

# Some Relationships Between Malted Barleys of Different Nitrogen Levels and the Wort Properties

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## ABSTRACT

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Barleys containing different levels of total nitrogen seem to have similar initial patterns for endosperm modification during malting. The higher nitrogen barley had a slower rate of endosperm modification, whilst the lower nitrogen barley had a faster rate of endosperm modification as germination progressed. Although the higher nitrogen barley had slower rate of endosperm modification, it transferred more nitrogen materials to the roots and shoots, whilst the lower nitrogen barley transferred less nitrogen materials to the roots and shoots. The higher nitrogen barley produced a lower yield of extract, but released higher levels of soluble nitrogen, free amino nitrogen (FAN) and peptides in the extract. The lower nitrogen barley produced a higher yield of extract and higher levels of carbohydrates (reducing sugars) in the extract. These results suggest that other important relationships exist between barleys of different nitrogen content. A drop in peptide nitrogen occurred on the same day of germination in both barley samples.

**Key words:** Barley, carbohydrates, malting, nitrogen, peptide, soluble nitrogen.

## INTRODUCTION

Over the past century, research studies aimed at gaining an in-depth knowledge of the physiological changes that occur during the malting of cereal grains, especially barley, have been on-going. One important aspect of barley and malt studies where opinions still differ amongst researchers/maltsters is the relationship between the level of nitrogen incorporated into barley during germination and growth in the field and the level of enzyme activities developed in barley during malting. While some researchers and maltsters believe that barleys containing higher nitrogen levels would produce higher enzyme levels, other workers seem to disagree with this concept<sup>2,4,7</sup>. Our earlier communication<sup>2,4</sup> showed that barleys having lower nitrogen content developed higher levels of amylolytic enzymes. The work reported in this study provides further

information with regard to other relationships between the level of nitrogen present in malting barley and the properties of their malt and wort.

## MATERIALS AND METHODS

### Sample collection and preparation

Puffin and Chariot barley samples used in this study were obtained from the Heriot-Watt University brewing store (UK harvest, 2000). Preliminary assessment of the barley samples, such as germination energy and germination potential were determined. Barley was also screened (>2.2 mm sieve) prior to malting.

### Total nitrogen determination

Total nitrogen of barley, malt, as well as nitrogen present in roots and shoots or endosperm, i.e. whole grain with the embryo and scutellum severed off<sup>1</sup>, was determined by the Kjeldahl method<sup>13</sup>. In brief, samples were milled using the Buhler Miag mill (setting 2). The milled sample (1.0–1.5 g) was digested on a heater block (Tecator Digestion System 1007 Digester). Distillation was effected in a Kjelttec distillation unit (Tecator Kjelttec System 1002 Distillation Unit), and titration of the resulting distillate was carried out using the Metrohm Herisau Multi-burette E485 system.

### Malting of barley

Barley (Puffin and Chariot), was steeped and germinated at 17°C in a Seeger Micro-Malting Steeping Unit (Seeger Maschinenfabrik Fellbach, West Germany). A standard steeping schedule (8 h wet steep at 17°C, 16 h air-rest followed by 24 h water steep) was adopted, so that an out-of-steam moisture of 45% was achieved. Germination was carried out for 5 days. Grains were kilned at 50°C for 16 h in a Seeger Kiln.

### Determination of malting loss through the roots and shoots

A known weight of germinated and kilned barley contained in plastic sample bags was rubbed between hands to remove the roots and shoots. The sample was then sieved and whole malted grains collected. Roots and shoots were collected and weighed. Percentage malting loss was determined from the relationship:

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$$\frac{W_3 \times 100}{W_1}$$

Where:

- $W_1$  = weight of malt + roots and shoots
- $W_2$  = weight of malt after rubbing between hands
- $W_3$  = weight of roots + shoots = ( $W_1 - W_2$ )

Note: the weight of the residual dust in the empty bags (although negligible) was also considered as part of the malting loss.

### Diastatic Power (DP) of malt

The Fehling's Solution Method as described in the Institute of Brewing Recommended Methods<sup>13</sup> was employed for the determination of malt diastatic activity.

### Mashing of barley malts

Barley malt flour (Buhler Miag mill setting at 2) was weighed into a stainless mashing beaker and equilibrated distilled water (65°C) was added and methodology followed the Institute of Brewing Recommended Methods<sup>13</sup>. Mashing was carried out in a BRF mashing bath (Crisp Malting Ltd., Great Ryburgh, UK) at 65°C for 1 h. Following volume adjustment and filtration, clear wort was obtained.

### Extract determination

Hot water extract was determined by feeding the wort sample obtained above into a density meter (Calculating Digital Density meter, Stanton Redcroft PAAR DMA 46). After conversion to specific gravity (SG), the hot water extract was calculated<sup>1</sup>.

### Soluble nitrogen determination

Soluble nitrogen present in the hot water extracts was determined using standard methods described by the IOB<sup>13</sup>.

### $\alpha$ -Amino nitrogen and peptide production

The  $\alpha$ -amino nitrogen was determined by two methods – using the reagents ninhydrin or 2,4,6-trinitrobenzene sulphonic acid (TNBS) as described elsewhere<sup>1</sup>. Peptide results were calculated as the difference between the two assay methods because the TNBS assay measures both free amino nitrogen (FAN) and peptide nitrogen, whilst the ninhydrin assay measures only the FAN product.

### Reducing sugar determination

The reducing sugars present in the hot water extracts were determined as described by the Robyt and Whelan method<sup>14</sup>, following a 200-fold dilution.

### Analyses of carbohydrates (HPLC)

The HPLC equipment used to analyse the sugar content of the hot water extracts was a Dionex Eluent Degas Module (Gilson Binary Conductivity HPLC system) with the following components: Gilson 305 HPLC pump, Gilson 306 HPLC pump, Gilson Manometric unit, Gilson Dynamic mixer, Rheodyne 7125 injection with 20  $\mu$ L loop and 7126 start/stop switch, Jones column heater, temperature probe, Dionex PAD electrochemical detector and Hitachi Merck D 2000 integrator. Diluted sample (200-fold)

was injected in the Rheodyne injector and run on File 1 at 1.0 to 1.2 Kpsi<sup>1</sup>. Variations in experimental results reported in this study did not exceed  $\pm 5\%$

## RESULTS AND DISCUSSION

In Table I, some properties of the two barley varieties studied are presented. Puffin was the higher nitrogen barley. Both barley samples studied had excellent germination potentials and therefore meet one important criterion required for malting barley. Detailed malting performances of these barley samples are discussed below. It can be seen from Fig. 1 that on the first day of germination, the malting loss through the roots and shoots was low and similar for both barley varieties. Differences in malting losses of these barley samples through the roots and shoots were however, observed as germination progressed. This is because from day 2 germination period, differences in malting loss occurred, especially on the day 5 germination period (Fig. 1), even though the barley samples were malted under similar conditions.

The roots and shoots of malted barleys are rich in hydrolysed proteins<sup>1,6</sup>. High malting loss would therefore result in higher loss of hydrolysed proteins through the roots and shoots. The proteins present in the roots and shoots of these malted barley samples were determined. The results (Fig. 2) indicate that Puffin barley transferred more nitrogen materials to the roots and shoots than the corresponding Chariot barley, and further confirm results reported elsewhere<sup>1,6</sup> and also the results of the malting loss as shown in Fig. 1. Similar levels of protein materials were again, found in the roots and shoots of malted Puffin and Chariot barleys on day 1 germination period (Fig. 2), as

Table I. Some properties of Puffin and Chariot barleys.

	Puffin	Chariot
Moisture (%)	12.6	12.8
Germination energy (%)	98.0	99.0
Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> ) test (%)	100	100
Total nitrogen (%)	1.9	1.7
Crude protein (N $\times$ 6.25) (%)	11.9	10.6

TN and crude determined as % d.m.

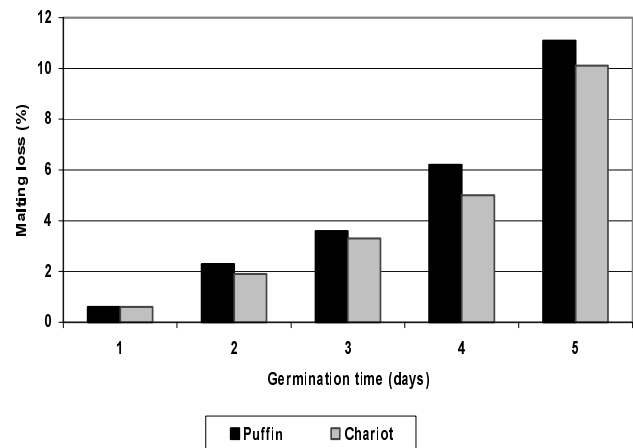


Fig. 1. Malting loss through roots and shoots during malting of barley. (Malting loss was calculated on dry weight basis.)

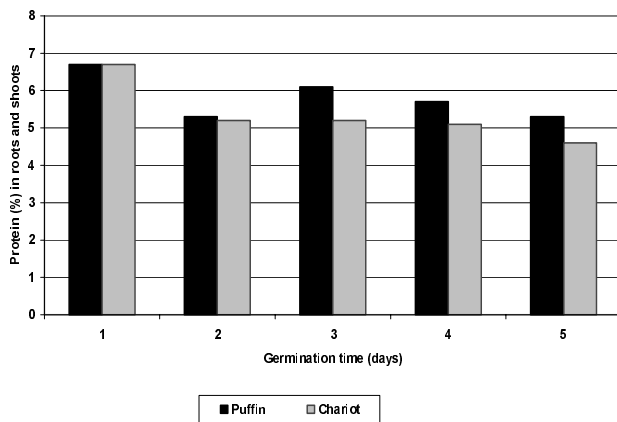


Fig. 2. Proteins transferred to the roots and shoots during malting of barley. (Proteins present in the roots and shoots were calculated on dry weight basis.)

was the case with the results of malting loss (Fig. 1). Results shown in Figs. 1 and 2 confirm that similar patterns for protein hydrolysis are operating in both barley varieties during the initial stage of germination. The nitrogen content of barley will affect the extent of protein hydrolysis and hence endosperm modification achieved during the malting of barley<sup>10,11</sup>. This is clearly seen in Table II where Puffin, with the higher nitrogen content achieved less degree of protein hydrolysis because it retained more proteins in the endosperm. In contrast Chariot, with the lower nitrogen content, achieved higher degree of protein hydrolysis because it retained a lower level of protein in the endosperm after 4–5 days of germination (Table II).

In regard to the relationship between the level of nitrogen present in barley and enzyme development during the malting of barley, Chariot had a lower nitrogen content but developed marginally higher levels of diastatic enzyme activity than the Puffin barley of higher nitrogen content (Fig. 3). It could be argued that the barley samples studied were different varieties, and genetic factors might be playing some role. Varietal differences could outweigh differences in total nitrogen with respect to free amino nitrogen and soluble nitrogen production. In this regard, it is worth mentioning that barleys such as Chariot can produce lower levels of soluble nitrogen and free amino nitrogen than other barleys, but can support similar yeast performance during fermentation<sup>3</sup>. Earlier communications<sup>2,4</sup> showed that when the same barley varieties, containing different nitrogen levels, were malted under similar conditions, they produced hydrolytic enzymes at different levels, with the

Table II. Nitrogen (%) present in the endosperm during the malting of barley.

Germination time (days)	Puffin		Chariot	
	TN (%)	(%) Hydrolysed	TN (%)	(%) Hydrolysed
1	1.8	5.3	1.6	5.9
2	1.7	10.5	1.4	17.6
3	1.6	15.8	1.2	29.4
4	1.6	15.8	1.1	35.3
5	1.3	31.6	1.0	41.2

TN determined as % d.m.

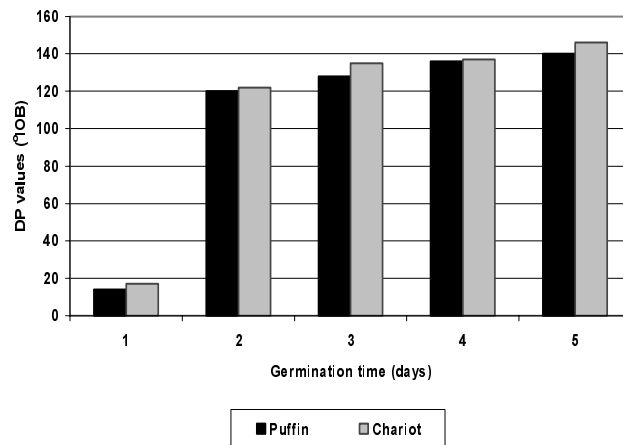


Fig. 3. Enzyme development of Puffin and Chariot barleys during malting.

lower nitrogen barleys developing higher enzyme levels<sup>2,4,10</sup>.

Proteolysis is important during malting because the soluble nitrogen pool required for enzyme synthesis is produced when proteolysis is optimal<sup>5,9,12</sup>. This is more so when most of the soluble nitrogen pool is retained within the grain rather than transferred, and/or lost through the roots and shoots. Optimal proteolysis will also result in the release of bound  $\beta$ -amylase enzymes<sup>9</sup>. Adequate release of bound  $\beta$ -amylase will in turn lead to higher diastatic activity of malt, especially in the Fehling's solution assay method used in this study. It is not clear at present, whether the higher level of proteolysis achieved with Chariot barley during malting (Table II), in addition to the lower malting loss (Fig. 1), and/or lower protein found in the roots and shoots (Fig. 2) may be linked to Chariot having slightly higher diastatic activity than Puffin (Fig. 3). This requires further investigation.

When the malts made from these barley samples were mashed in a similar manner<sup>13</sup>, Chariot (the lower nitrogen barley) produced higher extract yield than Puffin (the higher nitrogen barley) from day 1 germination period (Table III). The extract results therefore confirmed that a greater degree of endosperm modification had occurred in Chariot than in Puffin. The assertion, that a greater degree of endosperm modification occurred in Chariot than in Puffin, is clearly illustrated in the results in Table III. Hydrolysis of protein to produce soluble nitrogen peaked on the day 4 germination period for Chariot, but continued up to day 5 or more for Puffin. However, the extract results

Table III. Properties of hot water extracts of Puffin and Chariot barley malts.

Germination period (days)	Extract (L°/kg)		TSN (%)	
	Puffin	Chariot	Puffin	Chariot
1	294	301	0.40	0.41
2	307	313	0.60	0.51
3	310	320	0.76	0.60
4	313	322	0.87	0.64
5	310	323	0.92	0.65

Extract determined "as is"; TSN determined as % d.m.

presented in Tables III and IV show that Puffin produced more soluble nitrogen (Table III) whilst Chariot produced more reducing sugars (Table IV). It is interesting to note that on day 1, germination both Puffin and Chariot barley malts produced much higher maltose than glucose sugars in their worts (Table IV). Although both barley samples produced satisfactory levels of maltose sugars in their worts, Chariot barley produced more maltose sugars than Puffin barley (Tables IV). The maltose sugars in both samples of malt decreased as germination progressed, reaching an approximately similar maltose to glucose ratio by day 4 or 5 germination period. Furthermore, the sugar profiles (HPLC) showed that Chariot produced a wider spectrum of sugars than Puffin (Table V).

On the other hand, the higher nitrogen barley (Puffin) not only developed more soluble nitrogen during malting and mashing it also produced higher levels of  $\alpha$ -amino nitrogen (FAN) by both the ninhydrin and TNBS assay methods (Table VI). Puffin barley also released more peptides than Chariot barley. One interesting observation was the peptide release pattern of both barley samples. Peptide release increased up to the day 3 germination period but dropped on day 4, rising again on day 5. Although the reason for the drop in peptide on day 4 is not clear, this drop in peptide on day 4 could be a result of peptide nitrogen migrating from the storage endosperm to the embryo and partial loss through the roots and shoots.

Table IV. Reducing sugar content and maltose to glucose of hot water extracts made from Puffin and Chariot barley malts.

Germination period (days)	Reducing sugars (mg/mL)		Maltose : glucose ratio	
	Puffin	Chariot	Puffin	Chariot
1	27.0	27.6	13:1	14:1
2	28.4	31.6	9:1	11:1
3	31.6	33.6	8:1	8:1
4	36.0	36.8	6:1	7:1
5	35.2	36.4	5:1	6:1

Table V. Fermentable sugar (g/L) content of the hot water extracts of 5 day malts of Puffin and Chariot.

	Puffin	Chariot
Glucose	3.7 (6.8)	3.48 (6.0)
Fructose	—	0.36 (0.6)
Sucrose	—	0.38 (0.7)
Maltose	47.6 (87.5)	50.40 (87.0)
Maltotriose	3.1 (5.7)	3.34 (5.8)

Values in brackets represent percentages of the various sugars present in the extract.

Table VI.  $\alpha$ -Amino nitrogen and peptide content of worts from Puffin and Chariot malts.

Germination period (days)	FAN (mg/L) (TNBS)		FAN (mg/L) (ninhydrin)		Peptides (mg/L) (difference)	
	Puffin	Chariot	Puffin	Chariot	Puffin	Chariot
1	68	62	47	48	21	14
2	153	129	88	95	65	34
3	196	164	122	117	74	47
4	233	182	193	142	40	40
5	279	192	196	140	83	52

## CONCLUSIONS

The results obtained from this study have shown that when malting barley contained a high level of nitrogen, more proteins were lost through the roots and shoots. Limited modification of endosperm materials also occurred resulting in lower extract yield. Whilst proteolytic activity appeared to peak on day 4 germination for Chariot, proteolytic activity continued up to the day 5 germination period for Puffin. This suggested that Puffin may require more than 5 days germination period for proteolytic activity to peak. Higher nitrogen barleys however, are likely to produce extracts that are rich in soluble nitrogen, FAN and peptide nitrogen. In contrast, lower nitrogen barleys are likely to produce extracts that are rich in carbohydrates. It is not clear how the different levels of soluble nitrogen or carbohydrates present in the different worts produced in this study affect fermentation, as this was not investigated, especially in regard to fermentation rate or alcohol production<sup>8</sup>. This requires further investigation. The results presented in this paper revealed that the nitrogen content of malting barley was not only linked to enzyme production during malting, but also related to the carbohydrates/soluble nitrogen present in the wort when the malted barley was mashed.

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