# Malting Performance of Normal Huskless and Acid-Dehusked Barley Samples

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#### ABSTRACT

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Sulphuric acid dehusked barley had a higher germinative energy and lower microbial infection than normal huskless (naked) barley, suggesting that the pericarp layer harboured microbial infection which may have limited the germination rate. Dehusking the normal huskless barley using sulphuric acid resulted in lower microbial infection, and increased germinative energy. The normal huskless barley sample had a higher β-glucan content than the acid-dehusked barley and had slower  $\beta$ -glucan breakdown during malting. This resulted in the release of seven times more  $\beta$ -glucan during mashing, and the production of wort of higher viscosity. The normal huskless barley sample had a higher total nitrogen content than the acid-dehusked barley but both samples produced similar levels of amylolytic ( $\alpha$ - and  $\beta$ -amylase) activity over the same malting period. No direct correlation was found between barley total nitrogen level and the amylolytic activity of the malt. When barley loses its husk at harvest, the embryo is exposed and may be damaged. This may result in uneven germination which can reduce malting performance and hence malt quality.

Key words: Acid-dehusked,  $\alpha$ -amylase,  $\beta$ -amylase, barley,  $\beta$ -glucan, normal huskless, malting.

### INTRODUCTION

Malting research on different kinds of cereals has a long tradition<sup>11</sup>. This has led to a better understanding of the malting process<sup>24</sup>. Nevertheless, this understanding pertains mainly to normal commercially cultivated barley (*Hordeum vulgare*), which contains an outer layer of husk material. In contrast, the malting potential of acid-dehusked barleys has been subjected to minimal observation<sup>3</sup> while that of normal huskless (naked) barley has had even less attention. The purpose of this study was to investigate differences between normal huskless (naked) and acid-dehusked barleys to assess the influences which acid and normal dehusking can have on malting and microbial properties of each type of grain. Structurally, the pericarp layer is present in normal huskless (naked) barleys, but it is removed by treatment with 50% sulphuric acid.

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## MATERIALS AND METHODS

The normal huskless barley sample (blue aleurone layer) was a Himalayan barley (*Hordeum* sp) whereas the variety Halcyon (*Hordeum vulgare* cv), a United Kingdom barley variety which also has a blue aleurone layer, was obtained from the University Brewing store and was dehusked using cold 50%  $H_2SO_4$ , as described previously<sup>3</sup>.

## Assessment of microflora of husked, aciddehusked and normal huskless barley

*Media preparations.* A direct plating method was employed in the investigation of barley microflora. Potato dextrose agar (PDA, Oxoid) was prepared as described elsewhere<sup>4,14,16</sup>. To assess microbial contamination, fine forceps, sterilised between transfers by dipping in ethanol and burning off were used to plate out eight corns equidistantly round the circumference of the 9 cm agar plates, and two grains were placed towards the centre to provide maximum spacing. The PDA plates were incubated for 7 days at  $25^{\circ}C^{14}$ .

# Total nitrogen and total soluble nitrogen determination

The total nitrogen content of barley and total soluble nitrogen content of malt wort were determined using the Kjeldahl method using a Tecator System 2020 digester and a Tecator Kjeltec System 1002 Distillation unit. The titration unit was a Metrohm Herisau Multiburette E485 System<sup>4</sup>.

#### β-Glucan measurement

Barley or malt were milled using the Buhler Miag mill at setting 5. Exactly 0.5 g of barley or 1.0 g of malt was used for the assay as described in the Megazyme mixed-

TABLE I. Pr	operties of the	normal h	uskless and	acid-dehusked	barley
samples.					

	Normal huskless	Acid- dehusked normal huskless	Acid- dehusked standard barley
Moisture (%)	10.6	$ND^1$	10.2
Total nitrogen <sup>2</sup> (%)	2.5	ND	1.7
$\beta$ -Glucan <sup>3</sup> (w/w%)	4.6	ND	2.7
G.E. (4ml test %)	90.0	94.0	97.0
$H_2O_2$ test (%)	93.0	96.0	100
Aleurone colour	blue	blue	blue

<sup>1</sup>Not determined.

<sup>2</sup>Total nitrogen (d.m.).

<sup>3</sup>β-Glucan (as is).

<sup>&</sup>lt;sup>1</sup> The Extract Factory, Scotmalt Ltd, Kirkliston, West Lothian, Edinburgh, Scotland

linked  $\beta$ -glucan assay procedure<sup>20</sup>. The absorbance of the reaction mixture, blank, and standard were read at 510 nm using a Philips PU 8730 UV/VIS Scanning spectro-photometer attached to colour plotter. The  $\beta$ -glucan content of wort was determined by the Megazyme assay procedure also<sup>21,26</sup>.

### Malting of barley

Standard pilot plant malting procedure for barley. Barley samples were screened, steeped and germinated in a Seeger micro-malting plant (Seeger Machinenfabrik, Fellbach, West Germany). Samples were steeped at 16°C by immersion for 8 h, followed by 16 h air-rest, followed by 24 h immersion. Grain was germinated at 16°C for 5 days. Samples were kilned at 50°C for 16 h and de-rooted by hand to give the finished malt.

Selective pilot plant malting procedure for barley. Because of the differences in germination of the normal huskless (naked) barley in the hydrogen peroxide  $(H_2O_2)$ test compared with the 4 ml test, which suggested that up to 7% of the embryos were damaged or dead, an unusual malting procedure was adopted. After steeping only the chitted samples of both normal huskless and acid-dehusked barley were separated out and allowed to germinate for 4 or 5 days. The aim was to ascertain how both barleys would behave when maximum germination was achieved during the malting process.



PLATE 1. Barley with husk plated out on PDA plates.

### $\alpha$ -Amylase and $\beta$ -amylase extraction and assay

 $\alpha$ -Amylase activity of the malt was determined using the Megazyme assay<sup>22</sup>, as described elsewhere<sup>2</sup>.  $\beta$ -Amylase was similarly extracted and assayed using the Megazyme procedure for this enzyme<sup>19</sup> as previously described<sup>2</sup>.

#### Mashing and extract determination

Malts were milled using the Buhler- Miag mill at setting 2 and mashed in the BRF mashing bath (Crisp Malting Ltd., Great Ryburgh, UK) at 65°C for 1 h as described elsewhere<sup>1</sup>.

#### **Extract determination**

Extract was calculated from the specific gravity of the filtered wort<sup>27</sup>.

#### Wort viscosity

Wort samples were equilibrated in a Julabo water bath attached to a Brookfield Digital Viscometer (Cone and Plate Viscometer LVDC 1 + CTE 40 spindle) at 20°C. After equilibration, 1 ml of wort sample was injected twice into the Viscometer using a syringe and the viscosity calculated using a correction factor.

#### $\alpha$ -Amino nitrogen

Wort  $\alpha$ -amino nitrogen was determined by a modification of the Ninhydrin<sup>27</sup> method as described previously<sup>1</sup>. Variations between experimental results did not exceed 5%.





PLATE 2. Dehusked barley plated out on PDA plates.

FIG. 1. Alpha amylase activity of germinated barley.



PLATE 3. Huskless barley plated out on PDA plates.

## **RESULTS AND DISCUSSION**

## General properties of the barley samples used and the role of the husk

The moisture levels of the normal huskless barley and the sulphuric acid-dehusked barley were similar (Table I). The total nitrogen and  $\beta$ -glucan content of the normal huskless barley were higher than those of the aciddehusked sample. Plates 1 and 3 show that the husk and pericarp of barleys carry microbial infection. This is confirmed in Plate 2, because the acid-dehusked barley samples (husk and pericarp absent) showed minimal level of infection. Similarly, when acid-dehusked normal huskless barley was plated out on PDA medium, microbial infection was minimal (results not shown). This further confirms that microbial infection of the barley resided mainly on the husk and pericarp. These results may be linked to the lower germinative energies reported in Table I for the normal huskless barley because microbial infection can inhibit germination<sup>3-5</sup> by limiting oxygen availability to the embryo<sup>3,5,7,8,9,10,12,13,17,18</sup>.



FIG. 2. Beta amylase activity of germinated barley.



FIG. 3. Alpha amylase development in 100% germinated normal huskless (NH) and 100% acid-dehusked (AD) barley samples.

In order to investigate this further, the normal huskless barley was also subjected to the dehusking procedure. This resulted in an increase in germinative energy (Table I) although full germination was not achieved. During the dehusking process the pericarp is removed together with the husk. When the normal huskless (naked) barley was dehusked, only the pericarp was removed. The increase in the germinative energy of the dehusked normal huskless (naked) barley would indicate, therefore, that the pericarp contributed to the dormancy because the pericarp can inhibit oxygen uptake by the embryo. However, the inability of the dehusked normal huskless barley to achieve 100% germination might be due to embryo damage, which may have occurred during harvesting.

## Development of enzymes and endosperm modification

Figs. 1 and 2 show the patterns of amylolytic activity development when the standard procedure was used to malt the normal huskless (naked) and acid-dehusked barleys. It is clear from Fig. 1 that both barley types followed similar patterns in the development of  $\alpha$ -amylase activity during malting and that both developed similar levels of the enzymes, differences in their germination potential notwithstanding (see Table I). However, using the standard malting procedure, the normal huskless barley developed marginally more  $\beta$ -amylase activity than the aciddehusked barley (Fig. 2). In order to assess their full potential for enzyme both barley types were malted using the



FIG. 4. Beta amylase development in 100% germinated normal huskless (NH) and 100% acid-dehusked (AD) barley samples.



FIG. 5. Pattern of beta glucan degradation in normal huskless and acid-dehusked barley samples during malting.

selective procedure (see Methods). It is clear that when 100% germinated samples of normal huskless and aciddehusked barley were assayed, they developed higher levels of  $\alpha$ -amylase (Fig. 3) than when they were malted in the standard way (Fig. 1). It is important to note that the  $\alpha$ -amylase levels of the 100% germinated samples of both types of barley were similar (Figs. 1 and 3). The 100% germinated normal huskless (naked) barley developed marginally more  $\beta$ -amylase than the corresponding aciddehusked barley (Fig. 4).

#### β-Glucan breakdown and extract recovery

In Table II and Fig. 5 it can be seen that in the normal, huskless (naked) and acid-dehusked barley,  $\beta$ -glucan degradation proceeded progressively over the germination period. Only 85% of the  $\beta$ -glucan was broken down in the normal, huskless grain by day 5 of germination compared with 93%  $\beta$ -glucan breakdown in the acid-dehusked over the same period.  $\beta$ -Glucan degradation was higher when the 100% germinated samples were assessed (Fig. 6). The



FIG. 6. Beta glucan breakdown in 100% germinated normal huskless (NH) and 100% germinated acid-dehusked (AD) barley samples.



FIG. 7. Pattern of development of alpha amino nitrogen during germination.

high  $\beta$ -glucan content of barley contributes to a high residual level of  $\beta$ -glucan in the malt. This is important because maximum breakdown of  $\beta$ -glucan is essential during the malting process as  $\beta$ -glucanase enzymes are virtually inactive during mashing<sup>26</sup>.

When the malts from the samples of normal, huskless (naked) and acid-dehusked barleys were mashed, their extract development increased progressively from day 2 to day 5 (Table III). It is important to note that both types of barley malts developed high extract yield on day 5 germination. The higher extract yield of the malt of aciddehusked Halcyon barley over the same period of germination could have resulted from a difference in the total nitrogen content as well as varietal difference of both samples<sup>6</sup> (Table I). The higher residual  $\beta$ -glucan in the malt of the normal huskless barley reported in Table II is further reflected in both the higher wort viscosity and wort  $\beta$ -glucan shown in Table IV. In contrast, the acid-dehusked Halcyon barley which had lower total nitrogen and β-glucan content (Table I), had lower residual  $\beta$ -glucan in the malt (Table 2), and produced wort with lower viscosity and  $\beta$ glucan (Table 4). The  $\beta$ -glucan content of the wort from the normal, huskless (naked) malt was over 7 times that of acid-dehusked malted barley.

Although the acid-dehusked Halcyon barley malt had an initial faster rate of FAN production up to day 2 germi-

TABLE II. Percentage content and degradation of  $\beta$ -glucan (w/w %) during germination for 5 days.

	Normal huskless %		Acid-dehusked %	
Sample β-glucan <sup>1</sup>	Barley	Break- down	Standard barley	Break- down
Barley	4.6	0	2.7	0
Day 1	3.7	20	1.06	61
Day 2	2.1	54	0.49	82
Day 3	1.4	70	0.31	89
Day 4	0.96	79	0.22	92
Day 5	0.71	85	0.18	93

<sup>1</sup> $\beta$ -Glucan (as is).

TABLE III. Extract (l°/Kg) development pattern during germination for 5 days.  $^{1}\,$ 

Germination time	Normal huskless barley	Acid-dehusked barley	
Day 1	ND	ND	
Day 2	236 (62)	294 (77)	
Day 3	269 (71)	300 (79)	
Day 4	277 (73)	309 (81)	
Day 5	289 (76)	307 (81)	

<sup>1</sup>Values in brackets are percentage extract yield (d.m. ASBC).

TABLE IV. Properties of wort derived from normal huskless and aciddehusked malted barley.

	Normal huskless barley		Acid-dehusked barley	
	Day 4	Day 5	Day 4	Day 5
HWE (l°/Kg)	277	289	309	307
TSN (%)	0.67	0.77	0.62	0.64
FAN (mg/L)	136	148	105	104
SNR (%)	0.27	0.31	0.37	0.39
Viscosity (cP)	1.41	1.31	1.15	1.15
β-glucan (mg/L)	685	650	94	90

nation, the malt of normal huskless barley produced more FAN products towards the end of the germination period (Fig. 7). This is probably because the normal huskless barley had more total nitrogen to start with. It is, however, interesting to note that the malt of the acid-dehusked barley produced a high level of soluble nitrogen (Table IV) and FAN products (Fig. 7). This may be because barley with a lower total nitrogen content may achieve a more uniform and greater degree of modification than barley which is higher in total nitrogen. This results in higher soluble nitrogen ratios<sup>6,25,28</sup>. With regard to the work reported here, another interesting observation was found in the relationship between total nitrogen content of barley and level of FAN products of the malt. The normal huskless barley contained 30% more nitrogen than the aciddehusked barley (Table I). When malted for 5 days, and mashed in a similar way, the normal huskless barley produced 30% more FAN products in the wort than the aciddehusked barley (Table IV). Such differences in soluble nitrogen production were not correlated with differences in the development of amylolytic activity<sup>6</sup>.

## CONCLUSION

The work reported in this study shows that the husk and pericarp of barley are major sources of microbial infection. The husk, in contrast to the pericarp, protects the barley embryo from damage. The embryo of the normal huskless barley was largely exposed and may cause great damage during harvesting. Damage of the embryo of normal huskless barley may cause poor germination during malting and produce malt of poor quality. Poor germination will, in turn, result in inadequate modification of the endosperm materials and poor development of hydrolytic enzymes. This is evident because when the barley samples with dormant or dead grains were malted, amylolytic activity levels were reduced compared with malt from 100% germinated barley. The lower level of amylolytic enzymes developed in the barley samples, which contained small percentages of ungerminated grains, resulted in lower levels of  $\beta$ -amylase when the grain was malted. As reported previously<sup>6,15,23</sup>, β-amylase development is more correlated with malt modification than with nitrogen levels alone. The higher FAN products in the wort of the higher nitrogen (normal huskless) barley suggest that there is a good correlation between barley nitrogen and  $\alpha$ -amino nitrogen production. The high nitrogen of the normal huskless barley also limited the extent of endosperm modification of normal huskless barley, and hence the extract recovery. The high  $\beta$ -glucan content of the wort of normal huskless barley produced wort that was more viscous.

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