

Application of Biological Acidification to Improve the Quality and Processability of Wort Produced from 50% Raw Barley

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ABSTRACT

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In this study four strains of lactic acid bacteria (LAB) were chosen to bioacidify a mash containing 50% barley and 50% malt. The strains were isolated from malted and unmalted barley and assayed for extracellular enzymatic activities (proteases, amylases, β -glucanases). The biologically acidified mash was compared to a chemically acidified mash, 100% malt mash unacidified and 50% malt and 50% barley mash unacidified. Characteristics such as pH, extract, colour, viscosity, total soluble nitrogen (TSN), free amino nitrogen (FAN), apparent fermentability, β -glucan and lautering performance of the resultant worts were determined. A model lautering system replicating one used in a brewery was designed and built in University College Cork (UCC) to measure the lautering performance of the bioacidified mashes. The new system was compared to the filtration method used in EBC method 4.5.1. Overall the addition of LAB to bioacidify a mash of 50% barley and 50% malt resulted in faster filtration times, which correlated with decreased β -glucan levels. Proteolytic LAB had a positive influence on the quality of wort and resulted in increased FAN levels. Lighter colour worts were observed along with increased extract levels.

Key words: β -Glucan, biological acidification, lactic acid bacteria, lautering, malted barley, unmalted barley.

INTRODUCTION

Barley malt has traditionally been the grain of choice in the brewing industry. Barley malt is preferred because among other reasons, it has a high potential for extract development for yeast growth and fermentation. Mash filtration is aided by the presence of the husk, whilst the high amylolytic potential due to the thick layer of aleurone cells gives barley malt the winning advantage⁸. However it is not always economically viable to brew with 100% malted barley, and today's breweries are forced to minimise their costs without changing the quality or the character of their beers. Unmalted barley is often em-

ployed in the brewing industry as an inexpensive source of extract. The application of brewing adjuncts does have an impact on beer flavour and processability compared to beer brewed with 100% malted barley. The use of unmalted barley leads to problems in the brewing industry such as low extract yields, high wort viscosity, decreased rate of lautering and haze formation in beer^{34,39}. These effects are caused by a low level of cytolytic, proteolytic and amylolytic enzymes followed by decreased brewhouse yield, apparent fermentability, unbalanced nitrogen ratios and flavour defects^{8,19,20}. Unmalted barley contains a high level of β -glucan. β -Glucans are complex carbohydrates composed of mixed linkage (1 \rightarrow 3),(1 \rightarrow 4) β -D-glucose polymers. They are nonstarchy polysaccharides and are usually concentrated in the inner aleurone cell walls and subaleurone endosperm cell walls of barley¹⁸. Many of the problems associated with barley β -glucans in the brewing industry are due to the high propensity of the polysaccharide to form aqueous solutions of high viscosity¹⁶. Bamforth and Barclay³ reported incomplete cell wall degradation, diminished mobilization of starch and proteins for hydrolysis and lower malt extract values, as a result of high levels of β -glucan in malt. Undegraded β -glucans from malt or from cereal adjuncts can have a detrimental effect on the chemical stability of beer during storage due to the association of β -glucans with polyphenols and proteins forming chill haze². Brewing with unmalted barley leads to higher wort viscosities due to incompletely digested cell wall fragments (e.g. hemicelluloses; pentosans) or undigested small starch granules in the mash. These are important elements that impede the passage of liquid through the grain filter bed resulting in increased lautering times^{6,26,35}, while high wort viscosities have also been reported to impair beer filtration^{4,15,28,29}. Nielsen³⁰ found in general fermentability of barley wort is lower than that of 100% malt wort, while Pfenninger³¹ reported barley beers had lighter colour, higher bitter content, and a better foam and haze stability. Furthermore, excess cell wall β -glucan has a negative impact on malt extraction, leading to prolonged filtration times and a decrease in brewhouse yield¹². To avoid these disadvantages of adjunct brewing, acidification of mash and wort is employed to intensify the overall enzymatic degradation. Reduction of pH to 5.4 for mash and pH 5.2 for wort by biological acidification (BA) allows the important enzymes (glucanolytic, proteolytic and amylolytic) to reach their optimum activity, thus resulting in improved processing and better quality beer.

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The advantages of BA using lactic acid bacteria (LAB) have been well documented. Pittner and Back³² reported a fuller and smoother flavour profile, a pleasant bitterness and a lower risk of protein haze due to extensive protein breakdown. Similarly, Lewis and Young²¹ observed improved organoleptic qualities of beer, a shorter mashing program, and more efficient enzymatic processes when uniformly modified malt was acidified. Better diacetyl reduction during fermentation due to fast pH drop, paler beer colour, increased tannoid content as well as noticeable improvement of taste stability were noted by Daumen *et al.*¹⁰

In this study four strains of LAB were chosen to biologically acidify mashes containing 50% malt and 50% barley. Bacterial cultures were chosen as a result of their enzymatic (proteolytic/amylolytic) activity, and ability to produce lactic acid in wort. Bacterial cultures were also assayed for β -glucanase activity, however results showed each strain tested negative. Their effects on wort characteristics particularly the lautering performances and β -glucan levels were investigated and compared to that of 100% malt. A chemical food grade (FG) lactic acid was also used to acidify the mash for comparison purposes. A lautering system designed and built in University College Cork (UCC) was used to measure the lauter performance of the mash; this was compared to the EBC method.

MATERIALS AND METHODS

Applied cereals

Unmalted barley (variety Optic, harvest 2002) and malted barley (Optic, 2002) were obtained from the Malt-ing Company of Ireland (Table I). Mains water was used for mashing liquor.

Strains and culture conditions

Lactobacillus plantarum FST 1.7, *Lactobacillus amylo-vorus* FST 1.1, and *Weissella confusa* FST 1.31 were obtained from the culture collections of the Department of Microbiology at UCC. *Lactobacillus plantarum* TMW 1.460 was obtained from the Institute of Technical Micro-biology at the Technical University of Munich–Weihen-

stephan (TMW), Germany. These bacteria isolated from malted barley were maintained as frozen stocks in 50% glycerol at -80°C and were propagated twice in MRS broth for 16 h before experimental use. Strains were cul-tured on MRS agar plates (MRSa, Oxoid Ltd) at 30°C for 48 h. Propagation of strains for BA of the laboratory scale mash trials (MT) were prepared as follows: an overnight culture of LAB was subcultured with 1% inoculum in 5 mL of MRS-broth for 24 h at 30°C . 1 mL of the culture was transferred into 100 mL of unhopped wort-broth, made of first runnings of 50% malt and 50% barley brew, and inoculated for 48 h at 30°C . The ability of the bacteria to reduce the pH of the wort and an increment of optical density indicated that the bacteria were able to grow in the wort environment.

Determination of growth of LAB in wort broth

Cell growth was determined by measuring the optical density in wort broths. Wort broths used for the cultivation of the LAB were prepared using a laboratory-mashing pro-gram as described in “preparation of the biological acidified wort broth”. Worts were diluted to 10% (w/w) extract before autoclaving at 121°C for 10 min. Wort broth was inoculated at 1% (w/w) with the test strains and incubated at 30°C for 24 h. Optical density of the worts was deter-mined by measuring absorbance using a spectrophotome-ter at 600 nm.

Preparation of the biological acidified wort broth

Wort broth was produced using 50% malt and 50% bar-ley according to a laboratory-mashing program. Barley (60 g) and malt (60 g) were milled and weighed into stainless steel cylinders. The grist was mashed in with 480 mL distilled water at 45°C . The mashing program chosen to adapt to the higher barley content was as follows: 50°C for 30 min, 62°C for 40 min, 72°C for 30 min. Mashing off was carried out at 78°C . After lautering using a pilot scale lautering system the wort-broth remained unhopped. The wort-broth was filled in a sterilized 5 L cylinder and stored at 5°C until use. The extract of this wort-broth was at 14.95% (w/w). Wort broth samples (50 mL) were fer-mented by LAB and lactic acid production was deter-mined by titration with 0.1 M NaOH to pH 7.0 after 48 h according to Kunze¹⁹. As a control, chemically acidified wort was prepared adding 0.80% (w/w) of FG lactic acid to non-fermented wort-broth. Set volumes of acidified wort broths (see experimental set up) were added to the mashing beakers to lower the pH to 5.4 before mashing.

Determination of proteolytic activity of LAB

Proteolytic activity was determined as described by Fransen *et al.*¹⁴, using skim milk agar (SMA) and caseinate agar (CA). CA was made up using 1.5% agar (Difco), 0.5% tryptone (Difco), 0.25% yeast extract (Difco), 0.1% glucose (Difco), 1.0% sodium caseinate and 20 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Difco) in a 0.15 M trisodium citrate solution (Dif-co) at pH 6.9. After autoclaving the agar at 121.1°C for 15 min plates were poured. SMA was made by adding 15 g of agar (Difco) to 700 mL distilled water and autoclaving at 115°C for 10 min. The 10% skim milk agar was formu-lated in distilled H_2O and this media was autoclaved for

Table I. Characteristics of barley/malt samples.

Characteristics (units)	Barley	Malt
Variety	Optic	Optic
Harvest	Ireland 2002	Ireland 2002
Homogeneity (%)		98.5
Friability (%)		96.3
Moisture (%)	11.78	3.18
Total N (%)	1.6	1.52
Total protein (%)	9.97	9.5
Soluble protein (%)	2.67*	3.71
Diastatic power (WK)		205
β -Glucan (%)	2.84	0.27
Filtration time	Slow*	Normal
Colour (EBC)	5.16*	5.62
Extract (DB) (%)	79.45*	80.5
Viscosity (mPa s)	1.99*	1.77
Kolbach index	26.17*	36

* = EBC Congress mashing method performed on a grist of 50% un-malted barley and 50% malted barley.
DB = dry basis

Table II. Acidified wort broth (mL) added to reach pH 5.4 in mashes.

Grist composition	Acidification method	Wort broth (mL)	Lactic acid conc. (%)
100% malt	None	0	0
50% malt 50% barley	FG lactic acid	3.7	0.80
50% malt 50% barley	<i>L. plantarum</i> TMW 1.460	3.2	0.93
50% malt 50% barley	<i>L. plantarum</i> FST 1.7	3.0	0.90
50% malt 50% barley	<i>L. amylovorus</i> FST 1.1	6.0	0.78
50% malt 50% barley	<i>W. confusa</i> FST 1.31	11.9	0.66
50% malt 50% barley	None	0	0

10 min at 115°C, and 300 mL of this skim milk solution was aseptically added to 700 mL of the liquefied agar (50°C) and plates were poured. The volume in the plates was 40 mL each. Wells measuring approximately 4.6 mm were aseptically extracted from the solid agar. The fermentation broth was centrifuged (10 min 14,000 × g) to obtain a cell free supernatant. Separate wells were filled with 50 µl of a 24 h culture and 50 µl of the cell free supernatant onto both CA and SMA plates. To inhibit any further growth of LAB, chloramphenicol was added to the cultures (20 mg L⁻¹). Plates were incubated at 37°C for 48 h. Clearing zones (halos) around the wells indicated proteolytic activity.

Determination of α-amylase activity of LAB

The production of α-amylase activity was tested on starch plates (SP). SP were formulated by addition of corn starch (Sigma Aldrich Co. Ltd.) at a concentration of 3 g L⁻¹ to mMRS4 (containing 15 mL L⁻¹ of 50% (w/w) maltose in water solution and 7.5 mL L⁻¹ of 50% (w/w) fructose solution). For a qualitative α-amylase plate test, SP were flooded with iodine solution (5 mL) for 30 min. Undegraded starch stains blue/black using the method of Daniels *et al.*⁹, while the presence of a clear halo around a tested colony was taken as an indication of starch degradation and therefore the production of α-amylase activity. α-Amylase activity was additionally proven by a colorimetric assay using an α-amylase test kit obtained from Megazyme.

Determination of β-glucanase activity

The presence of β-glucanase activity was determined using the beta glucanase test kit obtained from Megazyme.

Experimental set-up

A grist composition of 60 g malt and 60 g barley mixed with 320 mL water was assessed in this study. Seven grist combinations with and without acidification were carried out. Each grist composition was mashed four times and then filtered and lautered using both techniques in duplicate. Wort analysis was repeated three times per sample. The results quoted are the means of the repeated experiments and have been calculated to an extract of 11% (w/w).

1. 100% malt, unacidified
2. 50% barley and 50% malt acidified with FG lactic acid
3. 50% barley and 50% malt acidified with *L. plantarum* TMW 1.460

4. 50% barley and 50% malt acidified with *L. plantarum* FST 1.7
5. 50% barley and 50% malt acidified with *L. amylovorus* FST 1.1
6. 50% barley and 50% malt acidified with *W. confusa* FST 1.31
7. 50% barley and 50% malt, unacidified

Milling

Barley and malt were milled using a lab scale disc mill (Bühler) set at a milling gap of 1.0 mm for barley and 1.4 mm for malt.

Mashing

Milled malt and barley were weighed into stainless steel mashing beakers (total grist weight of 120 g) and mixed with mash-in liquor (320 mL at 50°C). Fermented wort broth was titrated into beakers until a pH of 5.4 was achieved (Table II). The pH of controls 100% malt and 50:50 unacidified malt and barley were not adjusted. Mash in liquor was then added until a total liquor:grist ratio of 3:1 was obtained. The mashing was carried out in an EBC mash bath (Funke–Dr. N. Gerber Instruments). The mashing program chosen to adapt to the higher barley content was as follows: 50°C for 30 min, 62°C for 40 min, 72°C for 30 min. Mashing off was carried out at 78°C. The total weight of the mash was made up to 600 g such that a final liquor:grist ratio was 4:1 using distilled water preheated at 78°C. Mashing of each sample was carried out four times. Each sample was filtered and lautered using both techniques in duplicate. Wort analysis was repeated three times per sample. The results quoted are the means of the repeated experiments and have been calculated to an extract of 11% (w/w).

Determination of filterability and lautering performance

Filtration or lautering was carried out according to different techniques:

1. The mash was filtered according to EBC 4.5.1, through filter paper into graduated cylinders. The amount of filtrate recovered was recorded every 15 min over a 90 min period.
2. A pilot scale lautering system replicating industry scale was designed and built in UCC. Lautering containers were connected via rubber tube to 60 mL syringes. Water was added to the syringe and flowed through the tube and up the base of the cylinder, thus ensuring the system was air free. The mash beakers were stirred and added quickly to the lautering cylinders. A lauter break of 25 min was carried out to allow a filter cake of husks to

form on the bottom of the lautering cylinders. For 45 min the wort was lautered and recirculated back to the top of the cylinder. The time taken for each 60 mL syringe to fill was recorded. After 45 min, first runnings (250 mL) were collected and transferred to a flask. Sparging was carried out by the addition of 400 mL of distilled water to the cylinders and second runnings were collected in a 500 mL flask. First and second runnings were then mixed together.

Wort analyses

All analyses were carried out in duplicate using EBC¹¹ and MEBAK²⁵ methods. Specific gravity and extract of the wort samples were measured with an automatic beer analyser (SCABA) according to EBC 9.2.2. pH of the wort was measured according to EBC 8.17 and FAN levels were determined according to EBC 8.10. Determination of the fermentability of the wort was carried out according to EBC 8.6. TSN of the wort was determined by using the Dumas combustion method (EBC 8.9.2) for nitrogen determination (LECO Corporation). Wort viscosity was measured using a falling ball viscometer at 20°C (Haake) according to MEBAK I, 4.1.4.4.1. The β-glucan content was determined using the mixed linkage β-glucan kit (Megazyme) according to EBC 8.13.1. Colour was determined in accordance with EBC 8.5.

RESULTS AND DISCUSSION

Approximately 50,000 colonies of LAB from the brewing and malting environment were isolated and checked for extracellular enzymatic activity (proteases, amylases, and β-glucanases) at UCC. Only 20 isolates expressed multiple enzymatic activity. These twenty isolates were further screened for their suitability to be used as starter cultures for BA. The cultures that were chosen were *L. amylovorus* FST 1.1, *L. plantarum* TMW 1.460, *L. plantarum* FST 1.7, and *W. confusa* FST 1.31. These strains expressed either a positive reaction on skim milk, caseinate or starch agar plates, showed good pH reduction, and grew well in wort (Table III). Strains showed no β-glucanase activity when tested with a β-glucanase test kit. Once selected these strains were applied for BA of laboratory-scale mashes. The effects of the LAB were challenged in a mash containing 50% malt and 50% barley. The biologically acidified mashes were compared to a chemically acidified mash, 100% malt unacidified and to a 50%/50% mash not acidified. Table IV shows the wort analysis for pH, extract, colour, FAN, TSN, fermentability, and vis-

Table III. Characteristics of LAB.

Strain	Growth in wort	Lactic acid production	Protease activity	Amylase activity
<i>Lactobacillus amylovorus</i> FST 1.1	+++	++	++	++
<i>Weissella confusa</i> FST 1.31	++	+	+	+
<i>Lactobacillus plantarum</i> FST 1.7	+++	+++	-	-
<i>Lactobacillus plantarum</i> TMW 1.460	+++	++	-	-

All strains tested negative for β-glucanase activity.

+ = fair
++ = good
+++ = very good

cosity. Filterability, lautering performance and β-glucan content were also measured as shown in Figs. 1, 2, and 3, respectively. The filterability of the worts (Fig. 1) was compared to the lautering system (Fig. 2). Results showed both methods repeatedly showed the same order of filterability, which correlated with β-glucan levels (Fig. 3). Results of the trial are outlined below.

Mash and wort pH

The amount of lactic acid used was determined according to Kunze¹⁹. The concentration of lactic acid after fermentation according to Table II ranged between 0.66% (w/w) and 0.93% (w/w). Certain amounts of the biological culture were added to the samples to reach a pH value of 5.4 in the mash (Table II). The extracts and pH values decreased to 14.35 ± 0.15% (w/w) and 3.28 ± 0.02, respectively, in all wort broth samples. The pH at the beginning of mashing is of extreme importance, as each of the mash enzymes possesses an optimum pH value. Normal malt mashing begins at a pH of 5.7, which is, with the exception of α-amylase, above that required to promote the activity of the most important polysaccharide degrading enzymes e.g. β-glucanase (pH 4.5–4.8) and β-amylase (pH 5.4–5.6)^{7,20}. At a mash-in pH lower than 5.7, enzyme activity is greater, which leads to a more extensive breakdown of mash polysaccharides. The result is a reduction in viscosity and therefore rapid lautering³². Proteolytic enzymes such as carboxypeptidase and endopeptidase show increased activity at a pH of less than 5.6³⁸, the resulting effect is an increase in TSN and better break formation has been recorded²⁷. Also levels of FAN, which are very

Table IV. Wort analysis.

	100% Malt	50:50 + FG lactic acid	50:50 + <i>L. plantarum</i> TMW 1.460	50:50 + <i>L. plantarum</i> FST 1.7	50:50 + <i>L. amylovorus</i> FST 1.1	50:50 + <i>W. confusa</i> FST 1.31	50:50 Malt:barley
pH mash at beginning [50°]	6.0 ± 0.0	5.4 ± 0.0	5.4 ± 0.0	5.4 ± 0.0	5.4 ± 0.0	5.4 ± 0.0	6.0 ± 0.0
pH wort [20°C]	6.00 ± 0.03	5.80 ± 0.0	5.90 ± 0.01	5.80 ± 0.00	5.90 ± 0.00	5.90 ± 0.00	5.90 ± 0.01
Extract [%]	11.2 ± 0.3	10.3 ± 0.3	10.3 ± 0.3	10.7 ± 0.1	10.0 ± 0.4	10.4 ± 0.1	9.6 ± 0.2
Colour [EBC]	4.10 ± 0.00	3.20 ± 0.00	3.30 ± 0.00	3.10 ± 0.00	3.10 ± 0.02	3.00 ± 0.03	3.30 ± 0.03
Viscosity [mPa s]	1.73* ± 0.06	1.91* ± 0.04	1.90* ± 0.03	1.86* ± 0.02	1.93* ± 0.02	1.88* ± 0.04	2.03* ± 0.02
TSN [mg L ⁻¹]	695* ± 25	675* ± 18	678* ± 16	672* ± 6	669* ± 13	684* ± 5	673* ± 22
FAN [mg L ⁻¹]	166* ± 10	128* ± 1	123* ± 9	131* ± 9	135* ± 2	130* ± 5	115* ± 13
Apparent fermentability [%]	83 ± 1	72 ± 2	74 ± 1	76 ± 2	74 ± 1	76 ± 2	70 ± 3

Shown are the calculated means of two independent experiments.

* = Calculated results at an extract of 11%, ± = standard deviation.

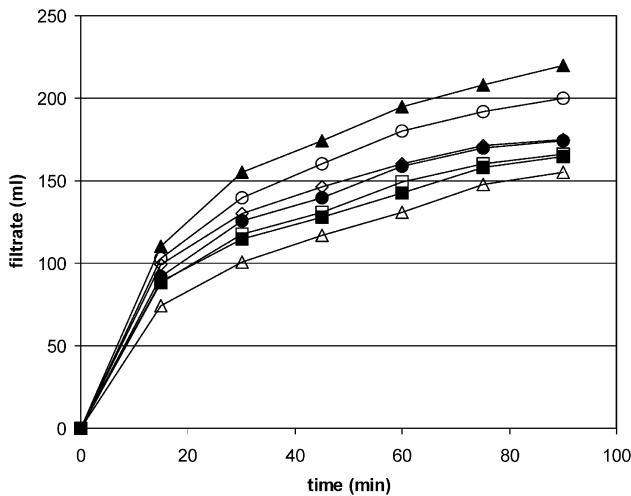


Fig. 1. Filtration performance shown in volume-time dependency of mashes using filter paper. (FG LA, food grade lactic acid.) Shown are the samples 100% malt (▲), FG LA (■), FST 1.7 (□), FST 1.31 (●), TMW 1.460 (○), FST 1.1 (◇), 50:50 unacidified (△).

important for the fermentative and biosynthetic performance of brewers' yeast, are said to increase at this low pH. These results correspond with the results obtained at UCC particularly when the wort was acidified with *L. amylovorus* FST 1.1, due to the additional presence of proteolytic enzymes. The pH of 100% malt and 50/50% unacidified malt and barley were not adjusted. The pH of the remaining mashes was adjusted to 5.4. This served to activate the mash enzymes, and hence degrade the starch compounds, solubilising them to give increased extract. Results showed a slightly lower wort pH when raw barley was used. This is unusual but may be due to higher activity of phosphatases in raw barley compared to malt. Buffer substances are released which compensate the addition of lactic acid. The pH of the acidified worts ranged between 5.8 and 5.9 at the end of the mashing process.

Extract

Considerable differences can be seen between acidified and non-acidified mashes. Mashes acidified either chemically or biologically show a higher extract. This can be explained by a higher level of amyolytic and proteolytic activity at a lower pH. As expected 100% malt showed highest extract level as during the malting process, the mobilization of enzymes in the starchy endosperm modify it such that during mashing and brewing a high amount of fermentable sugars can be obtained without difficulty. *L. plantarum* FST 1.7 showed highest extract levels, this culture also showed high lactic acid production; only 2.9 mL of lactic acid was needed to reduce the pH to 5.4. This was followed by *W. confusa* FST 1.31, which demonstrated an extract of 10.4% and exhibited excellent amyolytic activity leading to the high extract value. This however is a heterofermentative strain, which explains why almost 12 mL of fermented broth was needed to reduce the pH to 5.4. Mashes biologically acidified in general had a higher extract level owing to improved enzymatic activity of the LAB.

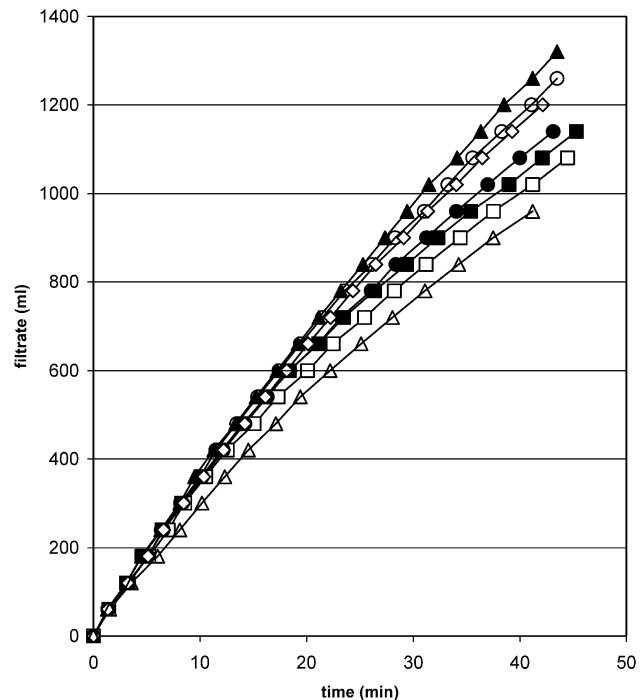


Fig. 2. Lautering performance shown in volume-time dependency of mashes using lautering system. (FG LA, food grade lactic acid.) Shown are the samples 100% malt (▲), FG LA (■), FST 1.7 (□), FST 1.31 (●), TMW 1.460 (○), FST 1.1 (◇), 50:50 unacidified (△).

Colour

The general trend showed that mashes containing 50% unmalted barley revealed lower colour values ranging from 3.0–3.3 EBC units compared to 100% malt which showed a colour value of 4.1 EBC units. Colour formation can be attributed to the malting process, where green malt is kilned at temperatures around 80°C. Kilning is responsible for the production of high levels of Maillard precursors⁷, which are later solubilised in the brewhouse and give the worts their colour and characteristic aroma. Wort used for cultivating the bacteria was autoclaved at 121°C for 10 min, these high temperatures would also lead to Maillard browning, this added wort extract however seemed to make no impact on the overall colour compared to the unacidified wort.

Nitrogenous fractions

The method used for determining FAN gives an estimate of the amount of amino acids, ammonia, and in addition, the terminal α -amino nitrogen groups of peptides and proteins. The formation of FAN peptides and polypeptides influences yeast growth and the foam and haze properties of derived beers^{17,22}. A slight increase in the TSN and FAN as a result of acidification correlates with the findings of Lewis²⁰. This may be due to the decreased mash pH corresponding with the optima pH of proteases. The action of the protease enzyme "carboxypeptidase" accounts for 80% of all the amino acids set free in mashing, and has an optimum pH of 5.2³⁸. Carboxypeptidases are regarded as the most important exopeptidases; they attack from the carboxyl end of the peptide chain to produce

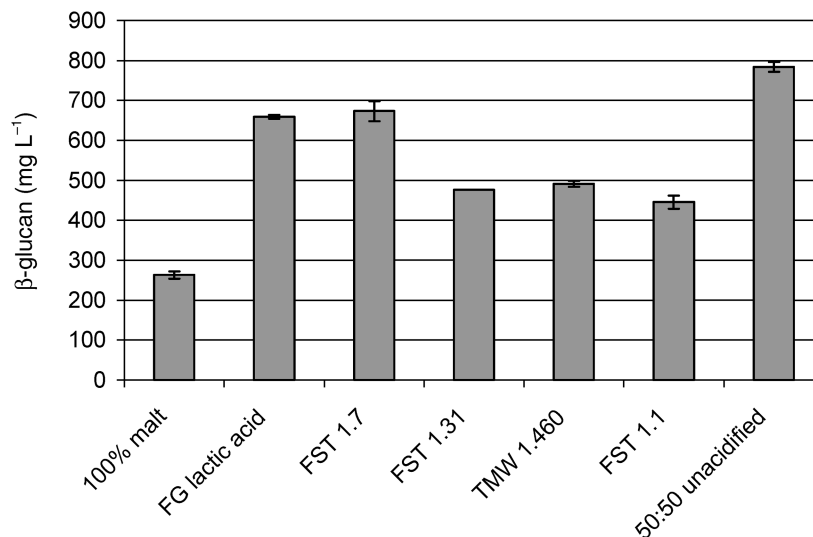


Fig. 3. β-Glucan content of worts at 11% extract.

amino acids and a wide range of simple and complex peptides. Carboxypeptidases act with endopeptidases to hydrolyse hordein, the storage protein present in barley²³. With regard to FAN levels, acidification is shown to increase the levels of FAN in worts. According to Power³³, 120–150 mg L⁻¹ FAN needs to be available for yeast at the start of fermentation in order for an efficient fermentation and acceptable flavour for beer. With the exception of the unacidified 50/50% malt and barley, all worts fell within the recommended level. It was also observed that in general, the bioacidified worts show slightly higher levels of FAN than the chemically acidified wort, indicating that the bacterial cultures played a small role in the solubilisation of nitrogen from the malt. It was also observed that *L. amylovorus* FST 1.1, the strain with the highest proteolytic activity, showed the highest FAN level.

Apparent degree of fermentability

With regard to attenuation limit, slight differences were observed. The lower enzyme pool and pH of unacidified grist containing 50% malt and 50% barley has led to a decrease of up to 5.8% in apparent fermentability compared to biologically acidified worts and 12.3% decrease compared to 100% malt. The trend in general showed that BA gave higher levels of sugars available for fermentation (73.6%–76.0%), while FG lactic acid could only reach fermentability levels of 71.8%. This seems to indicate that apparent fermentability levels are not only increased as a result of the lactic acid produced by the cultures but rather more as a result of the combination of lactic acid produced in conjunction with extracellular enzyme activity of the cultures.

Viscosity

When malt is replaced with unmalted barley the first difficulties are encountered in the runoff of the wort. An increase in barley level over 20–25% leads to an increase in wort viscosity and therefore a longer run off time in the lauter tun or mash filter. High wort viscosity is generally due to the presence of undegraded starch and cell wall materials such as β-glucans and arabinoxylans. This can

lead to problems in wort and final beer filtration⁴¹. Table IV shows the results of wort viscosity at an extract of 11%. Highest viscosity was as expected from 50/50 unacidified malt and barley. This was followed by *L. amylovorus* FST 1.1. One might expect however a slightly lower viscosity value for the latter due to the presence of amylolytic activity within the strain. One explanation, which might be considered, is the release of pentosans and arabinoxylans due to proteinase/amylase activity. This would serve to increase viscosity however no literature to our knowledge has supported this idea. In general it was observed that chemical acidification was less effective at reducing the viscosity, than BA. It was also noted that viscosity levels did not correlate with β-glucan levels, this may be due to the absence of malt endogenous enzymes resulting in poorer starch, protein, glucan and arabinoxylan breakdown, similar results were observed by Vis and Lorenz⁴⁰. Other constituents such as α-glucans, protein complexes or other dissolved solids may also be responsible for at least a part of the viscosity present.

Filtration and β-glucan

Wort purification was carried out according to two different techniques (Figs. 1 and 2). Both techniques demonstrated similar lautering performances in the order in which the mashes filtered, the speed at which they filtered also correlated to the level of β-glucan present. Highest filterability in both cases was shown by 100% malt as expected, this showed lowest β-glucan levels (Fig. 3). This is a result of adequate breakdown of barley endosperm cell wall during malting and mashing. This ensures maximum exposure of cellular starch and protein to hydrolytic enzymes, a minimum release of high molecular weight β-glucan during mashing and an insignificant number of endosperm fragments. These endosperm fragments are reported to coalesce during lautering resulting in a sealed off mash bed and hindering lautering performance⁵. This malt activity results ultimately in increased filterability of wort and lowest β-glucan levels (262 mg L⁻¹). Slowest lautering in both cases was demonstrated by unacidified 50% malt and 50% barley, followed by *L. plantarum* FST

1.7. The resultant worts also contained the highest levels of β -glucans (784 mg L⁻¹) and (673 mg L⁻¹), respectively. Some arabinoxylans are solubilised from the cell walls but are not extensively degraded by endogenous enzymes during malting. Solubilised arabinoxylans are therefore responsible for the high viscosity of the malt-extract, which may also be a factor of diminished rate of wort filtration¹³. Bioacidified mashers in general showed better filterability and lower β -glucan levels than chemically acidified mashers. In both cases *L. plantarum* TMW 1.460 had highest filtering ability coinciding with low β -glucan levels (490 mg L⁻¹). *L. plantarum* TMW 1.460 also showed the high lactic acid production after 24 h. FG lactic acid demonstrated a slow lautering ability, which can be explained by a β -glucan level of almost 660 mg L⁻¹, this underlines the fact that biological cultures play a role in reducing viscosity, and increasing filtration rates. The amyolytic and proteolytic activity of *L. amylovorus* FST 1.1 is also portrayed in the lautering performance. Its enzyme activity resulted in greater breakdown of both polysaccharides and proteins aiding the filtration process. Both lautering systems resulted in a fast wort filtration where *L. amylovorus* FST 1.1 was applied.

CONCLUSIONS

The work reported in this paper shows that BA with *L. plantarum* TMW 1.460, *L. plantarum* FST 1.7, *L. amylovorus* FST 1.1, and *W. confusa* FST 1.31 can compensate for reduced enzyme activity in a mash containing 50% barley. In comparison to the control mash of 100% malt, the volume of filtrate of unacidified malt and barley mashers were approximately 30% lower in the case of EBC filtering and 24% lower in the lautering system. When *L. plantarum* TMW 1.460 was applied the reduction in volume of filtrate was narrowed to approximately 10% and 5%, respectively. With regard to the lautering performance of the mashers acidified biologically, *L. plantarum* TMW 1.460 demonstrated the best lautering performance in both filtration systems. This confirms the work of Sjöholm *et al.*³⁶ who found that starter cultures *L. plantarum* and *Pediococcus pentosaceus* increased the rate of mash filterability when measured by the Büchner filtration test. The addition of *L. plantarum* TMW 1.460 also resulted in a significant reduction of β -glucan, which has long been recognised as having the potential to cause a number of problems in the brewing process. These include poor mash conversion, retarded and inefficient wort and beer filtration, and non-bacterial colloidal hazes in the final product¹. Throughout the trial little or no correlation was found between the viscosity and β -glucan levels of the wort. This may be due to the absence of malt endogenous enzymes resulting in poorer starch, protein, glucan and arabinoxylan breakdown. Although Vis and Lorenz⁴⁰ observed similar results, many authors have found wort viscosity is the best indicator of the presence of β -glucan^{24,26,28,36,37}. *L. amylovorus* FST 1.1, exhibited excellent proteolytic and amyolytic activity. This was reflected in its good lautering performance and increased FAN levels. This indicates that there is a relationship between the enzymatic activity of the bacterial culture and the solubilisation of nitrogen from the malt resulting in increased FAN levels, and increased

breakdown of polysaccharides leading to faster filtration times. This strain also showed the lowest β -glucan levels. With the exception of the unacidified mash all worts fell within the recommended FAN level.

Mashes acidified chemically using FG lactic acid were also compared to biologically acidified mashers. It was concluded that both chemical and BA resulted in a higher wort extract than non-acidified mashers due to the higher amyolytic activity at the lower pH. However due to the improved enzymatic activities coming from the strains it was generally observed that BA was slightly more effective than chemical acidification particularly in relation to the lautering ability of the mashers. As previously mentioned a strong correlation was observed between the lautering performance of the mashers and the β -glucan levels of the resultant worts. The slowest lautering performance was exhibited by strain *L. plantarum* FST 1.7 followed by the chemically acidified mash; highest β -glucan levels in both cases were measured in the resultant worts.

With regard to apparent fermentability, it was concluded that biological or chemical acidification increased the level of fermentable sugars by 5.8% in comparison to unacidified mashers. However the effect of the lower enzyme pool was observed as 100% malt reached fermentability levels of up to 6.5% higher than acidified worts. The worts that showed the highest apparent fermentability levels were that of *W. confusa* FST 1.31 and *L. plantarum* FST 1.7, similarly both worts demonstrated highest extract values.

In conclusion it can be said that BA with enzyme active LAB can be used to overcome the reduced enzyme content of a mash containing 50% unmalted barley. Overall it was observed that proteolytic and amyolytic LAB had a positive influence on the quality and hence the processability of the wort.

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