

# The Influence of Lactic Acid Bacteria on the Quality of Malt

Deirdre P. Lowe<sup>1,2</sup>, Elke K. Arendt<sup>1,4</sup>, Almudena M. Soriano<sup>3</sup> and Helge M. Ulmer<sup>1,2</sup>

## ABSTRACT

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In this study three strains of lactic acid bacteria were applied during the malting process to evaluate the impact on malt and wort quality. The trials were performed in a micromalting plant simulating an industrial malting programme. The samples were compared to chemically acidified as well as non-acidified malt. Bacterial cultures were chosen with reference to their enzymatic (proteolytic/amyolytic) activity, or their good acidifying properties. The effects of lactic acid bacteria on wort characteristics were investigated and compared to wort produced from 100% unacidified malt. A chemical food grade lactic acid was also used to acidify the barley for comparison purposes. Characteristics such as pH, extract, colour, viscosity, total soluble nitrogen, free amino nitrogen, apparent fermentability,  $\beta$ -glucan and lautering performance of the resultant worts were determined. Results showed improved levels of  $\beta$ -glucanase in the malt although reduced malt friability was observed where LAB was employed. An improved lautering performance, lower wort viscosity and elevated TSN levels were also reported where LAB exhibiting protease activity were applied.

**Key words:** Enzymatic activity, lactic acid bacteria, lautering rates, malting, wort quality.

## INTRODUCTION

Microbial proliferation is an indigenous component of the malting and brewing environment. Malted cereals contain high levels of various microorganisms representing a wide range of bacteria, yeast, and fungal species<sup>39</sup>. Douglas and Flannigan<sup>11</sup> found, that both the initial microorganism level of the barley and the malting process in operation influences the activity and the development of the microflora during malt production. Microbial spoilage as a result of these microorganisms often leads to technological impediments in the malting and brewing processes including raw material spoilage, filtration problems, and deleterious effects on both the fermentation process and final beer. These include turbidity of the beer and the formation of undesirable flavours and aromas<sup>19</sup>. LAB omni-

present on the surface of malt barley have previously been exploited for the biological improvement of the malting and brewing process by preventing the growth of harmful microorganisms and favouring beneficial ones<sup>38</sup>. Aerobic bacteria on and in the outer layers of the barley kernel are known to compete with grain tissue for oxygen due to their substantial oxygen uptake rates<sup>23</sup>. In this respect the reduction of grain bacterial flora can be regarded as advantageous. Boivin and Malanda<sup>7</sup> demonstrated how the application of *Geotrichum candidum* as a starter culture in malting could inhibit the growth of undesirable moulds and the production of mycotoxins, when applied to steeping water. In the same study they found that the organoleptic qualities of the “starter” beers were excellent. Previous to this *Geotrichum candidum* and lactic bacteria starter cultures have been investigated in micromalting plants to better control the malting process and improve the quality and safety of the malt<sup>8,19,20</sup>. Haikara et al.<sup>20</sup> employed starter cultures of *Lactobacillus plantarum* and *Pediococcus pentosaceus* during malting to restrict the growth of *Fusarium* moulds and their mycotoxins in order to prevent gushing. Gushing of beer is regarded as one of the most negative consequences of moulds with respect to the quality of malt and beer<sup>1</sup>. Gushing may be defined as the quick uncontrolled spontaneous over-foaming immediately when opening a bottle or can<sup>1</sup>. *Fusarium graminearum*, *Fusarium culmorum*, and *Fusarium poae* have all been named as active gushing inducers<sup>18,35,37,43</sup>. The addition of starter cultures in the early stage of malting is important due to the intensive growth of Fusaria during the very first hours of steeping<sup>19</sup>. Certain *Lactobacillus plantarum* and *Pediococcus pentosaceus* cultures have been reported as especially efficient when added to the steeping waters of barley at the level of about  $10^7$  cells g<sup>-1</sup><sup>19</sup>. Beneficial effects on the malting process included an improved modification, lower viscosity and  $\beta$ -glucan levels in wort, and increased malt yields. A pronounced improvement of mash filtration and wort filterability has also been recorded due to the addition of LAB<sup>19</sup>. Laitila et al.<sup>26</sup> added LAB to naturally contaminated barley in the steeping process. This resulted in a diminished level of *Fusarium* contamination along with lower levels of the mycotoxins deoxynivalenol and zearalenol during the malting process.

In this study three strains of LAB were applied during the malting process to evaluate the impact on malt quality. The trials were performed in a micromalting plant simulating an industrial malting programme. Malt produced from the acidified barley samples was compared to malt produced from untreated and chemically acidified barley. The effects of the LAB on wort characteristics were inves-

<sup>1</sup>Department of Food and Nutritional Sciences, National University of Ireland, University College Cork, Ireland.

<sup>2</sup>National Food Biotechnology Centre, National University of Ireland, University College Cork, Ireland.

<sup>3</sup>Department of Analytical Chemistry and Food Technology, University of Castilla-La Mancha, Ciudad Real, Spain.

<sup>4</sup>Corresponding author. E-mail: e.arendt@ucc.ie

**Table I.** Barley characteristics.

Characteristics (units)	Barley
Variety	Optic
Harvest	Ireland 2002
Moisture (%)	11.78
Total N (%)	1.6
Total protein (%)	9.97
Soluble protein (%)	2.67*
$\beta$ -Glucan (%)	2.84
Filtration Time	Slow*
Colour (EBC)	5.16*
Extract (DB) (%)	79.45*
Viscosity (mPa s)	1.99*
Kolbach index	26.17*

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

tigated and compared to wort produced from 100% untreated malt. A chemical food grade lactic acid (FG LA) was also used to acidify the barley for comparison purposes.

## MATERIALS AND METHODS

### Applied cereals

Unmalted barley and malt (variety: Optic; harvest: 2002) was obtained from the Malting Company of Ireland (Table I). The barley was stored in airtight drums at room temperature. Mains water was used for mashing liquor.

### Strains and culture conditions

*Lactobacillus amylovorus* FST 1.1 was obtained from the Department of Food Science and Technology, University College Cork (UCC). *Lactobacillus plantarum* TMW 1.460 and *Lactobacillus amyolyticus* TMW 1.268 were obtained from the Institute of Technical Microbiology at the Technical University of Munich-Weihenstephan (TMW), Germany. These bacteria, isolated from malted barley were maintained as frozen stocks in 50% glycerol at  $-80^{\circ}\text{C}$  and were propagated twice in MRS broth for 16 h before experimental use. Strains were cultured on de

Man Rogosa Sharp Agar (MRSA) plates (Oxoid Ltd) at  $30^{\circ}\text{C}$  overnight before being transferred into 450 mL of modified MRS broth (mMRS4) (containing 15 mL  $\text{L}^{-1}$  of 50% (w/w) maltose in water solution and 7.5 mL  $\text{L}^{-1}$  of 50% (w/w) fructose solution) and incubated aerobically for 48 h at  $30^{\circ}\text{C}$ .

### Detection of proteolytic activity of LAB

The proteolytic activity of *L. amylovorus* FST 1.1, *Lactobacillus plantarum* TMW 1.460 and *Lactobacillus amyolyticus* TMW 1.268 was determined on skim milk agar plates as described by Lowe et al.<sup>29</sup>. Clearing zones (halos) around the wells indicated proteolytic activity where a zone of 0.1–0.6 cm around the well indicated fair proteolytic activity, 0.6–1.5 cm around the well indicated good proteolytic activity and  $>1.5$  cm around the well indicated very good proteolytic activity. The protease enzymes were not determined.

### Detection of $\alpha$ -amylase activity of LAB

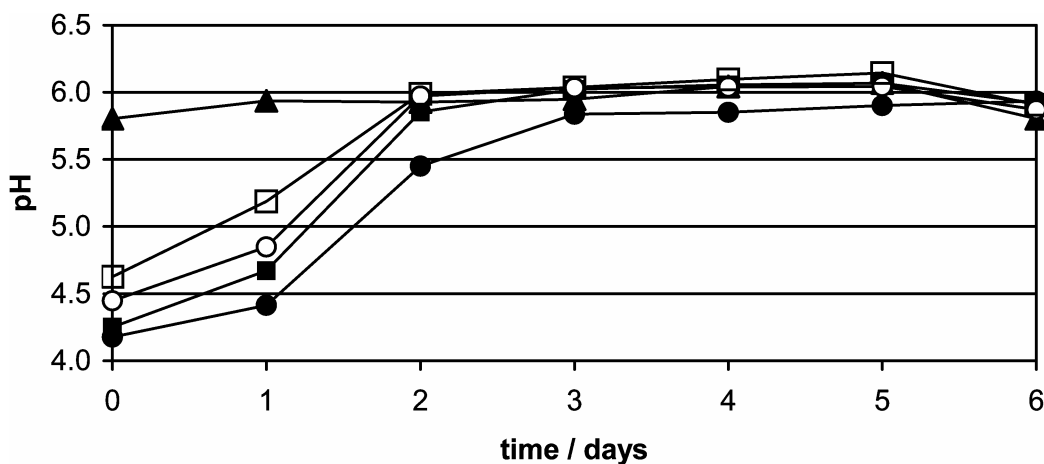
The production of  $\alpha$ -amylase activity was tested on starch plates as described by Lowe et al.<sup>29</sup>. The presence of a clear halo around a tested colony was taken as an indication of starch degradation and therefore the production of  $\alpha$ -amylase activity, where a zone of 0.1–0.6 cm around the colony indicated fair amylase activity, 0.6–1.5 cm around the colony indicated good amylase activity and  $>1.5$  cm around the colony indicated very good amylase activity. The amylase enzymes were not determined.

### Detection of $\beta$ -glucanase activity of LAB

$\beta$ -Glucanase activity was determined using a beta glucanase test kit obtained from Megazyme (Wicklow, Ireland).

### Inoculation of LAB during malting

Each LAB strain was inoculated into 450 mL of mMRS4 broth as described earlier before being decanted into 1 kg barley samples after steeping (Fig. 1). The 1 kg samples were then mixed to ensure an even distribution of inoculant. To compare biological acidified malt with



**Fig. 1.** pH development in washing water of the malt, (FG LA, food grade lactic acid). Shown are the malted samples from untreated barley (▲), as tested with TMW 1.268 (□), FST 1.1 (○), FG LA (■), TMW 1.460 (●).

chemical acidified malt, DL-lactic acid (Sigma) was added to one barley sample. Lactic acid production was determined by titration of 25 mL inoculated mMRS4 broth with 0.1 M NaOH to pH 7.0 after 48 h according to Kunze<sup>25</sup>. The highest concentration of lactic acid obtained in the different broths was taken as the concentration of chemical lactic acid to be added to the barley sample. Untreated barley was malted as an additional control.

### Malting programme

Malting was carried out in a micro-malting machine (Joe White Malting Systems, Australia). The temperatures and times of the malting programme are outlined in Table II.

### Analyses during malting

The pH of washings of germinating barley was measured on a daily basis by washing 5 g of green malt with 5 mL of sterile tap water. Samples for microbiological analysis were taken following steeping, throughout the germination process and after kilning. Ten grams of each sample was washed in 10 mL of sterile tap water and the colony forming units (cfu) per mL of washings were determined on MRS agar (Oxoid, UK). Throughout germination, the lengths (mm) of the rootlet and acrospire were measured using a vernier calliper. One hundred kernels of each sample were selected randomly, and the mean rootlet and acrospire length (mm) was determined.

### Malt analyses

Friability was measured using a Friabilimeter (Pfeuffer GmbH, Germany) according to Analytica EBC 4.15. Moisture content was determined according to the method of EBC 4.2. Total nitrogen was measured using the Dumas combustion method according to Analytica EBC 4.3.2.  $\beta$ -Glucanase,  $\alpha$ -amylase and  $\beta$ -amylase activity were measured in the final malt according to test kits obtained from Megazyme (Wicklow, Ireland).

### Milling

Prior to mashing, the malt was milled using a Bühler Miag laboratory scale disc mill (Bühler GmbH, Germany) set at a fine grind setting of 0.2 mm.

### Mashing

Mashing was carried out in an EBC mash bath (Funke-Dr.N.Gerber Instruments) using the standard Congress mashing method according to EBC 4.5.1.

### Filtration

The mash was filtered according to EBC 4.5.1, through filter paper (Schleicher & Schuell, Germany) into graduated cylinders. The amount of filtrate recovered was recorded every 15 min over a 90 min period.

Table II. Malting programme.

Steeping	Germination	Kilning
First wet 14°C × 6 h	17°C × 48 h	30°C × 4 h
First dry 16°C × 9 h	15°C × 24 h	55°C × 12 h
Second wet 14°C × 5 h	14°C × 24 h	62°C × 3 h
Second dry 16°C × 10 h	13°C × 12 h	72°C × 3 h
Third wet 14°C × 5 h	12°C × 12 h	78°C × 3 h
Third dry 16°C × 4 h		82°C × 3 h

### Wort analysis

All analyses were carried out in duplicate using EBC<sup>13</sup> or MEBAK<sup>33</sup> methods. Specific gravity and extract of the wort samples were measured with an automatic beer analyser (SCABA) according to EBC 9.2.2. Wort pH was measured according to EBC 8.17. 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 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  $\beta$ -glucan content was determined using the mixed linkage  $\beta$ -glucan kit (Megazyme) according to EBC 8.13.1. Colour was determined in accordance with EBC 8.5.

### Experimental procedure

The malting trial and wort analysis were carried out in duplicate. Results shown are the mean of two repeated malting trials and/or two repeated EBC Congress mashes, where  $\pm$  denotes the standard deviation.

## RESULTS AND DISCUSSION

In this study three strains of LAB were applied at the end of steeping during the malting process to evaluate their impact on malt quality. *L. amylovorus* FST 1.1 exhibited protease and amylase activity when tested on skim milk and starch agar plates, respectively (Table III). *L. amylolyticus* exhibited amylase activity when tested on starch agar plates, while *L. plantarum* TMW 1.460 demonstrated highest levels of lactic acid production in mMRS4 broth. LAB tested negative for  $\beta$ -glucanase activity. The influences of LAB were evaluated in pilot scale maltings and samples were compared to chemically acidified as well as non-acidified malt. The effects of the LAB and FG LA on wort characteristics were also investigated and compared to wort produced from 100% untreated malt. Results of the trial are discussed below.

### pH

The pH of the malting washing water was recorded over the germination period. Fig. 1 reflects that as a result of lactic acid production by LAB and the addition of FG LA, the pH is lower in these samples at the beginning of germination compared to the barley control. However, after two days of germination all samples have reached approximately the same pH. This pH increment is most

Table III. Enzymatic and lactic acid production properties of LAB.

LAB	Lactic acid production at 48 h (mg/mL)	Protease activity	Amylase activity
<i>L. amylovorus</i> FST 1.1	16.2	++	++
<i>L. plantarum</i> TMW 1.460	18.9	-	-
<i>L. amylolyticus</i> TMW 1.268	11.6	-	++

All strains tested negative for  $\beta$ -glucanase activity  
 + = fair activity, where halo measures 0.1–0.6 cm  
 ++ = good activity, where halo measures 0.6–1.5 cm  
 +++ = very good activity, where halo measures > 1.5 cm

**Table IV.** Acrospire length (mm) of barley samples during germination.

Day	Barley (untreated)	Barley + TMW 1.460	Barley + TMW 1.268	Barley + FST 1.1	Barley + FG LA
1	1.86 ± 0.09	1.84 ± 0.03	1.81 ± 0.07	1.96 ± 0.14	1.83 ± 0.01
2	2.41 ± 0.17	2.35 ± 0.16	2.31 ± 0.13	2.41 ± 0.16	2.18 ± 0.17
3	2.61 ± 0.16	2.45 ± 0.21	2.57 ± 0.16	2.55 ± 0.02	2.50 ± 0.08
4	2.75 ± 0.13	2.55 ± 0.04	2.59 ± 0.18	2.79 ± 0.04	2.69 ± 0.07
5	2.93 ± 0.21	2.89 ± 0.04	2.79 ± 0.17	2.85 ± 0.04	2.71 ± 0.18

(Shown are the mean values of two independent experiments), FG LA= food grade lactic acid, ± = standard deviation.

**Table V.** Root length (mm) development of barley samples during germination

Day	Barley (untreated)	Barley + TMW 1.460	Barley + TMW 1.268	Barley + FST 1.1	Barley + FG LA
1	6.01 ± 2.26	2.44 ± 0.45	4.08 ± 1.64	3.77 ± 0.33	2.42 ± 0.19
2	11.33 ± 0.59	6.94 ± 2.09	8.31 ± 2.53	8.58 ± 0.11	6.84 ± 1.07
3	11.47 ± 0.79	5.15 ± 0.72	10.20 ± 0.82	9.11 ± 0.64	7.41 ± 0.01
4	13.90 ± 1.14	7.39 ± 1.00	9.86 ± 1.19	10.48 ± 1.78	9.33 ± 0.18
5	14.80 ± 0.95	9.40 ± 1.53	11.44 ± 1.02	10.88 ± 1.30	8.96 ± 0.28

(Shown are the mean values of two independent experiments), FG LA = food grade lactic acid, ± = standard deviation.

likely due to the anaerobic respiration of the grain. This occurs when the grain is steeped, the oxygen dissolved in the water is rapidly utilised, resulting in the production of carbon dioxide and ethanol<sup>10</sup>. In addition, the LAB and natural microflora present on or in the grain compete for oxygen with the grain tissues causing substances such as phenolic compounds to leach from the surface layers during steeping<sup>10</sup>. As respiration continues, phytate is hydrolysed and inorganic phosphates are released with other mineral substances, lipids are hydrolysed and fatty acids are oxidised or converted to sugars<sup>10</sup> thus contributing to the gradual increase in pH again. Day 5 shows a slight drop in pH again, as some lactobacilli may oxidize lactate formed earlier during steeping and germination to yield formic, acetic and succinic acids during glucose limitation<sup>46</sup>.

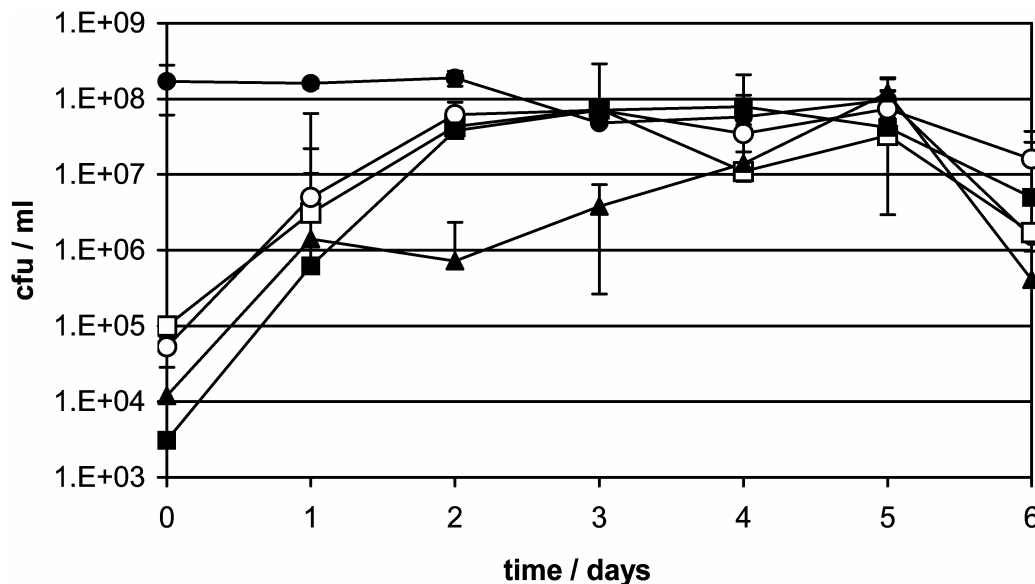
### Malt modification

The enzymic degradation of the cell wall is crucial to both the steeping and germinative phases. Most of the enzymes are supposed to be secreted from the aleurone layers; the cell walls prevent the access of these enzymes to their substrates within the starchy endosperm cells. As a result, the rapid early removal of the cell wall is of central importance both for the successful germination of the grain<sup>14</sup> and for the production of well-modified malt<sup>5</sup>. Tables IV and V show the development of the acrospires and rootlets over the germination period. The length of the acrospire in fully modified malt will typically be 75–100% of the seed length. Table IV shows the acrospire length of the untreated barley control throughout the germination period was slightly longer than all other barley samples. This may be an effect of lactic acid or as a result of additional bacteria, which may inhibit barley germination by physically impeding the entry of oxygen to the kernel<sup>17</sup>. Shorter acrospires may also be as a result of the increased level of microorganisms competing for oxygen resulting in an inhibition of the germination process<sup>17</sup>. Some microorganisms have also been reported to attack

the embryos, which may inhibit malt modification. The barley kernel treated with FG LA had the shortest acrospire length, which may be related to a slower rate of modification. The acrospires length of all germinating barley samples at day 5 ranged between 2.71 ± 0.18 – 2.93 ± 0.21 mm. Similarly, the same explanation can be offered with regard to the root length, where untreated barley exhibited the longest rootlets throughout germination. In addition, treatments that inhibit rootlet growth frequently cause an increase in soluble nitrogen levels, presumably because the nitrogen is not drained away into the roots. Thus elevated levels of TSN may be seen in the wort as discussed later. A difference of approximately 3–5 mm was observed on day 5 between the rootlet length of untreated barley kernels and kernels treated with either LAB or FG LA.

### Microbial growth during germination

The development of microbial growth on barley during malting is shown in Fig. 2. During the steeping stage, the moisture content of the grain is elevated resulting in conditions that are favourable for the multiplication of contaminants present on the grain. Aerobic, heterotrophic bacteria, mycelial fungi and yeasts develop and in general, the total numbers of microorganisms further develop during germination. Rapid proliferation of microorganisms was observed after steeping (Fig. 2, day 0) despite the low temperatures of the malting programme. This might be as a result of steep aeration<sup>23</sup>. Highest cfu/mL after steeping were exhibited by *L. plantarum* TMW 1.460. This LAB was isolated from malt and adapted well to the plant environment unlike *L. amylovorus* and *L. amylolyticus*, which did not adapt as readily. In addition *L. plantarum* TMW 1.460 grew to 10<sup>10</sup> cfu/mL in mMRS4 broth, while *L. amylovorus* and *L. amylolyticus* grew up to just 10<sup>8</sup> cfu/mL. Application of LAB usually results in a dilution of 2 log cycles; therefore the addition of the latter two LAB to the malt may have had an initial inactivating effect. After two days the colony forming units (cfu) per mL



**Fig. 2.** Mean values of cfu /mL of washings during malting, where day 6 is the sample after kilning. (FG LA, food grade lactic acid.) Shown are the mean values and their standard deviations of two independent experiments, where samples are untreated barley (▲), barley treated with TMW 1.268 (□), FST 1.1 (○), FG LA (■), TMW 1.460 (●).

**Table VI.** Results of enzyme activities in malt and malt analysis.

	Barley (unacidified)	Barley + TMW 1.460	Barley + TMW 1.268	Barley + FST 1.1	Barley + FG LA
β-Glucanase (U/kg)	429 ± 26	490 ± 37	540 ± 31	517 ± 21	584 ± 14
α-Amylase (CU/g)	182 ± 9	151 ± 9	180 ± 11	170 ± 12	180 ± 9
β-Amylase (U/kg)	626 ± 15	577 ± 3	622 ± 10	576 ± 1	583 ± 8
Friability (%)	89.2 ± 1.6	83.3 ± 1.7	90.0 ± 2.2	87.6 ± 2.8	88.0 ± 1.1
Total nitrogen (TN) (%)	1.43 ± 0.01	1.44 ± 0.01	1.42 ± 0.02	1.43 ± 0.01	1.42 ± 0.02

(Shown are the mean values of two independent experiments), FG LA = food grade lactic acid, ± = standard deviation.

of washings on MRS agar ranged between  $10^7$  and  $10^8$  whereas the microflora on the barley sample were 1–1.5 orders of magnitude lower. This may be attributed to the fact that mMRS4 broth was added in addition with LAB cells and lactic acid to all samples excluding untreated barley. The viable counts of bacteria reached a maximum during germination, similarly Petters et al.<sup>40</sup> found comparable results. The onset of microbial activity after steeping is also reflected by oxygen uptake activity associated with the husk and steep liquor where the availability of nutrients leads to further microbial proliferation<sup>9</sup>. The progressive increase in microbial population observed in all samples throughout the germination period may also be attributed to the release of readily metabolizable components as a result of enzymatic activity in the germinating kernels<sup>40</sup>. Results showed that LAB had the ability to grow by 2 log cycles on the malt and still survive kilning.

### Friability

Friability is a measure of the breakdown of malt endosperm cell wall components and so gives a measure of physical modification. Table VI show that the values of friability range between 87–89% with the exception of the malt acidified with *L. plantarum* TMW 1.460, which demonstrated a friability value of 83%. It was also noted that

malt acidified with *L. plantarum* TMW 1.460 had a higher glassy corn fraction than all other malt samples. Numerous studies have examined the relationship between malt friability and wort viscosity, and reported strong negative relationships between the aforementioned parameters<sup>6,47</sup>. Our results do not support these findings as malt acidified with *L. plantarum* TMW 1.460, had the lowest friability, and a low wort viscosity. In general it was found with the exception of malt produced from barley treated with TMW 1.268, which demonstrated a friability of  $90.0 \pm 2.2\%$ , all other acid treated malts were lower than the untreated malt, which had a friability of  $89.2 \pm 1.6\%$ . This differs from the results of Reinikainen et al.<sup>42</sup> who found that microbial starter cultures employed in the field resulted in improved malt modification in terms of friability. It should be noted however that inoculation of LAB during the malting stage differs from natural contamination in that the microorganisms are applied externally, while natural or artificial contamination in the field allows the microorganisms to settle within or beneath the husk<sup>46</sup>.

### Total nitrogen

Table VI shows the TN (%) values for the acidified and non-acidified malts. Original TN of barley was 1.6%. According to EBC<sup>13</sup> a TN content of 1.4–1.7% is recom-

**Table VII.** Results of wort analysis.

	Barley (untreated)	Barley + TMW 1.460	Barley + TMW 1.268	Barley + FST 1.1	Barley + FG LA
pH wort [20°C]	5.83 ± 0.02	5.84 ± 0.03	5.87 ± 0.02	5.86 ± 0.02	5.88 ± 0.03
Extract [% dm]	82.5 ± 0.6	82.4 ± 0.7	82.3 ± 0.6	82.4 ± 1.0	82.2 ± 0.8
Colour [EBC]	3.01 ± 0.01	2.99 ± 0.02	3.29 ± 0.09	3.29 ± 0.05	3.11 ± 0.05
TSN [mg L <sup>-1</sup> ]	624 ± 22	658 ± 22	688 ± 23	689 ± 28	677 ± 25
FAN [mg L <sup>-1</sup> ]	138 ± 2	132 ± 2	141 ± 4	136 ± 3	137 ± 4
Apparent fermentability [%]	86.0 ± 0.4	84.5 ± 0.4	84.2 ± 0.4	84.8 ± 0.4	84.2 ± 0.2
β-Glucan (mg L <sup>-1</sup> )	95 ± 20	163 ± 27	93 ± 14	119 ± 27	125 ± 23
Viscosity [mPas]	1.59 ± 0.03	1.53 ± 0.03	1.53 ± 0.02	1.53 ± 0.02	1.54 ± 0.03
Filtrate volume at 1 h (mL)	235 ± 21	277 ± 9	264 ± 5	300 ± 14	277 ± 12

(Shown are the mean values of two independent experiments), FG LA= food grade lactic acid, ± = standard deviation.

mended, greater than 1.9% TN may lead to the formation of beer haze along with mash off problems. All malts demonstrated a TN% between 1.42 ± 0.02% and 1.44 ± 0.01%.

### Enzymes in malt

Table III shows the activities of β-glucanases, α-amylases and β-amylases present in the malt samples before mashing. β-Glucan is a cell wall polysaccharide, which accounts for approximately 70% (w/w) of the endosperm cell walls in barley<sup>16,22</sup>. Malted barley contains several endogenous β-glucanases including at least two isoenzymes of (1-3,1-4)-β-D-glucan 4-glucohydrolase<sup>15,27,30,44</sup>. According to EtokAkpan<sup>12</sup>, the degradation of endosperm cell walls and subsequent associated changes in β-glucan levels during malting is, to a great extent, related to β-glucanase activity, which depolymerises β-glucan. Malted barley is also known to contain (1-3)-β-D-glucan 3-glucohydrolase<sup>21,31</sup> as well as low levels of (1-4)-β-D-glucan 4-glucohydrolase, which is known more commonly as cellulase<sup>49</sup>. Increased β-glucanase activities were found in malts where LAB and FG LA had been applied to the barley kernels (Table III). Lowest β-glucanase activity was observed in malt from untreated barley, this is indicative of problems, which may later arise in beer filtration. All biologically acidified malts exhibited higher β-glucanase activity compared to β-glucanase activity in malt produced from untreated barley. However highest β-glucanase activity was found in malt produced from chemically acidified barley, this would seem to indicate that it was the effect of lactic acid or the lower pH as a result of lactic acid at the start of germination (β-glucanase has an optimum pH of 4.5–5) that caused the increase in β-glucanase activity rather than the LAB themselves. With regard to α-amylase and β-amylase activities in the malt samples, it was concluded that higher α-amylase and β-amylase activities were found in malt from untreated barley (182 ± 9 CU/g and 626 ± 15 CU/g, respectively). This is most likely due to the higher pH in untreated barley at the start of germination (pH 5.8) as highest α-amylase and β-amylase activities are demonstrated at an optimum pH of approximately 5.7 and 5.4–5.6, respectively<sup>10</sup>. This was followed by malt, which had been produced from barley treated with *L. amylolyticus* TMW 1.268. This may have been due to its amylase activity, however *L. amylovorus* FST 1.1 also exhibited amylase activity but only demonstrated α-amylase and β-amylase activities of 170 ± 12 and 576 ± 1 CU/g, respectively. Lowest enzyme activities

were found in malt produced from barley treated with *L. plantarum* TMW 1.460, this malt also showed lowest friability (83.3 ± 1.7%).

### Wort analysis

**pH.** According to Kunze<sup>25</sup>, the pH of wort ranges between 5.6 and 5.9. Table VII shows that all wort samples fell within 5.83 ± 0.02 and 5.88 ± 0.03.

**Extract.** Extract levels of worts are shown in Table VII. No significant differences were observed between the extracts of worts from acid treated malts and untreated malt as all worts contained an extract in the range of 82.2–82.5 ± 0.8%.

**Apparent degree of fermentability.** With regard to the attenuation limit of the worts, the addition of LAB or FGLA imparted little or no effect. The apparent fermentability levels of worts ranged on average between 84.2–86% (Table VII), with wort from untreated malt exhibiting the highest level of available sugars.

**Nitrogenous fractions.** Table VII shows the level of TSN in worts. Results indicate that the addition of LAB or FG LA after steeping results in higher concentrations of TSN in resultant worts compared to wort derived from untreated malt. Highest levels of TSN were observed in worts where *L. amylovorus* FST 1.1 and *L. amylolyticus* TMW 1.268 had been applied to barley; this may be due to additional enzymatic activities (proteolytic/amyolytic) of the LAB. Apart from substances leached during steeping there is no loss of nitrogen from the grain during malting. However, respiratory losses of dry matter that occur tend to raise the nitrogen percentage in the final grain, while a substantial quantity of nitrogen moves into the roots and is removed after malting<sup>10</sup>. In this way LAB may have played a role in impeding the movement of nitrogen into the roots, resulting in an increased level of nitrogenous substances coming into solution during mashing and an overall increase in TSN. In general it was concluded, that worts obtained higher levels of TSN when barley was acidified biologically rather than chemically and when LAB exhibited additional extracellular enzymatic activities. In a study by Reinikainen<sup>42</sup>, increased levels of both soluble nitrogen and FAN were found when LAB cultures were applied in the field. With regard to FAN levels, only slight differences were observed between worts. According to Power<sup>41</sup>, 120–150 mg L<sup>-1</sup> FAN needs to be available for yeast at the start of fermentation in order for an efficient fermentation and acceptable flavour for beer. The formation of FAN peptides and poly-

peptides influences yeast growth and the foam and haze properties of derived beers<sup>24,28</sup>. Highest FAN levels were found in wort derived from malt where *L. amylolyticus* TMW 1.268 had been applied to the barley; this was followed by wort from untreated malt. *L. amylovorus* FST 1.1 which contains additional proteolytic activity contributed no positive impact on the FAN level. Similarly, FG LA exhibited lower FAN levels than wort prepared from untreated malt. All wort samples exhibited FAN levels within the recommended levels according to Power<sup>41</sup>.

**Colour.** Colour formation can be attributed to the malting process, where green malt is kilned at high temperatures of around 80°C. The numerous amino acids react with the reducing sugars to form intermediary products, which undergo further transformation to brown melanoidins giving worts their characteristic colour and aroma<sup>10</sup>. Results show (Table VII) that all worts ranged on average between  $2.99 \pm 0.02$  and  $3.29 \pm 0.05$  EBC units. Broth used for cultivating the bacteria was autoclaved at 121°C/10 min, these high temperatures would also lead to maillard browning, the addition of mMRS4 broth to barley may have imparted a small contribution to the wort colour compared to the wort colour from the untreated malt. In general it was concluded, that LAB added during the malting process had very little impact on the resultant wort colours. Similarly, only a marginal colour difference was seen between the control wort and chemically acidified wort.

**β-Glucan.** The major constituents of walls in the starchy endosperm of barley are the (1-3,1-4)-β-glucans and the arabinoxylans<sup>2,14</sup>. If cell walls are not adequately degraded during malting, malt extracts can contain high levels of the polysaccharides and attendant difficulties associated with the filtration of viscous extracts can significantly slow the brewing process<sup>4</sup>. The β-glucan contents of the worts are shown in Table VII. Lowest β-glucan level was measured in wort produced from malt where *L. amylolyticus* TMW 1.268 had been applied, this malt also showed high β-glucanase activity. A very high level of β-glucan was measured in wort where barley was acidified using *L. plantarum* TMW 1.460; this may be attributed to the very low level of β-glucanase activity exhibited by this malt. In general it was observed that LAB and FG LA served to increase the overall β-glucan content. With regard to the relationship between β-glucan and viscosity of the worts, no correlation was observed. Wort produced from untreated malt showed the highest viscosity level while also measuring very low levels of β-glucan. All worts derived from acid treated malts showed similar viscosity levels, however β-glucan levels of the corresponding worts ranged from  $93 \pm 14$  to  $163 \pm 27$  mPas. Similarly no correlation between β-glucan levels and wort viscosities were obtained by Vis and Lorenz<sup>48</sup> and Lowe et al.<sup>29</sup>.

**Viscosity and lautering performance.** Table VII shows the effects LAB had on the viscosity and lautering performance of the worts. Highest viscosity levels were observed in wort derived from the untreated malt, resulting in the lowest volume of filtrate after one hour. Fastest wort filtration occurred in wort where *L. amylovorus* FST 1.1 was applied, this might be due to the additional proteolytic and amylolytic activity of the strain, which may have

aided in breaking down the larger starch and protein components of the mash. In addition, a reduced wort viscosity was observed where *L. amylovorus* FST 1.1 was employed compared with wort viscosity from untreated malt. The addition of all strains of LAB resulted in higher volumes of filtrate after 1 h along with corresponding lower viscosity levels. This supports the work of Haikara and Laitila<sup>19</sup>, Reinikainen et al.<sup>42</sup> and Boivin and Malanda<sup>7</sup> who found that improved wort filtration was evident when starter cultures were applied either in the field or to the malting grain. Where FG LA was applied to barley an increase in the rate of wort filtration was measured, although a slightly higher wort viscosity was obtained.

## CONCLUSION

In this study three strains of LAB were added to malting grain to evaluate the impact on malt quality. The samples were compared to chemically acidified as well as non-acidified malt. The effects of the LAB and FG LA on wort characteristics were investigated and compared to wort produced from untreated malt. During barley germination, the degradation of the cell walls is the first step in the sequence of the hydrolytic processes, generally referred to as modification. In terms of physical modification, acrospire and rootlet length are sometimes used as indicators of the modification process. Both acrospire and rootlet length were found to be considerably shorter in acid treated malts compared with the acrospire and rootlet length of untreated green malt. This may have been linked to increased number of microorganisms, which are likely to impede the entrance of oxygen to the kernel and inhibit the germination process. The presence of both lactic acid starter cultures and FG LA in the malting process however resulted in improved levels of β-glucanase in the malt despite all LAB testing negative for β-glucanase activity. This is important for the breakdown of β-glucan, which is known to cause reduced recovery of extract by impeding enzyme access, reduced rates of lautering, reduced filter runs and formation of gels and hazes in the final beer<sup>3</sup>. Neither α-amylase nor β-amylase activities were improved by the presence of LAB or FG LA in the malting process. In terms of friability, it was found with the exception of malt produced from barley treated with *L. amylolyticus* TMW 1.268, which demonstrated a friability of  $90.0 \pm 2.2$  %, all other acid treated malts were lower than the untreated malt, which had a friability of  $89.2 \pm 1.6$ %. This is suggestive again of the fact that high numbers of LAB present concomitantly with the indigenous microflora of the grain may impede the modification process, as lowest friability was demonstrated in grain where *L. plantarum* TMW 1.460 had been inoculated. In addition the growth profile of *L. plantarum* TMW 1.460 after steeping showed the highest cfu/ mL of washings on MRS agar than all other grain samples. No difference was found between the TN % of untreated malts and acid treated malts despite *L. amylovorus* FST 1.1 exhibiting protease activity. The development of microflora during the malting process was related to the starter culture employed after the steeping process. LAB had the ability to grow by 2 log cycles on the malt and still survive kilning. Considerable improvements were noted in the lautering

performance of worts derived from acid treated malts compared with the lautering performance of wort from untreated malt. Fastest lautering rate was demonstrated by wort from malt acidified with *L. amylovorus* FST 1.1; this may be due to additional extracellular enzymatic activity of the strain. This supports the work of Boivin and Malanda<sup>7</sup>, and Haikara and Laitila<sup>19</sup>, who found improved mash filterability when starter cultures were added to the malting grain. The presence of LAB and FG LA during malting were also shown to increase the resultant  $\beta$ -glucan content of wort, with the exception of *L. amylolyticus* TMW 1.268, which had a slightly lower  $\beta$ -glucan ( $93 \pm 14$  mg/L) content than the wort from untreated malt. This may be related to the  $\beta$ -glucanase activity of the malt, however untreated malt showed very low  $\beta$ -glucanase activity while its wort contained a very low  $\beta$ -glucan content. In addition, no link was found between viscosity levels and  $\beta$ -glucan contents of the resultant worts from acid treated malts or untreated malts. Although Lowe et al.<sup>29</sup> and Vis and Lorenz<sup>48</sup> observed similar results, many authors have found wort viscosity is the best indicator of the presence of  $\beta$ -glucan<sup>32,34,36,45</sup>. The nitrogenous fractions of the worts were measured and elevated levels of TSN were reported in worts derived from acid treated malts. Highest levels of TSN were found in wort where *L. amylovorus* FST 1.1 was employed. This may be attributed to the protease activity of the strain. *L. amylovorus* FST 1.1 made no impact however on FAN levels. Similarly, neither LAB starter cultures nor FG LA showed any positive influence on fermentability or extract levels of resultant worts.

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