN in mg/L and the concentrations of glucose (Glu), maltose (M2), maltotriose (M3) and maltotetraose (M4) in g/L) of three mashing case studies with 13 kg 2-3 EBC malt and 2.5 kg corn flakes per 60 L mash. The three mashes were isothermal mashes at 60°C. Mashing time for mash B was 83 minutes. Mash A and AE had a mashing time of 165 minutes. An enzym mix of α-amylases, glucanases and proteases is added to the mash AE. The results of the industrial mash (with the same enzym mix) performed at the same day according to the industrial infusion temperature program are noted in the figures for the mash after filtration (filtrate).

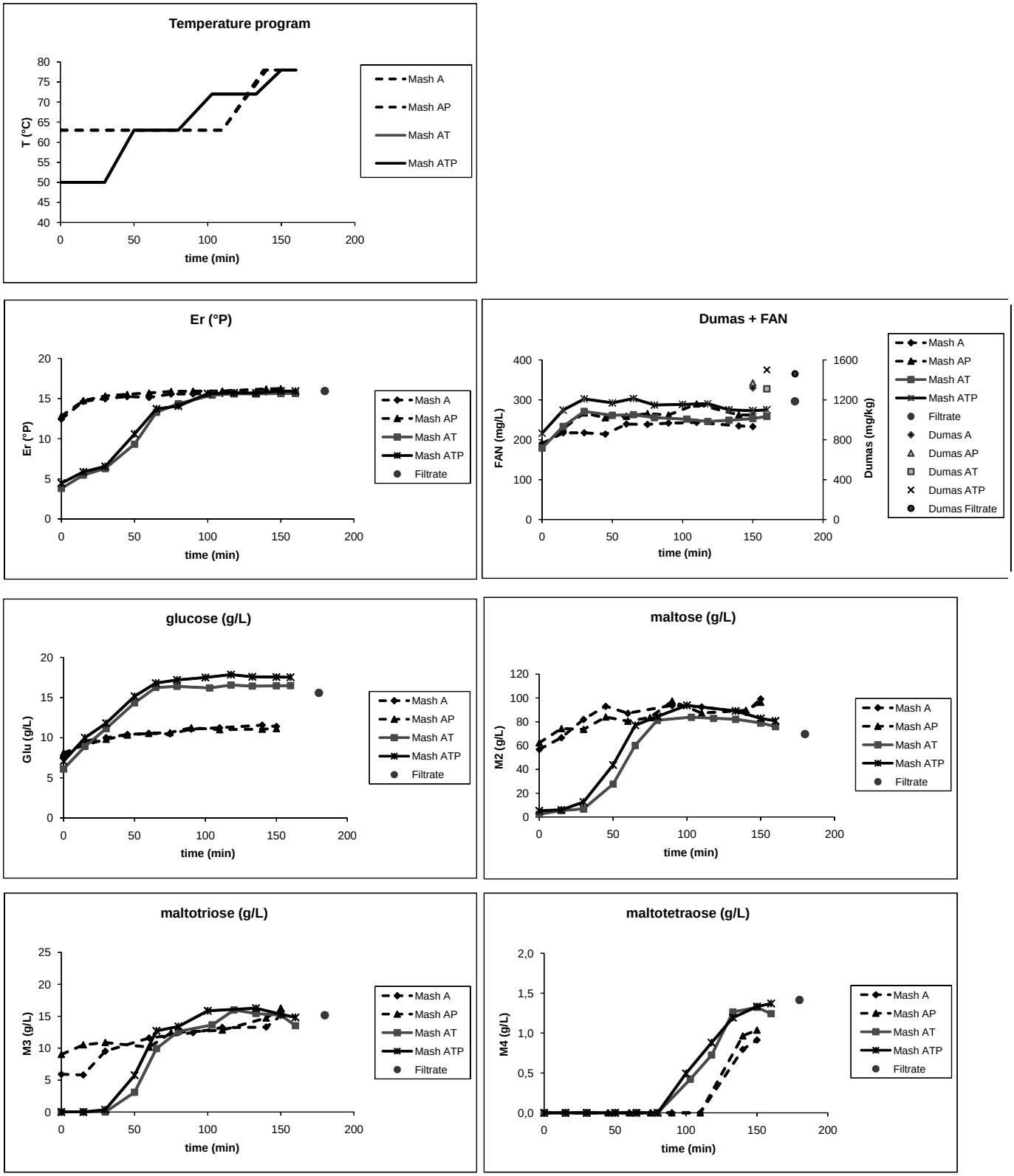

Figure 2: Results (extract expressed as Er in °P, FAN in mg/L, the concentrations of glucose (Glu) , maltose (M2), maltotriose (M3) and maltotetraose (M4) in g/L, and total N of the mash at the end of the mashing according to Dumas in mg/kg) of four mashing case studies with 15 kg pilsner malt/60 L mash. Mash A and AP were isothermal mashes at 63 °C. Mash AT and ATP were performed according to the infusion temperature program shown in the upper figure. In all cases was the total time for mashing and increase of the temperature to 78°C for filtration 160 minutes. Mash AP and ATP were adjusted to pH 5.4 with 20 mL 80% lactic acid at the start of the mashing. The results of the industrial mash performed at the same day according to the temperature program of mash AT are noted in the figures for the mash after filtration (filtrate).

CONCLUSIONS

To conclude, good quality malt nowadays allows isothermal mashing at 60-63°C (or even 65°C) and the mashing time to be shortened to 1.0-1.5 h. By mashing directly in a lauter tun, rather than a mash tun, extra time can be saved without reduction of the extract recovery.

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