



**PROJECT REPORT No. 238**

**DEVELOPMENT OF A QUALITY  
CONTROL METHOD BASED ON  
LIGHT TRANSMISSION FOR  
PREDICTING THE QUALITY OF  
BARLEY FOR MALTING**

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ON LIGHT TRANSMISSION FOR PREDICTING THE QUALITY  
OF BARLEY FOR MALTING**

By

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## **ABSTRACT**

A critical part of the malting process is the conversion of the grain endosperm from a hard, tightly packed reserve of protein and starch to a friable, easily digested material. This depends on the activity of the embryo and aleurone to generate the appropriate enzymes and an endosperm structure that will permit the passage of water and hence these enzymes to the cell walls and small starch granules. If either of these fails then the grain will not modify properly and the malt quality will not be such as to allow subsequent trouble free processing.

Although there are many ways to assess embryo quality there is no simple, robust and quantitative method which can identify the appropriate quality of endosperm structure. A meter has been designed and produced at BRI to quickly measure these features of endosperm structure. The principle is based on the transmission of light through whole husked grains. As a test of grain quality, the light transmission (LTm) meter is quick, simple, quantitative and robust with good repeatability and is non-destructive.

### **Conclusions**

- The LTm meter can measure the proportions of meal/steeliness in a barley grain with excellent correlation with visual assessment.
- There was no evidence that the LTm of barley could predict extract or friability but measurements during steeping did give an indication of water uptake.
- There was a poor correlation between barley LTm and malt calcofluor values.
- There were more significant simple correlations between malt LTm (especially the median value) and malt extract, friability and calcofluor modification.
- For a single variety, the LTm can show the proportion of chit malt and may be able to indicate extract potential.

### **Implications**

The LTm meter gives an indication of the structural qualities of grain, barley and malt, endosperm. Data logging techniques allow the analysis and recording of each individual grain separately so the homogeneity of the sample is also determined.

The LTm meter could be used in a malting laboratory as an analysis of endosperm structure in either barley or malt. It could possibly replace other methods which are time-consuming and rely



on visual judgement (*e.g.* farinator). An accurate measurement of the major fraction of the barley *i.e.* mealy or steely, will enable maltsters to provide optimal malting conditions for a grain type. The LTM meter is also able to assess malt endosperm structure in a similarly effective manner. Although all of the work here described is related to barley or malt quality, the method is also able to reveal internal structure in wheat.

## SUMMARY

### Endosperm structure

If a sample of barley grain is dehusked and examined on a light box, two types of grain are clearly visible. Scanning electron micrographs highlight these structures, Figures 1 and 2. One type of grain is opaque and appears dark, the other is translucent and appears to be much lighter. The dark grains are those with mealy character and the lighter grains are those with steely character. Figure 4 illustrates the principle of this effect. The mealy endosperm, containing much free space between starch granules, scatters light effectively. The steely grain, containing a considerable protein matrix, acts as a lens and the light is scattered much less but is also partially focused to the viewer.

### Instruments to monitor the mealy/steeliness of a grain

There are few instruments that will determine the mealy/steeliness of a grain. Traditionally the maltster has relied on the farinator. This is a device that cuts a sample of grain, in a transverse manner, to reveal a plane of the endosperm. This is examined visually and a judgement is made. The device has several drawbacks. It is only qualitative and does not provide any quantitative information. The final judgement generally lies with the experience of the operator. The farinator cuts across only one plane and, as shown in Figure 3, this may well give rise to misleading information. If the plane of the cut is in fact through a non-representative part of the grain then the wrong conclusion is drawn especially when grains are rarely of one type. This test is destructive. Other tests used to monitor this parameter are also destructive.

### The LTm Meter

The LTm meter monitors the difference in endosperm structure by the passage of light intensity through the grains. The method relies on quantitative measurement of the transmission of a laser light through the whole grain. Therefore the grain does not have to be dehusked. The system is non-destructive and the grain can be used for other purposes. Individual analysis of many (*e.g.* 100) single grains can be used to obtain information regarding the homogeneity of the bulk sample (Figures 5, 7, 8 and 9).

Husked grains are arranged in slots around the periphery of a carousel which is then inserted into the LTm meter. The carousel rotates and each grain is presented to the laser and light detector. Detection of light is converted to an electrical signal which is recorded. Data are then transferred

and displayed as required. Ranges for mealiness have been set so groups varying in amount of meali/steeliness can be seen. Also an overall value for the sample is given. The time taken to obtain information about the endosperm structure is fifteen minutes.

## **Key Results**

### **Barley analysis**

Barley samples were measured using the LTm meter and the same grains assessed by eye for percentage steeliness (97 grains each). Visual assessment of meali/steeliness is similar to using the farinator and therefore has the same limitations *i.e.* personal judgement of a single plane. A correlation coefficient of 0.99 was obtained when comparing the % steeliness and the barley LTm log median value (Figure 10). Thus the LTm meter was able to measure the proportion of mealiness/steeliness in a grain.

The LTm meter can give an indication of endosperm structure for barleys of different malting grade. Results have shown there can be variation in the proportions of mealiness within a sample of the same variety. Also samples of lower malting quality (as assessed by NIAB grading system), can have higher LTm values than those of higher malting quality (Figures 11, 12, 26 and 28-32). Barley LTm values were compared with TN and total  $\beta$ -glucan levels of the barley but no correlation was found (Figures 14, 27, 33 and 24).

### **Barley LTm as a predictor of malting performance**

The BRI micromaltings (Kelly 1987) was used to prepare malt samples of barley varieties of different malting grades. Samples (350g) were screened over a 2.8mm sieve. A three steep schedule was used, 7, 17, 7, 17, 1 hrs alternating wet/dry. Kilning was for 8 hrs at 45°C and 16 hrs at 65°C. Malt extract, friability, calcofluor modification and calcofluor homogeneity were compared with the LTm values taken from the barley. Also diastatic power, taken as a measure of enzyme potential, was included in some calculations. There was no evidence that barley LTm could predict extract or friability as simple or multiple regression (with diastatic power). There did appear to be a poor correlation between calcofluor values in the malt and the LTm values, particularly the log median, of the barley.

LTm measurements were taken on samples of varying malting grades at hourly intervals during steeping. Barley LTm values were compared with measurements of the same grains after being steeped (Figures 46-152, Table 10). The LTm meter was able to give an indication of the ability of a sample to distribute water across the endosperm.

### Malt analysis

LTm measurements were carried out on the malt samples prepared as above. These results were then compared with the malt extract, friability, calcofluor modification and calcofluor homogeneity. No correlation was found between the malt LTm value and the calcofluor homogeneity. However, there was significant correlation between the malt LTm (especially the log median value) and malt extract, friability and particularly calcofluor modification as shown in Figure 155. As is visible in the graph there is an outlier. This variety was found to have an unusual mealy/steely pattern which was measured more mealy by the LTm meter than by visual assessment. If this point was removed from the calculation the correlation between calcofluor modification and malt LTm was improved from 0.93 to 0.99

The LTm meter can give a quick quantitative measurement of modification. It may have value as a rapid alternative to the Carlsberg calcofluor slab method.

### Malt for brewing

Samples were prepared where there was a known difference in LTm measurement. This was achieved by adding a malt which had been germinated for a single day, known as a chit malt, in known proportions to the standard malt. The proportions were 0%, 5%, 10% (in duplicate) and 20%. LTm measurements were then carried out on these samples. The grist was then brewed using standard conditions and brewing performance was measured as extract and differential pressure across the lauter plate.

As expected, there is clear a relationship between extract and the percentage of chit malt in the grist as seen in Figure 156. The lower extracts had the higher percentage of chit malt. Malt LTm measurement, particularly log median value, correlated well with the proportion of chit malt in the grist, giving a correlation coefficient of 0.91.

Results show that the LTm meter can show the proportion of chit malt when used with a single variety and may be able to indicate extract potential.

### **Implications**

Significant correlations were found between the malt LTm values and malt extract, friability and calcofluor modification. The LTm meter could be used in a malting laboratory as a prediction of malt performance. It could possibly replace other methods which are time-consuming and rely on visual judgement.

Also for a single variety, the LTm can show the proportion of under-modified malt and may be able to indicate extract potential. An accurate measurement of the major fraction of the barley *i.e.* mealy or steely, will enable maltsters to provide optimal malting conditions for a grain type.

The LTm meter is a rapid, simple and robust device which can give a quantitative indication of grain, barley and malt endosperm quality in terms of mealiness and also homogeneity.

## INTRODUCTION

The conversion of barley to malt is a complex process but the two most important criteria are rapid and even hydration of the grain and uniform germination leading to homogeneously modified malt.

Uneven modification can result in problems that will be reflected in the performance of the malt in the brewhouse. This is exemplified by poor extracts, high wort viscosities (giving wort separation difficulties) and  $\beta$ -glucan hazes in the final product. (Bamforth and Barclay 1992).

Different structural areas within the barley endosperm can be seen by visual examination. These are described as mealy or steely (Chandra *et al* 1997, Chandra *et al* 1999). These differences occur due to the packing of endosperm material into the grain. The mealy grains are loosely packed with air-spaces between the starch granules whereas the steely grains are densely packed with large and small starch granules in a dense protein matrix. These differences are readily apparent when visualised by scanning electron microscopy. Figures 1 and 2 show a mealy and a steely endosperm structure respectively. The causes of these structural differences are not fully understood but are thought to be related to climatic conditions such as water and nutrient availability during grain filling.

Barleys with a high proportion of steely grains are often, but not invariably, associated with higher protein and  $\beta$ -glucan contents and uneven modification of the endosperm during malting. The mechanism by which endosperm structure can affect modification during malting is poorly understood. Structural differences might be expected to have significant effect on water, and consequently enzyme movements within the endosperm, thus limiting hydrolytic activity during malting.

The extent of mealiness and steeliness has been shown to be associated with differences in the proportion of endosperm components such as hordein proteins and  $\beta$ -glucan (Chandra *et al* 1999). These differences influence the distribution of water and of the cell wall degrading enzymes within the endosperm. Steely areas can also occur randomly within a mealy endosperm. Both the extent of steeliness and the distribution of steely areas within the grain can influence modification. It is therefore necessary to study the whole structure of the grain rather than individual sections.

When purchasing barley for malting, the Maltster must choose between many different batches of approved varieties. There are currently few tests which can be used to rapidly identify differences in malting quality between different batches of the same variety. These include staining tests (for viability), visual assessment (for mould infections) and measurement of nitrogen content. The importance of even distribution of water in the endosperm has been identified and a test devised called the BRFI Malting Index (Davies 1991).

Endosperm structure has been monitored using a farinator. This device cuts transversely through a sample of fifty grains. The endosperm of each grain can then be examined and classified as mealy, steely or as a mixture. The farinator has major limitations, however, being a visual inspection only and not providing quantitative measurements. In addition it only reveals a planar section of the grain and does not allow the whole endosperm to be assessed. It has been shown that the endosperm can vary very considerably in its structure both from end to end and across the grain (Chandra *et al* 1999). The farinator, cutting through a single plane, does not reveal these differences (Figure 3).

**The objective of this project was to develop a QC test which can be used to determine the quality of barley endosperm and relate this quality to malting performance.**

## MATERIALS AND METHODS

### MATERIALS

Winter barley varieties varying in malting grade and growing area were obtained from Nickerson Seeds Ltd, Rothwell, Lincoln. (Years 1 and 3). Twelve samples which were grown in Europe and two in the UK were used in Year 2. All barleys were sieved between 2.2 and 2.8mm.

Table 1 : Barley samples

Year	Site	Variety
1 and 3	Haughley and Woolpit-Suffolk, Navenby-South Lincoln, Rothwell- Lincoln, Wooton-N.E.Lincolnshire from Nickerson Seeds UK	Fanfare, Puffin, Regina, Rifle, Spice, Halcyon, Sunrise, Fighter, Intro, Pastoral.  Chariot, Fanfare, Epic, Optic
2	Continental  UK	Barke, Cadeau, Cecilia, Merian, Optic, Texane, Trebon Regina, Tiffany, Brite, Bartok, Lysiba Regina, Optic, Fanfare, Chariot, Epic

### METHODS

#### Barley analysis

Barleys were tested for germination energy (4ml), germination capacity (H<sub>2</sub>O<sub>2</sub>), moisture and Total Nitrogen according to the recommended methods of the Institute of Brewing.

#### β-Glucan analysis

β-Glucan activity was determined by the method of McCleary using an assay kit supplied by MEGAZYME, Sydney, Australia. After extraction with Lichenase the assay was performed at 40°C and Trinder reagent was used instead of GOPOD reagent.



## Dehusking of barley

### *Sulphuric acid*

Sulphuric acid (50% w/w) was used to dehusk the grains. The grains were exposed to the acid for 1 hour. After removing the husk the grains were washed thoroughly with water. The advantage of this method is the uniform removal of the husk, which makes the assessment of light transfectance easier.

### *Pearling machine*

Barley was dehusked using a small scale pearling machine driven by an electric motor. The main chamber of the machine was lined with an abrasive paper and grains were pearled against the abrasive surface with the help of a wire brush for several minutes depending on the husk.

## Analysis of endosperm structure

### *Light box*

The light box consisted of a glass plate, which was illuminated evenly from below. As the absorbance of light by the husk is too strong to differentiate the grains visually, whole corns of barley were dehusked. In a mealy endosperm the loosely packed starch absorbs light, causing the grain to appear dark and opaque, whereas the densely packed starch and protein matrix in a steely grain transmits light and appears translucent (Figure 4).

### *Single grain LTm meter*

The instrument developed at BRI contained a sample chamber, where a single husked grain is placed (Figure 5). A laser light at 670nm is passed through the grain. A photoelectric detector which was placed above the sample measured the transmitted light. The intensity was indicated by a LCD display. A direct measurement of the laser light showed a reading of 1000mV. An electronic safety switch, attached to the lid ensured that the laser source was only emitted when the chamber was closed. In the first year readings from the meter were then adjusted using the scoring system shown on Figure 6, to obtain an LTm value. (The higher the value the more mealy the sample).

### *Rapid LTm meter*

The further development of the single grain meter enabled an automatic measurement of either 50 or 97 husked grains (Figures 7 and 8 respectively). These were positioned in a carousel type sample holder, which is moved by a stepper motor. A schematic design is shown in Figure 9. The meter automatically located the first position. There were 3 controls (a dark, a light and a coloured filter) in the carousel used in the rapid transfectance meter for 97 grains. These provided a control for each analyses. A memory chip and a parallel port in the meter made it possible to transfer the data offline as an Excel spreadsheet using EasyLog for Windows after the measurements were completed. The values were represented in increasing order by the spreadsheet. The grains themselves were not ordered before analysis. The saved file was used for subsequent calculations. Low values obtained from these machines indicated a mealy structure.

### Moisture adjustments

Grain with varying moisture contents were prepared by two methods:

- a) Barley samples were maintained over water in a desiccator jar for a period of a week. After LTm analysis these were then dried again in a high air flow oven at 40°C for 20 hrs and analysed again. They were then dried for a further 15 hrs before final analysis. Sub-samples were taken for moisture determinations by the IOB Recommended Method.
- b) Barley samples were steamed using a steam generator, for 3 hrs, subsequent analysis and drying were as above.

### Determination of water distribution

The distribution of water in barley endosperm was studied using iodine vapour staining method as described by Davies (1991). Steeped grains were blotted dry and cut along the ventral furrow from the distal end to the embryo end. The cut surface was exposed to saturated iodine vapour in a closed petri dish for 60s. Only the hydrated areas of the endosperm react with iodine and stain purple.

To express the water distribution in the whole sample, the scoring system as shown in Figure 6 was used (Chandra, 1999). The iodine vapour score (IVS) was calculated from:

$$\text{IVS} = \Sigma (\text{Number of grains in each group} \times \text{water redistribution score}) \quad \text{for 100 grains}$$

### Micromalting

Barley was malted in the ratio of 350g:700ml tap water at 16°C, using an interrupted steeping schedule of wet (w) or air rests (a) periods: 7w/17a/7w/17a/1w. Germination was for four days at 16°C and green malts were dried in a forced draught oven for 8 hrs at 45°C followed by 16 hrs at 65°C, unless otherwise stated.

### Malt analysis

Malt analysis *i.e.*, extract, friability, DP were carried out as instructed in the IOB Recommended Methods.

### Malt modification and homogeneity

100 grains were glued onto a plastic slab with the embryo all pointing the same way and allowed to dry. They were then sanded half way down. The slab was immersed in Calcofluor stain (0.1%w/v) for 4 minutes and then the excess washed off with 70% ethanol. This was then counter-stained with fast green (0.1%w/v) and the excess blotted off. The slab was examined under UV light and scored according to the amount of fluorescence along the grain. A measurement of modification and homogeneity was obtained. Fluorescence indicated the presence of  $\beta$ -glucan cell wall and therefore poor modification.

### Brewhouse trials

Optic barley was germinated for one day at 16°C to produce a 'chit malt'. This was then carefully mixed with the standard Optic malt used in our brewhouse. The two samples were mixed in proportions 0%, 5%, 10% (all in duplicate) and 20% chit malt. All samples were analysed by LTm three times and averaged. The grist was brewed using standard conditions and brewing performance was measured as extract and differential pressure across the lauter plate.

## RESULTS AND DISCUSSION - YEAR 1 (1997/1998)

### Production of a single grain LTm meter

The principle of the light transmission method is shown in Figure 4. In a mealy structure light is absorbed by the endosperm possibly due to free spaces between the starch granules. However when the spaces were filled with solid matrix, as in a steely structure, the rays pass through the endosperm to produce a translucent glow. A red laser light source at 670nm was found to be the most suitable light source for its ability to penetrate the husk of barley grains. Figure 5 shows the single grain LTm meter. A photoelectric detector which was placed above the sample measured the transmitted light. The intensity was indicated by a LCD display. A direct measurement of the laser light showed a reading of 1000mV. An electronic safety switch, attached to the lid ensured that the laser source was only emitted when the chamber was closed.

### Barley analysis

All barley samples show good germination capacity and activity. The results show a wide range in total nitrogen (Tables 2 – 6). Total nitrogens for the samples from the Wooton site were higher than normal.

Table 2 : Barley analysis for samples from Haughley site, Suffolk

BRi No	Variety	Moisture %	Germinative Energy %	Germinative Capacity %	Total Nitrogen %	Total $\beta$ -glucan %
97/11	Fanfare	13.5	92	99	1.80	2.05
97/12	Fighter	13.4	95	99	1.95	3.34
97/13	Halcyon	13.7	99	99	1.85	2.88
97/14	Intro	13.4	97	96	1.80	3.21
97/15	Pastoral	13.4	92	100	1.80	2.89
97/16	Puffin	13.5	99	98	1.88	2.08
97/17	Regina	13.1	98	98	1.74	2.37
97/18	Rifle	13.3	96	98	1.78	2.28
97/19	Spice	13.3	98	99	1.59	2.46
97/20	Sunrise	13.2	94	98	1.79	1.94

**Table 3 : Barley Analysis for samples from Navenby site, S. Lincoln**

<b>BRi No</b>	<b>Variety</b>	<b>Moisture %</b>	<b>Germinative Energy %</b>	<b>Germinative Capacity %</b>	<b>Total Nitrogen %</b>	<b>Total β-glucan %</b>
97/21	Fanfare	13.6	92	97	1.84	1.74
97/22	Fighter	13.6	92	94	1.94	2.59
97/23	Halcyon	13.3	96	97	2.04	2.36
97/24	Intro	13.4	97	94	1.97	2.51
97/25	Pastoral	13.4	91	97	1.84	2.25
97/26	Puffin	13.3	98	98	1.98	2.03
97/27	Regina	13.4	93	97	1.81	2.64
97/28	Rifle	13.5	96	98	1.89	2.04
97/29	Spice	13.6	96	97	1.81	2.20
97/30	Sunrise	13.6	93	98	1.91	2.88

**Table 4 : Barley analysis for samples from Rothwell site, Lincoln**

<b>BRi No</b>	<b>Variety</b>	<b>Moisture %</b>	<b>Germinative Energy %</b>	<b>Germinative Capacity %</b>	<b>Total Nitrogen %</b>	<b>Total β-glucan %</b>
97/31	Fanfare	13.4	98	99	1.72	2.16
97/32	Fighter	13.1	96	98	1.76	2.51
97/33	Halcyon	13.3	97	99	1.88	2.24
97/34	Intro	13.0	93	98	1.96	2.69
97/35	Pastoral	13.4	91	98	1.90	2.54
97/36	Puffin	13.3	98	99	1.74	2.58
97/37	Regina	13.2	98	99	1.70	2.33
97/38	Rifle	13.1	98	99	1.75	2.21
97/39	Spice	13.4	99	100	1.60	2.01
97/40	Sunrise	13.4	97	99	1.79	2.19

Table 5 : Barley Analysis for samples from Wooton, N.E. Lincolnshire

BRi No	Variety	Moisture %	Germinative Energy %	Germinative Capacity %	Total Nitrogen %	Total β-glucan %
97/41	Fanfare	13.5	97	100	2.11	1.79
97/42	Fighter	13.4	92	99	1.98	1.83
97/43	Halcyon	13.2	99	100	2.25	1.86
97/44	Intro	13.2	98	99	2.12	2.45
97/45	Pastoral	13.0	96	99	2.08	2.26
97/46	Puffin	13.2	97	98	2.16	1.89
97/47	Regina	13.0	98	99	2.06	2.20
97/48	Rifle	13.5	96	99	2.11	2.01
97/49	Spice	13.6	97	99	2.05	1.97
97/50	Sunrise	13.4	92	99	2.10	1.83

Table 6 : Barley analysis for samples from Woolpit, Suffolk

BRi No	Variety	Moisture %	Germinative Energy %	Germinative Capacity %	Total Nitrogen %	Total β-glucan %
97/51	Fanfare	12.8	95	94	1.62	2.71
97/52	Fighter	12.8	97	95	2.06	3.77
97/53	Halcyon	13.0	96	95	1.93	3.32
97/54	Intro	13.1	96	95	1.61	4.11
97/55	Pastoral	12.8	93	89	1.76	3.00
97/56	Puffin	12.8	96	93	1.92	2.78
97/57	Regina	12.8	95	96	1.75	2.34
97/58	Rifle	12.9	95	93	1.65	1.98
97/59	Spice	13.0	98	92	1.63	2.25
97/60	Sunrise	12.8	96	93	1.93	1.61

### Barley assessment (visual vs LTm)

Figure 10 shows the correlation between percentage steeliness assessed by eye and the log of the LTm reading for the same grain. Limitations of this experiment were that the degree of mealiness was assessed visually which can be variable and that this was measured in a single plane, similar to the farinator. In the graph the regression line is drawn between five points and gives a correlation coefficient of 0.99. The outlier was a variety which when assessed by eye had an unusual mealy/steely pattern where a layer of mealiness surrounded the middle section of steely character. The LTm meter measured this as mealy while by visual assessment a more steely character was concluded. Nevertheless the LTm meter was able to give a good indication of the proportion of mealy/steeliness in a grain, however in some special cases the meter could give erroneous readings.

### LTm measurements to assess barley endosperm structure

LTm readings measured using the single grain meter are adjusted using the scoring system shown in Figure 6. Sample size was 50 or 100 grains. Each reading was converted into a score and then multiplied by the total number of grains with this score. This parallels the same analysis of water distribution calculation (Chandra 1999). The total of all the groups is the LTm value. The higher the value the more mealy the endosperm structure.

Figures 11 and 12 illustrate the LTm values for ten barley varieties of varying NIAB grades from four sites. The LTm meter is able to measure differences in the barley varieties. LTm values seemed to be dependant on both environmental and variety as shown by the variation in results. Some varieties classed as feed grade had LTm values the same or even better than some of the malting grade varieties. This suggests that it was not the endosperm structure of that variety which caused poor malting performance but perhaps an enzymatic problem.

### Water distribution

It is well known that during the malting process the distribution of water in the endosperm is of prime importance for uniform modification. A measure of this can be obtained by the iodine vapour scoring method. This measures the extent of hydration during the early part of steeping, normally at the end of first air-rest (7wet/17air-rest). By this time the whole of the endosperm should be hydrated, resulting in higher values for samples having greater water distribution in the endosperm. No correlation between the measurement of endosperm water distribution and the LTm values was found as shown in Figure 13. This suggests that the data from the LTm not only

estimates the penetration of water into the endosperm but also measures the contribution made by its structure.

#### Influence of $\beta$ -glucan content

The Light Transmission effect seen in the endosperm structure incorporates the protein matrix, the starch granules and the air pockets. As part of the cell wall material is  $\beta$ -glucan this could have an influence on the LTm measurement. Forty barley sample were measured for total  $\beta$ -glucan levels and compared with the LTm values but no correlation was found as shown in Figure 14.

#### Comparison of NIR examination and LTm measurement

A stand-alone device dedicated to the analysis of mealy/steeliness in grain endosperm has several attractions. Nevertheless there was a possibility that the same analysis could be conducted using existing equipment. Hence Near Infrared Spectroscopy was investigated as an indicator of endosperm structure.

Barley samples of defined mealy/steely endosperm structure were supplied to Foss UK. They kindly conducted NIR examination of these grains and a correlation was sought. We are grateful to Dr Fiona Bury and Dr Ian Cowe of Foss UK for conducting this analysis. See Appendix A.

Preliminary results indicated that there was indeed a relationship between mealy/steely endosperm structure and NIR spectra. however the procedure would require development and, after discussion, it was decided that a standalone device would be cheaper and this was the alternative that was pursued in the second year.



## RESULTS AND DISCUSSION - YEAR 2 (1998/1999)

### Barley analysis

All samples showed good germination capacity and activity. There was also a wide range in total nitrogen and moisture content.

Table 7 : Barley Analysis (Year 2 samples)

Variety	Germinative Energy (%)	Germinative Capacity (%)	Total Nitrogen (%)	Moisture (%)
Barke	100	100	1.41	13.9
Cadeau	100	100	1.51	16.0
Cecilia	100	99	1.72	17.0
Merian	98	96	1.63	16.0
Optic 1	97	96	2.00	11.3
Texane	100	99	1.45	12.5
Trebon	99	100	1.52	12.4
Regina 1	99	99	2.08	15.4
Tiffany	98	99	2.08	14.6
Brite	99	97	1.48	11.4
Bartok	100	100	1.46	15.2
Lysiba	100	100	1.37	16.3
Regina 2	100	98	1.61	15.7
Optic 2	97	98	1.56	16.0
Fanfare	99	99	1.77	11.9
Chariot	98	not done	1.73	14.0
Epic	97	not done	2.56	13.0

### Calibration of the single LTM for husked and dehusked barley

To see the light transmission effect on a light box and divide a sample into groups of different mealiness, dehusked grains were used. To be able to detect the same effect using the single grain

meter, a suitable method for dehusking grains had to be chosen. To exclude any potential influence of the husk both sulphuric acid and pearling methods were used to establish the effect of dehusking on light transfectance values. Actual readings from the machine were used in the following experiments i.e. they have not been adjusted using the scoring system shown in Figure 6.

A sample of Fanfare was screened and 100 grains were measured on the light box and subsequently divided into mealy and steely fractions. These fractions were then used to calibrate the LTm meter. Figures 15 and 16 show the different groups within each sample detected on the light box and using the LTm machine.

Due to harsh conditions used in pearling, grains were damaged resulting in uneven grain surface. Some seeds were broken, many were completely abraded at their tips, and therefore they could not be used for steeping trials. The H<sub>2</sub>SO<sub>4</sub> method gave more consistent dehusking, resulting in good correlation between the light box and the LTm score. So this method was chosen for dehusking grains.

To establish the LTm score for husked barley samples one hundred whole grains were measured. The same grains were dehusked and measured again to calibrate the instrument. Therefore, for both husked and dehusked samples, grains were classified and the LTm values were set accordingly (Figure 17). To obtain the same distribution of mealiness within the sample the range of each group was altered for husked grains and thus adjusted for the husk contribution.

#### LTm measurements to assess the endosperm structure of Chariot and Epic

Using the single grain meter, differences in the mealiness of a feed and a malting grade variety could be detected. Chariot was found to be very mealy (75%), whereas Epic was very steely, as illustrated in Figure 18. In fact no mealy grains were detected in Epic. This is reflected in the quality of Chariot since it is a very good malting variety.

#### Malting trials to correlate measurement of husked and huskless grains

A formula known as the LTm Index was used to discriminate the samples. The LTm Index indicates the mealy character of the endosperm taking into account the amount of steeliness of the grain. The higher the Index the better the mealiness of the endosperm. The cut-off point for no steeliness was 200 for husked grains and 400 for dehusked grains. Thus:

$$\text{LTm Index} = \frac{(1 - \text{LTm value})}{200} \quad \text{for husked grains}$$

$$\text{LTm Index} = \frac{(1 - \text{LTm value})}{400} \quad \text{for huskless grains}$$

The structural changes which occur due to the malting process were measured using this Index to give an indication of modification, so that control points could be set during steeping and germination for various samples.

The LTm Index of Chariot and Epic with and without husk, were measured during the malting process. In figure 19, it can be seen that Epic started at a low LTm Index which increased after the beginning of the steep to a higher value by the end of malting. This indicates a rapid change in the barley endosperm soon after the water uptake. The LTm Index of Chariot is maintained during malting with just a slight increase in the malt. The dehusked samples in Epic show an even faster change in the endosperm structure than the grains with husk due to faster water uptake.

#### **Development of the rapid LTm meter (50 grains)**

Development of the machine increased the sample size to 50 grains at once by means of a carousel and a stepper motor (Figure 7). The LTm values obtained from the datalogger were transferred offline after the measurements were completed. The saved file could then be used for calculations and visualisation of results. The run time to analyse 50 grains was 3 minutes.

#### **Grain classification using the rapid LTm meter**

By using the values obtained from the single grain analyser for Chariot and Epic, the ranges of mealiness using the rapid LTm were calibrated to give the same values. Readings less than 100mV were used to indicate the very mealy grains, <200mV mealy grains and those higher than 400mV were classified as very steely. Figure 20 shows the differences between the varieties Chariot, Epic and Fanfare. Visual representation allows a quick assessment of the mealiness of the sample, the lower the values the more mealy, and also the homogeneity of the sample tested was shown by the slope of the graphs.

The LTm Index calculation was slightly different for the rapid LTm:

$$\text{LTm Index} = \frac{(1 - \text{LTm value})}{400} \quad \text{for husked grains}$$

### Repeatability tests

To establish the minimum number which represents the variation in endosperm structure, 300 grains from one barley sample were analysed in sets of 50. Figure 21 shows the differences for each of the sets, which were analysed in a single run. When these readings were averaged, it was determined that at least 100 grains are required to represent the endosperm characteristic of the sample (Figure 22).

The repeatability of readings was determined by measuring 50 grains 5 times *i.e.* the same 50 grains (Figure 23). There were slight differences in the readings between each run. The reason for this could be due to the position of the grains in the sample holder. This could be caused by the vibration of the stepper motor which moved the carousel.

### Influence of moisture content on LTm values

Samples of Chariot and Epic barley varieties were treated with either water or steam to increase moisture content and then dried for two periods in a drying oven. The resulting barleys covered a range of moistures from 8.3% to 21% w/w. Within this range the moisture content of the grain had no effect on the transmission value obtained by the LTm meter, illustrated in Figure 24. If the barleys were then steeped and allowed to absorb significant amounts of water (>25%) then small changes in LTm value were observed. It was concluded that within the normal range encountered for barley moisture, this parameter had no influence on the LTm value.

### LTm measurements during the malting process

The LTm Index of Epic and Chariot were measured during malting. Samples taken were assessed before and after drying for 12 hours at 40°C. As expected the apparent mealiness increased during the malting process, shown in Figure 25. The steely variety Epic showed a dramatic change of the endosperm structure, but at the end of the process, as malt, it did not reach the mealiness of Chariot. The LTm Index depends on the free space between the starch granules. As Chariot has this space already in the initial barley, its LTm Index did not change very much. A slight decrease in LTm Index was noted for the dried samples compared to the undried samples, moreso in the Epic variety. Although the LTm Indexes in the final malt were very similar.

Differences in the LTm Index in a particular variety did not represent modification but were merely an indication of change.

#### LTm measurements of different barley varieties

Barley varieties of different quality were analysed with the rapid LTm. Figure 26 shows the LTm Index and distribution of meali/steeliness within each sample. It can be seen that there is a range in the number of mealy grains in each of these varieties, which affects the LTm Index. Also the variation in samples of the same variety can be seen. The continental sample of Regina and the sample grown in the UK, show a similar degree of mealiness. In contrast, Optic differs between samples from these two areas. There were some malting varieties which showed a lower LTm Index than the feed varieties Lysiba and Bartok, as noted previously.

When LTm Index was compared with the total nitrogen content there was no significant correlation (Figure 27). This shows that a high nitrogen level does not necessarily mean that the sample is steely.

The barley samples from Nickersons Seeds UK were analysed again using the rapid LTm meter. Figures 28-32 show the distribution of mealiness within each sample of the 10 varieties from 5 sites. The first two columns give an indication of % mealy *i.e.* readings <200mV. Again this work shows that there is variation in the distribution of mealiness in a variety grown in different areas. And that some barley samples graded as feed material have LTm values similar if not better than some malting grade varieties.

When the mealiness of the barley sample was compared with either total nitrogen or total  $\beta$ -glucan levels in the barley, no correlation was found (Figures 33 and 34).

## RESULTS AND DISCUSSION – YEAR 3 (1999/2000)

### Comparison of LTm values of mixtures of barley varieties and malt calcofluor

To compare of the groupings of the LTm values and the corresponding Calcofluor measurement, Chariot and Epic barley grains were mixed together in the proportions listed below:

Table 8 : Mixtures of Chariot and Epic barleys

Mixture	Chariot (%)	Epic (%)
1	100	0
2	80	20
3	60	40
4	40	60
5	20	80
6	0	100

The LTm values on the barley mixtures were measured using the 50 grain transfectance meter. They were then malted using an 8.16.24 schedule at 16°C in a sterilin tube. After drying the samples under normal conditions used at BRI, the transfectance values were measured again and then a calcofluor assessment carried out on the malts.

As expected, the higher the percentage of Chariot grains in the mixture the higher the LTm Index, *i.e.* the more mealy the sample (Figure 35). The Chariot was a homogeneously mealy sample with most LTm values below 100mV whilst the Epic was a heterogeneous steely sample with a range of LTm values. This is highlighted in the figures 36-41 by the single column for the homogeneous sample from both measurements while a number of smaller columns represent the heterogeneous samples. During malting the apparent mealiness of each sample increases, shown by the shift in the grey column from the right to the left hand side of the graph.

Results from the LTm meter shows the homogeneity within a barley sample and indicate a similarity with the groupings obtained from the corresponding malt calcofluor assessment.

### Development of the rapid LTm meter (97 grains)

Further development of the LTm meter meant that the sample size increased to 97 grains (Figure 8). Incorporated into the carousel were three filters: a dark, a light and a coloured filter with a known LTm reading. This allowed the meter to be monitored to check it was functioning properly on each run. The meter was now able to measure malt samples by adjusting the sensitivity of the detectors. Other improvements were the smoother motion of the stepper motor and a slight increase in the size of the hole to contain the grain. Also a display on the LTm meter showed instructions to run a sample and had an updated display to show grain number under analysis. Run time to analyse 97 grains was 6 minutes.

### Barley LTm as a predictor of malting performance

LTm measurements were carried out on barley samples of varying malting grades. The barleys were malted using the standard regime used at BRI micromaltings. The subsequent malts were then assessed for extract, friability, calcofluor modification and homogeneity. Diastatic power was also measured as an indication of enzyme potential. These were then compared with the barley LTm values but no evidence was found that LTm could predict these parameters. Figure 42 shows a poor correlation between calcofluor values in the malt and the barley LTm.

Table 9 : Malt Analysis

Variety	Grade	Friability (%)	Extract l <sup>o</sup> /kg	DP °IOB	Calcofluor Modification (%)	Calcofluor Homogeneity (%)
Chariot	9	93	320	118	97.3	86.5
Fanfare	9	84	313	70	80.6	67.0
Regina	9	64	300	126	91.5	72.6
Halcyon	8	61	292	148	75.6	56.3
Pastoral	2	54	292	94	64.6	49.9
Epic	1	27	272	100	51.0	59.7

## Steeping Efficiency Prediction Test

### *Iodine Vapour Staining*

Single grains of Chariot barley were measured using the single grain analyser. They were then steeped for 6 hours as instructed in the Iodine vapour staining method. After 18 hours air rest, 50 grains were blotted dry and cut along the furrow. They were then measured using the LTm meter, stained in Iodine for 1 minute and measured again. Figure 43 shows the values before staining and the same grain after staining indicating a difference. This shows that the staining of the grain could be detected by the transfectance meter as the LTm value was reduced i.e. become darker.

This experiment was then repeated using both Chariot and Epic barleys. This time, samples (10 grains) were taken at hourly intervals during air rest to see if any change in water uptake could be detected (Figures 44 and 45). Chariot, as previously mentioned, was very mealy and remains in this state during the length of the experiment, while Epic was initially steely becoming slightly more mealy as time progresses, highlighted by the reduction in LTm value.

Although the principle of the Iodine Vapour staining procedure worked, using an adaptation of this technique in conjunction with the Light Transmission Method showed no more added information to the normal LTm result.

### *LTm measurement during steeping*

Barley samples (50 grains) were measured using the rapid LTm meter (50 grains). These were then micro-malted in sterilin tubes using an 8.16.24 schedule. At hourly intervals during steeping one set of grains was removed and blotted dry. The LTm values were then measured again. Figures 46-63 and 64-80 illustrate the LTm values of the initial barley grains and the same grains after steeping for Chariot and Epic respectively. There was an initial increase in the LTm value which could be due to the grain becoming translucent i.e. appearing more steely. It was noted that the time taken for the LTm values to return to their initial readings was quicker for Chariot than for Epic.

Other varieties, Fanfare, Fighter, Halcyon and Regina, were then measured using the rapid LTm meter (97 grains). Figures 81-152 show the LTm measurements for the barley and the steeped grain during the time course study. Table 10 shows the time taken for the LTm values of the steeped grain to return to that of the barley LTm value.



Table 10 : LTm measurement during steeping

Variety	Malting grade	Time (hrs)
Regina	9	24
Fanfare	9	24
Chariot	9	26
Halcyon	8	26
Fighter	6	31
Epic	1	27

In general, the higher the malting grade the quicker the time taken for the LTm values of the steeped grain to return to their original values. Graph 153 shows % apparent mealiness of these samples during the time course study. After air rest, there is a difference in the values of the different varieties which could relate to water uptake. Estimation of water distribution was carried out using the iodine vapour scoring method (Davies 1991, Chandra 1999). A poor correlation between iodine vapour score and % apparent mealiness after 24 hours was noted (Figure 154).

The rapid LTm meter was able to give an indication of the ability of a sample to distribute water across the endosperm.

#### Malt assessment

LTm measurements were carried out on malt samples produced using the regime routinely used at BRI. These results were then compared with malt analysis shown in Table 9. Malt homogeneity by calcofluor did not show any correlation with malt LTM value. There was however significant correlation between malt LTm (especially log median value) and malt extract, friability and particularly calcofluor modification (Figure 155). Multiple regressions including diastatic power did not improve these correlations.

The LTm gives a quantitative measurement of modification and may have value as a rapid determination of calcofluor value.

### Brewhouse trials

A chit malt (germinated for one day) was prepared using Optic barley. This was then carefully mixed in with the standard Optic malt used in proportions 0%, 5%, 10% (all in duplicate) and 20%. All samples were analysed by LTm three times and averaged. The grist was brewed using standard conditions and brewing performance was measured as extract and differential pressure across the lauter plate.

There is a clear relationship between extract and the percentage of chit malt in the grist as expected, shown in Figure 156. The higher the proportion of chit malt in the grist the lower the extract. Several indicators from the LTm show a linear relationship with the proportion of chit malt (Figure 157). This result indicates that the LTm can show the proportion of chit malt when used with a single variety. Graph 158 relates the extract obtained to a value obtained from the LTm. The greater the median value of the LTm the lower the extract. The quality of the relationship is good not excellent, nevertheless the LTm may be able to indicate extract potential for a single variety.

No clear relationship was found when comparing the differential pressure and the percentage of chit malt.

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*Bar chart showing proportions of mealiness of barley, using the rapid LTm meter (50 grains), and calcofluor of the corresponding malt of a sample of 100% Chariot. Malted using 8.16.24 schedule.*

- 37           **Distribution of barley LTm values and malt calcofluor scores on a mixture of mealy and steely varieties - Chariot 80% : Epic 20%**  
*Bar chart showing proportions of mealiness of barley, using the rapid LTm meter (50 grains), and calcofluor of the corresponding malt of a sample of Chariot 80% : Epic 20%. Malted using 8.16.24 schedule.*
- 38           **Distribution of barley LTm values and malt calcofluor scores on a mixture of mealy and steely varieties - Chariot 60% : Epic 40%**  
*Bar chart showing proportions of mealiness of barley, using the rapid LTm meter (50 grains), and calcofluor of the corresponding malt of a sample of Chariot 60% : Epic 40%. Malted using 8.16.24 schedule.*
- 39           **Distribution of barley LTm values and malt calcofluor scores on a mixture of mealy and steely varieties - Chariot 40% : Epic 60%**  
*Bar chart showing proportions of mealiness of barley, using the rapid LTm meter (50 grains), and calcofluor of the corresponding malt of a sample of Chariot 40% : Epic 60%. Malted using 8.16.24 schedule.*
- 40           **Distribution of barley LTm values and malt calcofluor scores on a mixture of mealy and steely varieties – Chariot 20% : Epic 80%**  
*Bar chart showing proportions of mealiness of barley, using the rapid LTm meter (50 grains), and calcofluor of the corresponding malt of a sample of Chariot 20% : Epic 80%. Malted using 8.16.24 schedule.*
- 41           **Distribution of barley LTm values and malt calcofluor scores on a mixture of mealy and steely varieties – Epic 100%**  
*Bar chart showing proportions of mealiness of barley, using the rapid LTm meter (50 grains), and calcofluor of the corresponding malt of a sample of Epic 100%. malted using 8.16.24 schedule.*
- 42           **Relationship between barley LTm and calcofluor modification**  
*Scatter diagram showing comparison of LTm log median of barley and calcofluor modification of malt, using the rapid LTm meter (97 grains). Barleys assessed were Chariot, Regina, Halcyon, Fighter, Pastoral and Epic.*
- 43           **Detection of water uptake (Chariot)**  
*Graph showing LTm values of Chariot before and after staining with iodine vapour (after 6 hr steep and 18 hrs air rest), using the single grain LTm meter.*

- 44**            **Detection of water uptake during 1<sup>st</sup> air rest (Chariot)**  
*Graph showing LTm value of Chariot before and after staining with iodine vapour at hourly intervals during 1<sup>st</sup> air rest after 6 hr steep, using the single grain LTm meter. Point is average of ten grains.*
- 45**            **Detection of water uptake during 1<sup>st</sup> air rest (Epic)**  
*Graph showing LTm value of Epic before and after staining with iodine vapour at hourly intervals during 1<sup>st</sup> air rest after 6 hr steep, using the single grain LTm meter. Point is average of ten grains.*
- 46 – 63**       **LTm measurement during steeping (Chariot)**  
*Graphs showing LTm measurement of Chariot barley and corresponding 'steeped' grain at hourly intervals during 1<sup>st</sup> and 2<sup>nd</sup> steeps on an 8.16.24 schedule, using the rapid LTm meter (50 grains).*
- 64 – 80**       **LTm measurement during steeping (Epic)**  
*Graphs showing LTm measurement of Epic barley and corresponding 'steeped' grain at hourly intervals during 1<sup>st</sup> and 2<sup>nd</sup> steeps on an 8.16.24 schedule, using the rapid LTm meter (50 grains).*
- 81 – 98**       **LTm measurement during steeping (Fanfare)**  
*Graphs showing LTm measurement of Fanfare barley and corresponding 'steeped' grain at hourly intervals during 1<sup>st</sup> and 2<sup>nd</sup> steeps on an 8.16.24 schedule, using the rapid LTm meter (97 grains).*
- 99 – 116**      **LTm measurement during steeping (Fighter)**  
*Graphs showing LTm measurement of Fighter barley and corresponding 'steeped' grain at hourly intervals during 1<sup>st</sup> and 2<sup>nd</sup> steeps on an 8.16.24 schedule, using the rapid LTm meter (97 grains).*
- 117 - 134**     **LTm measurement during steeping (Halcyon)**  
*Graphs showing LTm measurement of Halcyon barley and corresponding 'steeped' grain at hourly intervals during 1<sup>st</sup> and 2<sup>nd</sup> steeps on an 8.16.24 schedule, using the rapid LTm meter (97 grains).*
- 135 – 152**     **LTm measurement during steeping (Regina)**  
*Graphs showing LTm measurement of Regina barley and corresponding 'steeped' grain at hourly intervals during 1<sup>st</sup> and 2<sup>nd</sup> steeps on an 8.16.24 schedule, using the rapid LTm meter (97 grains).*

- 153        **LTm measurement during steeping**  
*Graph showing % apparent mealiness at time points during steeping. Varieties assessed were Chariot, Fanfare, Regina, Halcyon, Fighter and Epic.*
- 154        **Relationship between % apparent mealiness and Iodine vapour score**  
*Scatter diagram showing the correlation between % apparent mealiness and iodine vapour score after 8 hrs steep then 16 hrs air rest (time elapsed 24 hrs). Varieties assessed were Chariot, Fanfare, Halcyon, Fighter and Epic.*
- 155        **Relationship between malt LTm and Calcofluor modification**  
*Scatter diagram showing comparison of LTm log median of malt and calcofluor modification, using the rapid LTm meter (97 grains). Barleys assessed were Chariot, Regina, Halcyon, Fighter, Pastoral and Epic.*
- 156        **Relationship between Extract and the proportion of chit malt**  
*Scatter diagram showing the correlation between extract and the proportion of chit malt used in brewhouse trials. Proportions used were 0%, 5%, 10% and 20%.*
- 157        **Relationship between malt LTm and the proportion of chit malt**  
*Scatter diagram showing the correlation between the LTm log median of malt, using the rapid LTm meter (97 grains), and the proportion of chit malt used in brewhouse trials.*
- 158        **Relationship between malt LTm and Extract**  
*Scatter diagram showing the correlation between the LTm log median of malt, using the rapid LTm meter (97 grains), and the extract of malt used in brewhouse trials.*

Figure 1 : Scanning electron micrograph of a mealy endosperm structure

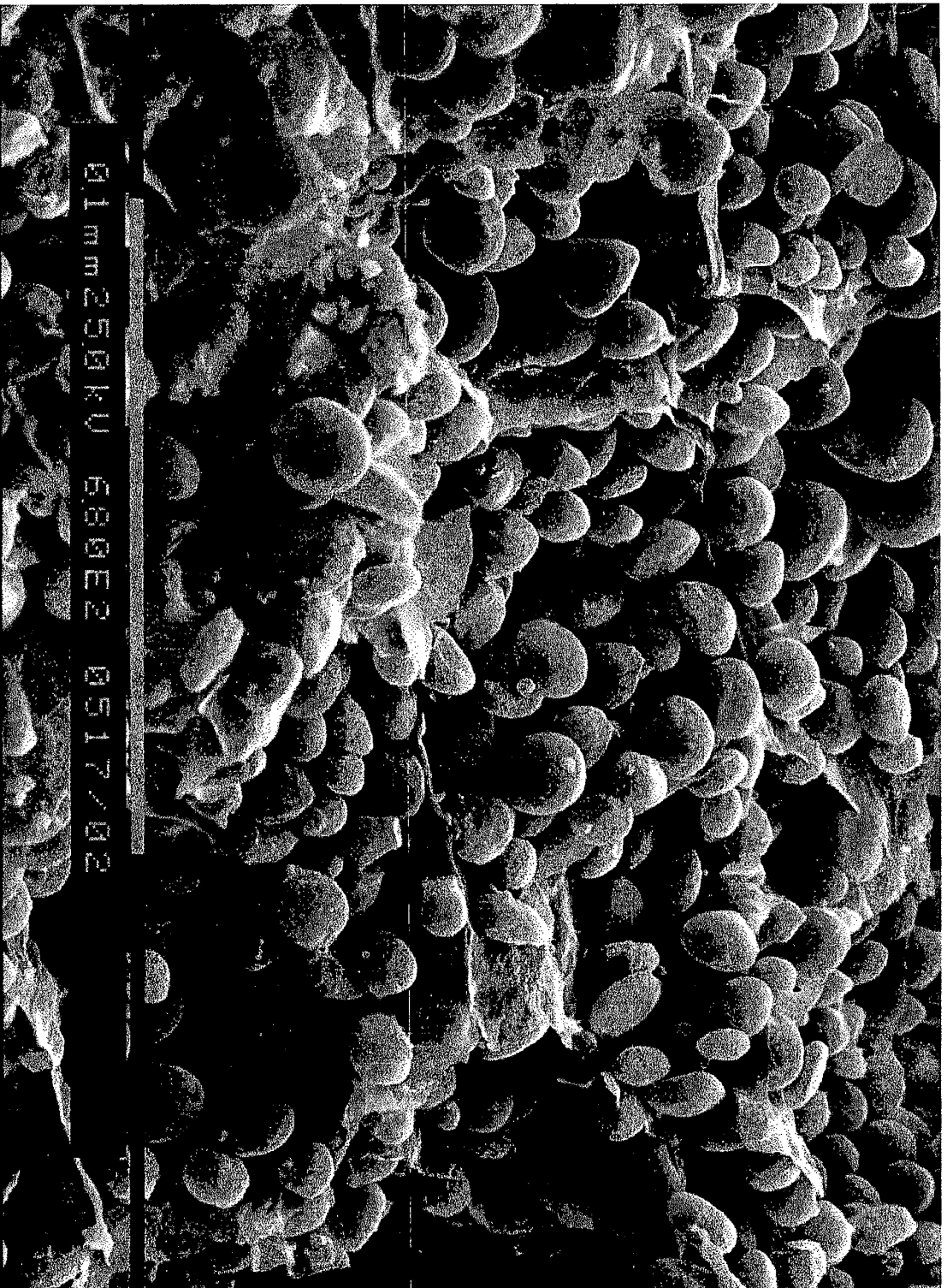


Figure 2 : Scanning electron micrograph of a steely endosperm structure

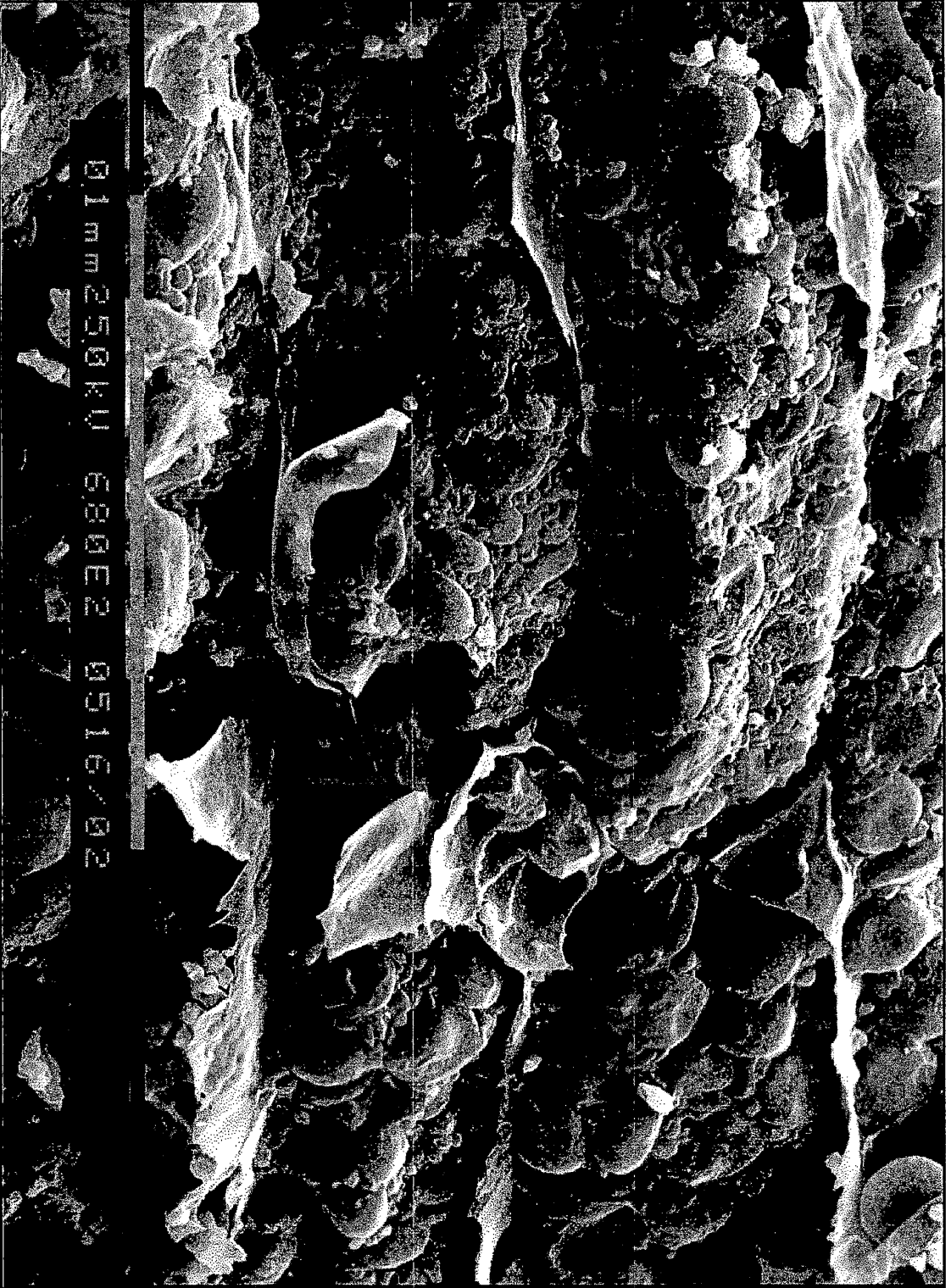
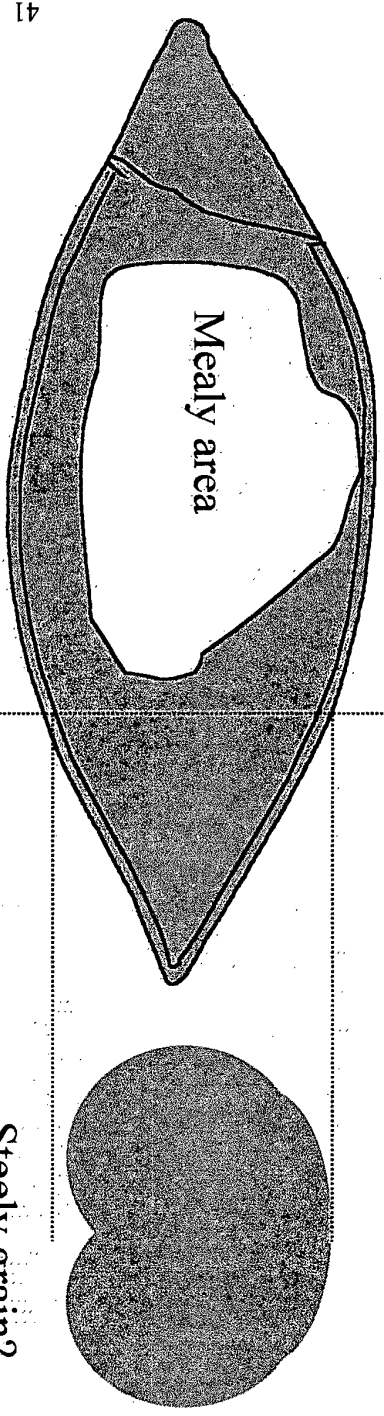
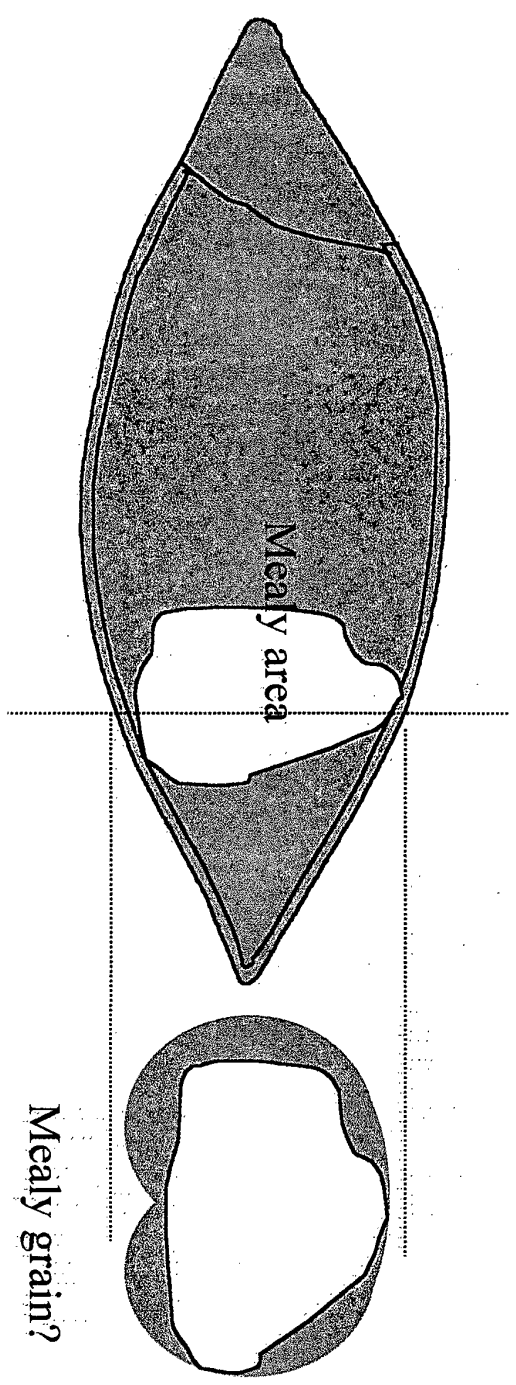


Figure 3 : Possible conclusions when using a Farinator to assess endosperm structure



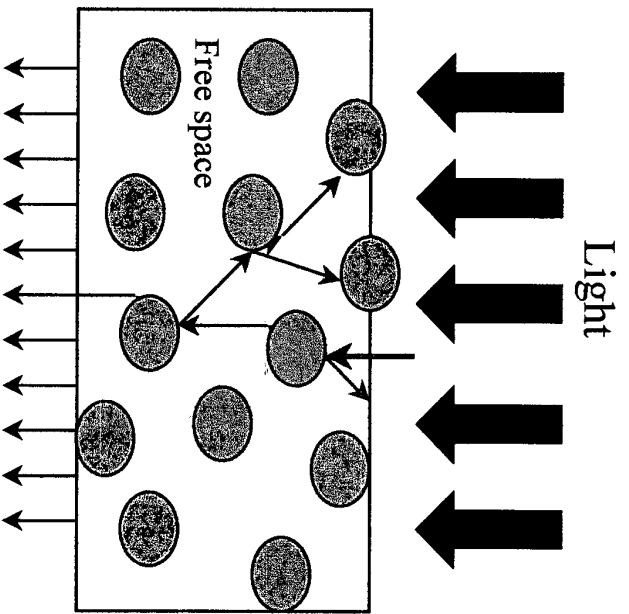
41



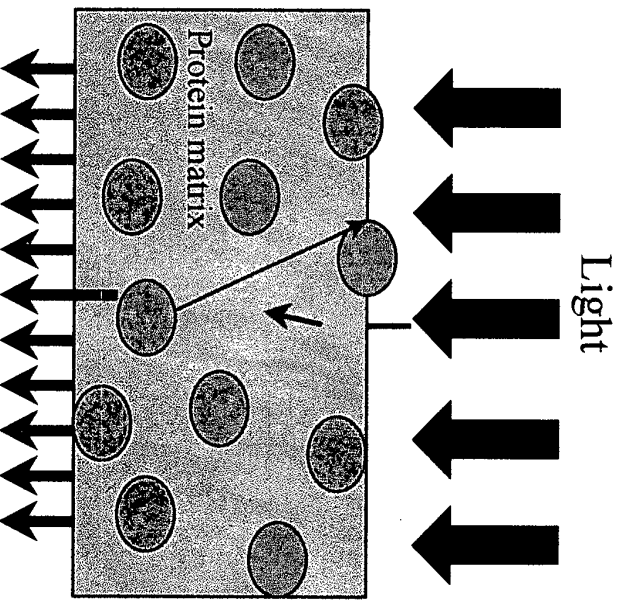
Mealy grain?



Figure 4 : Light transmission effect



Light absorbed by  
mealy endosperm  
→ **dark**



Light transmitted by  
steely endosperm  
→ *translucent*

Figure 5 : Single Grain LTm meter

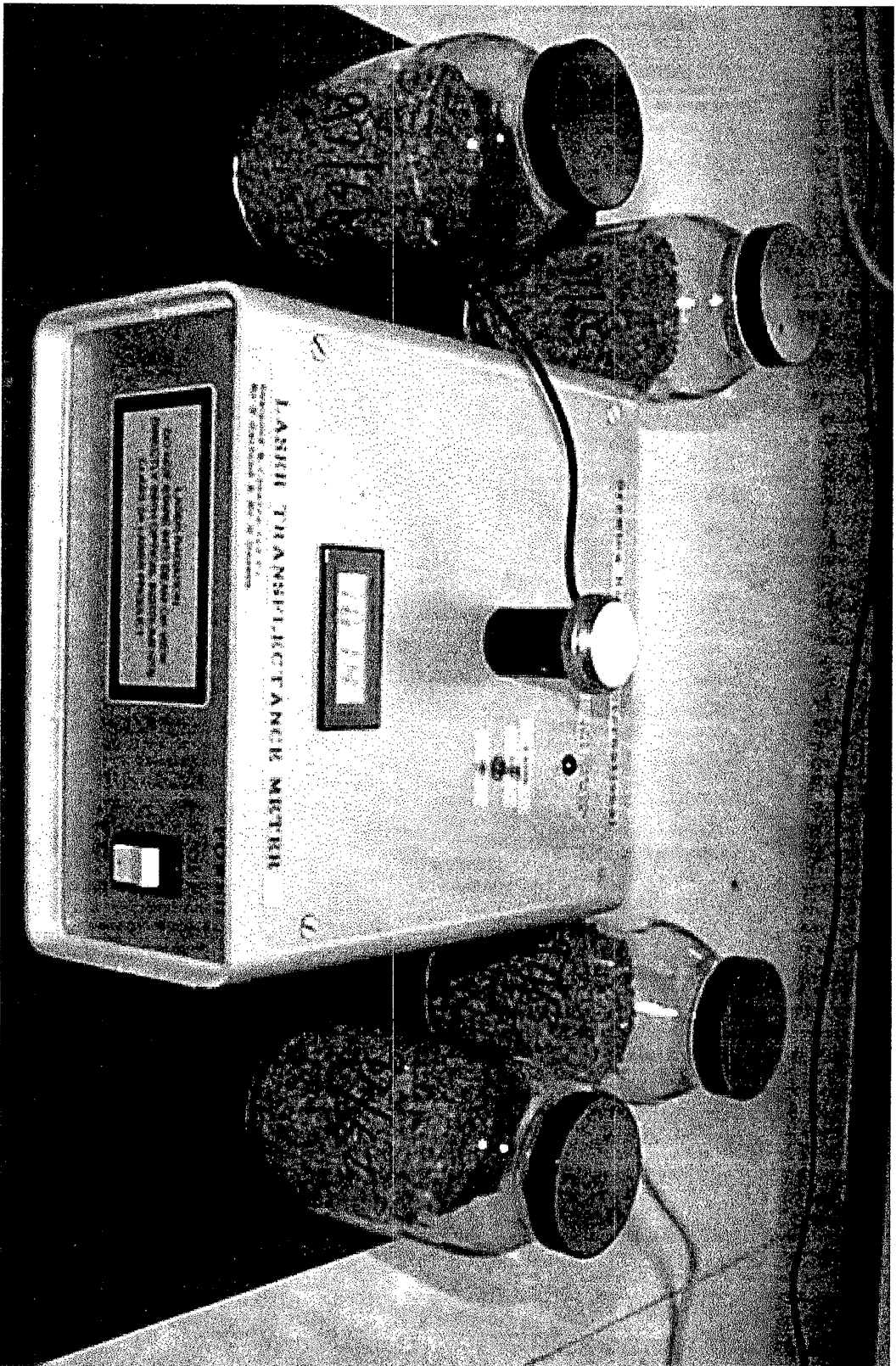
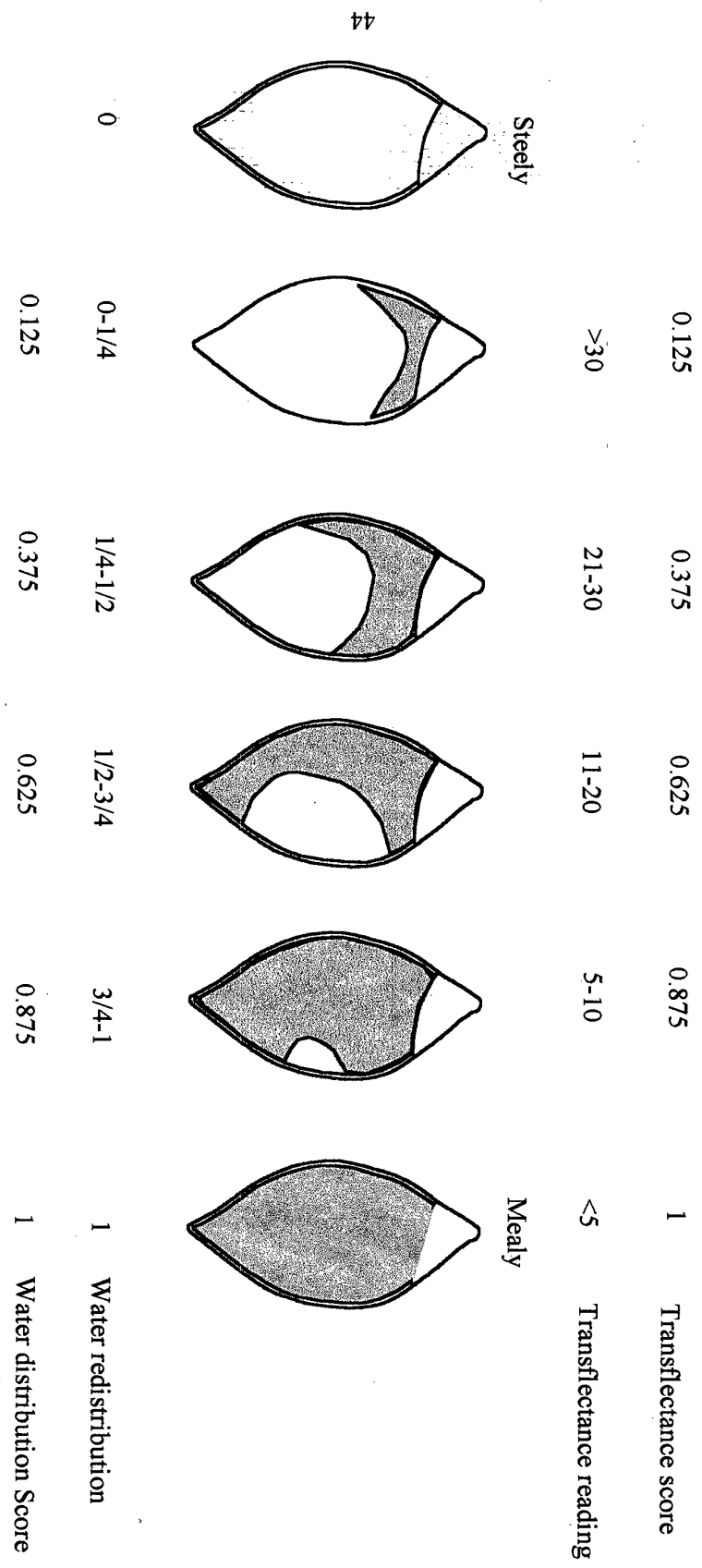
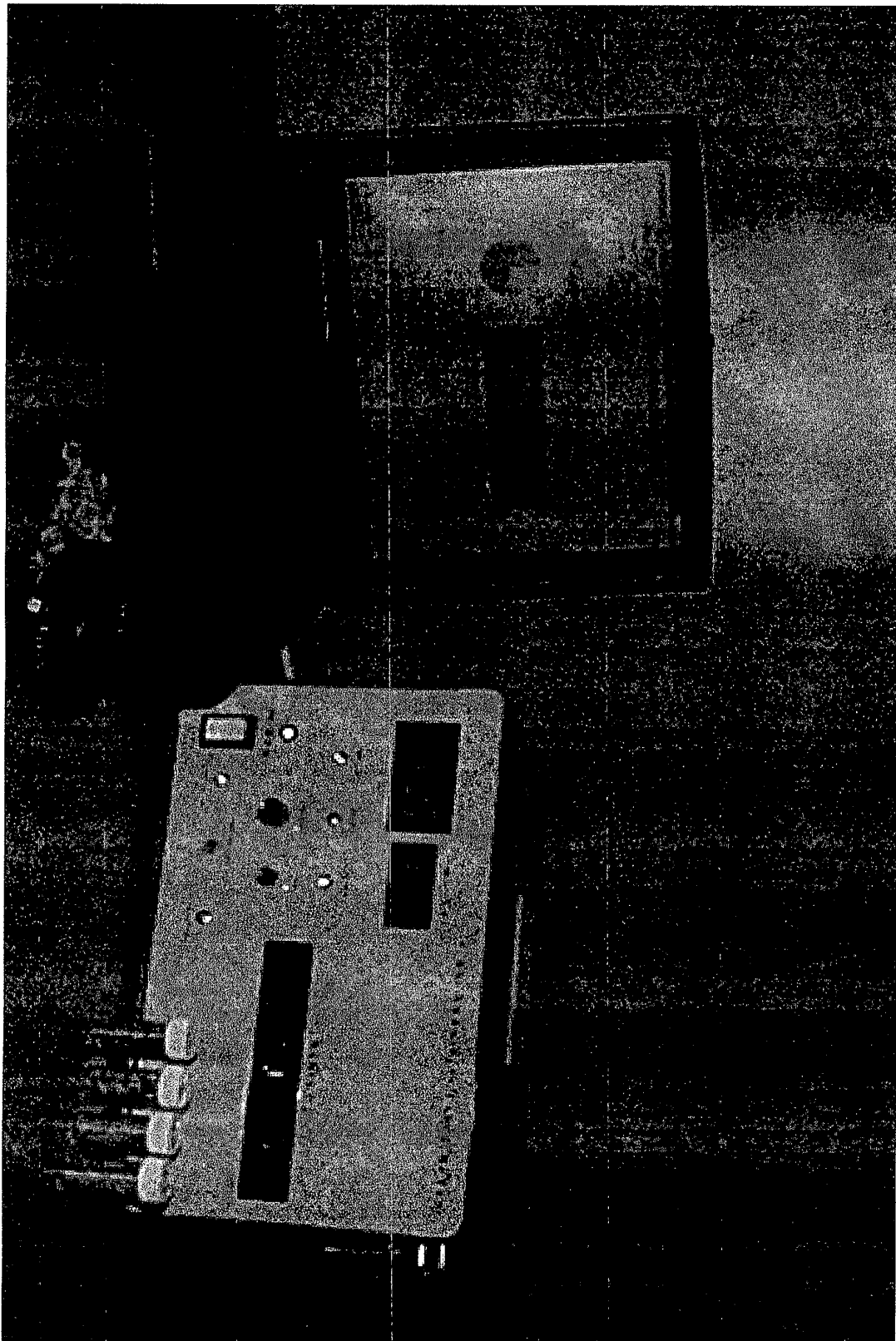


Figure 6 : Scoring method for light transmission and iodine vapour stain



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Figure 7 : Rapid L<sub>T</sub>m meter (50 grains)



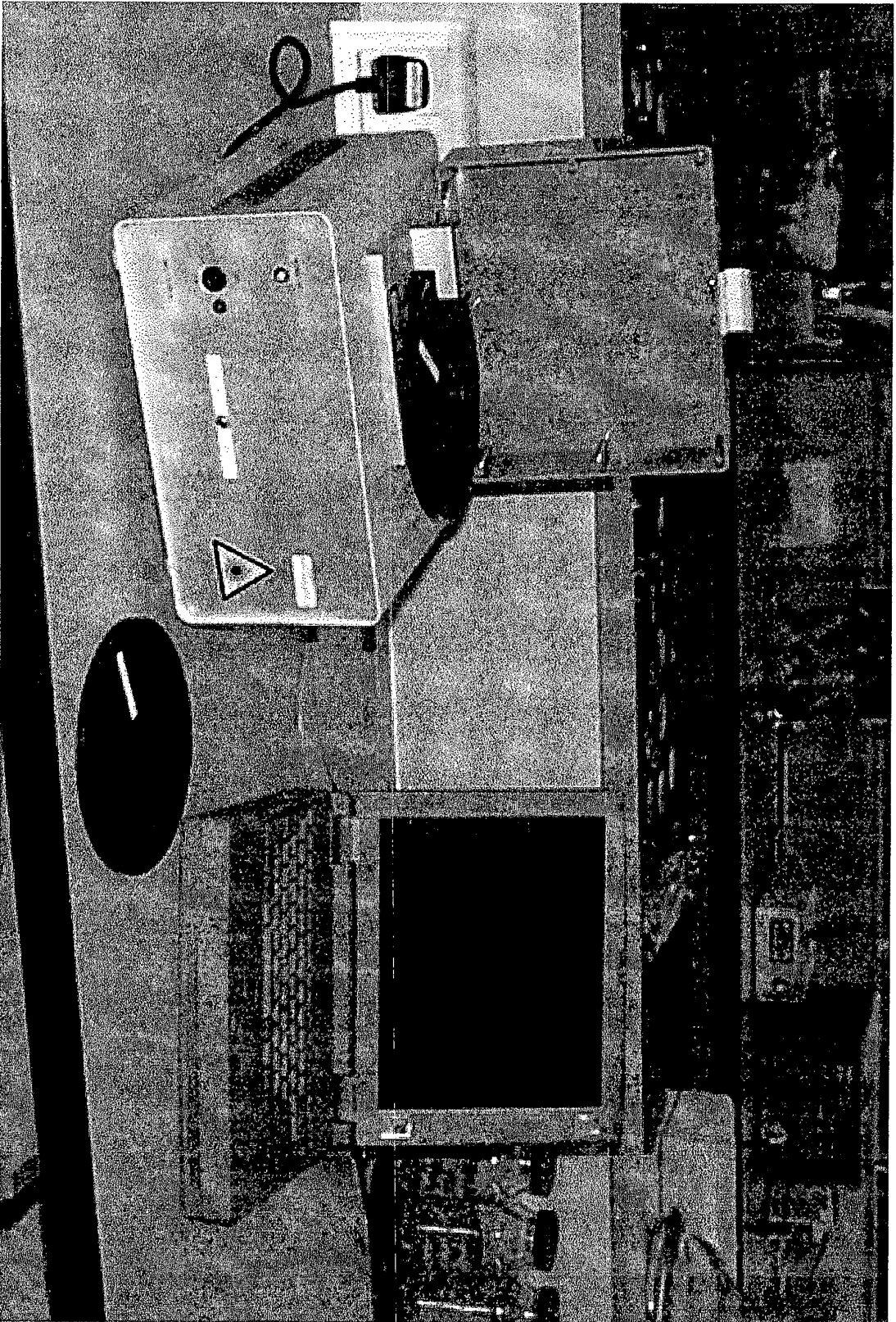
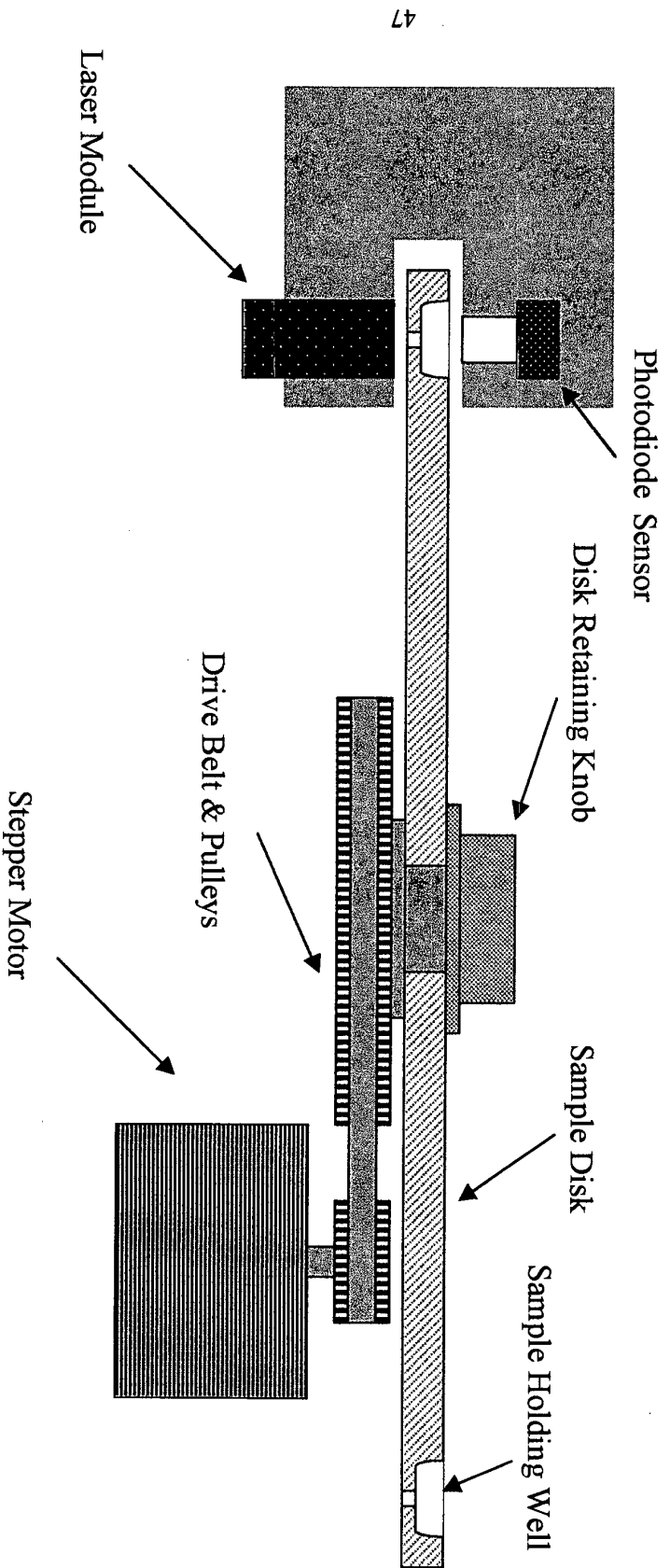


Figure 8 : Rapid LTM meter (97 grains)

Figure 9 : Schematic diagram of the L<sub>T</sub>m Meter



Designed By S G Garland & G J Gasson

Drawn by S G Garland July 2000

Figure 10 : Relationship between mealiness assessed visually and by LTm

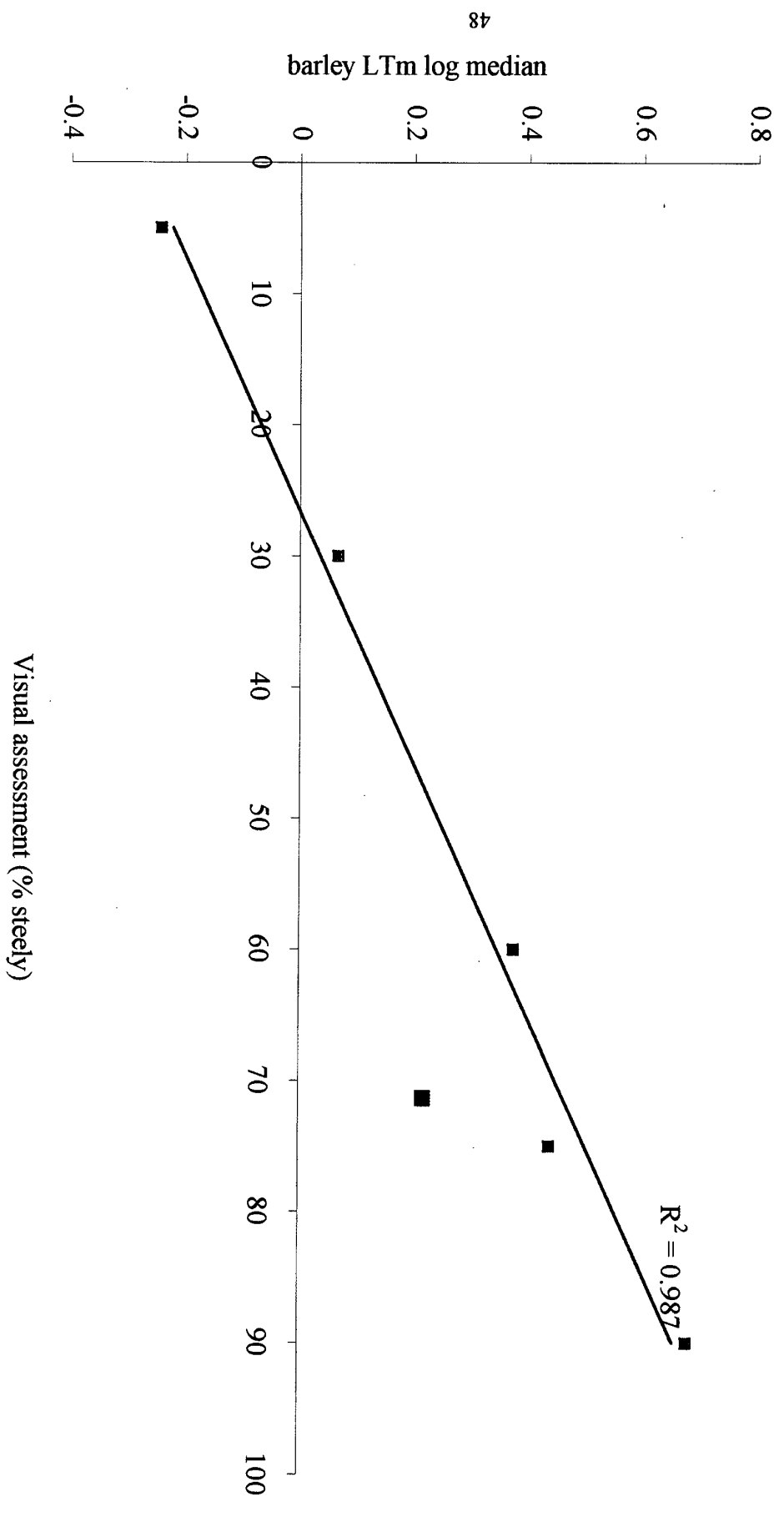


Figure 11 : LTm scores of malting grade barley varieties

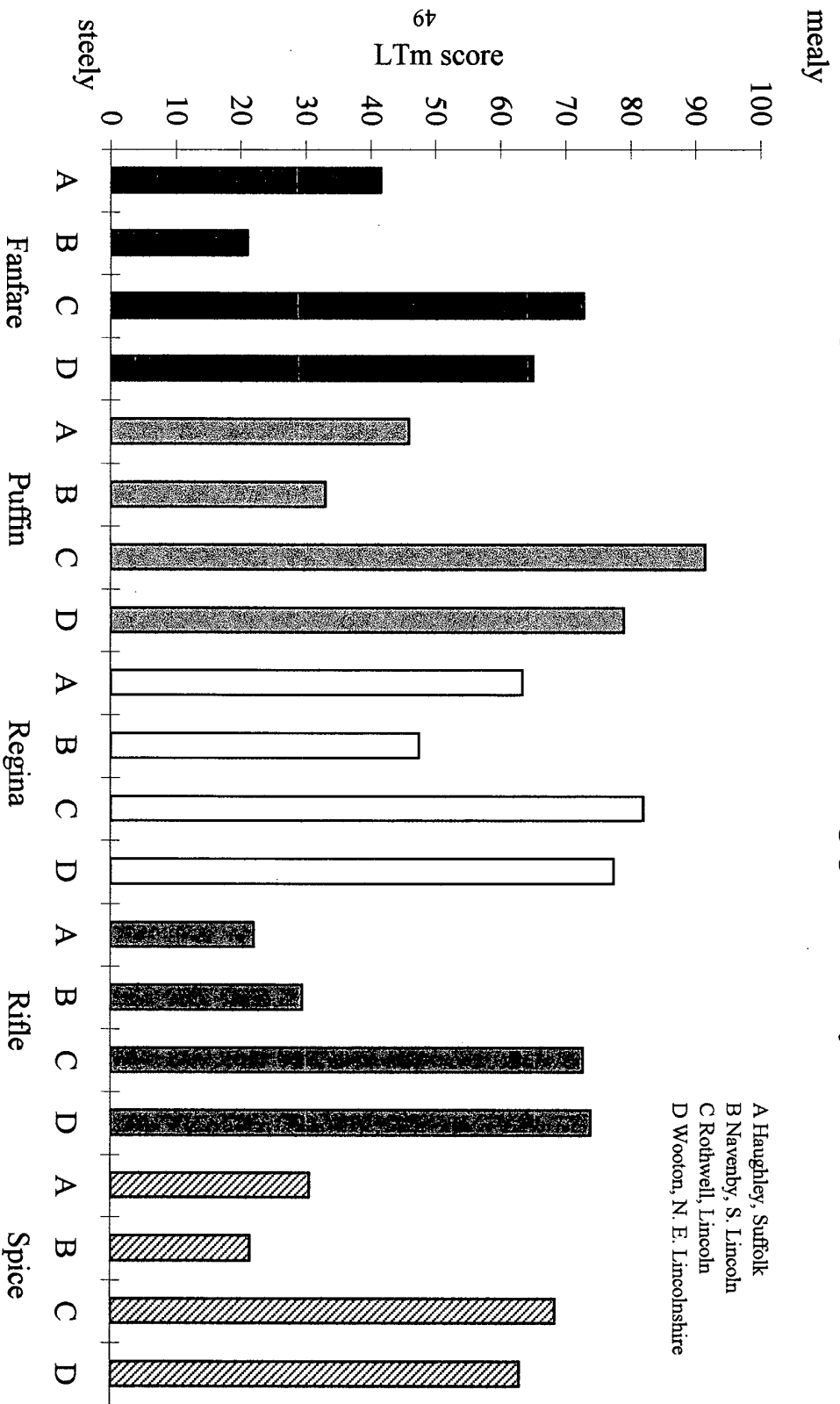
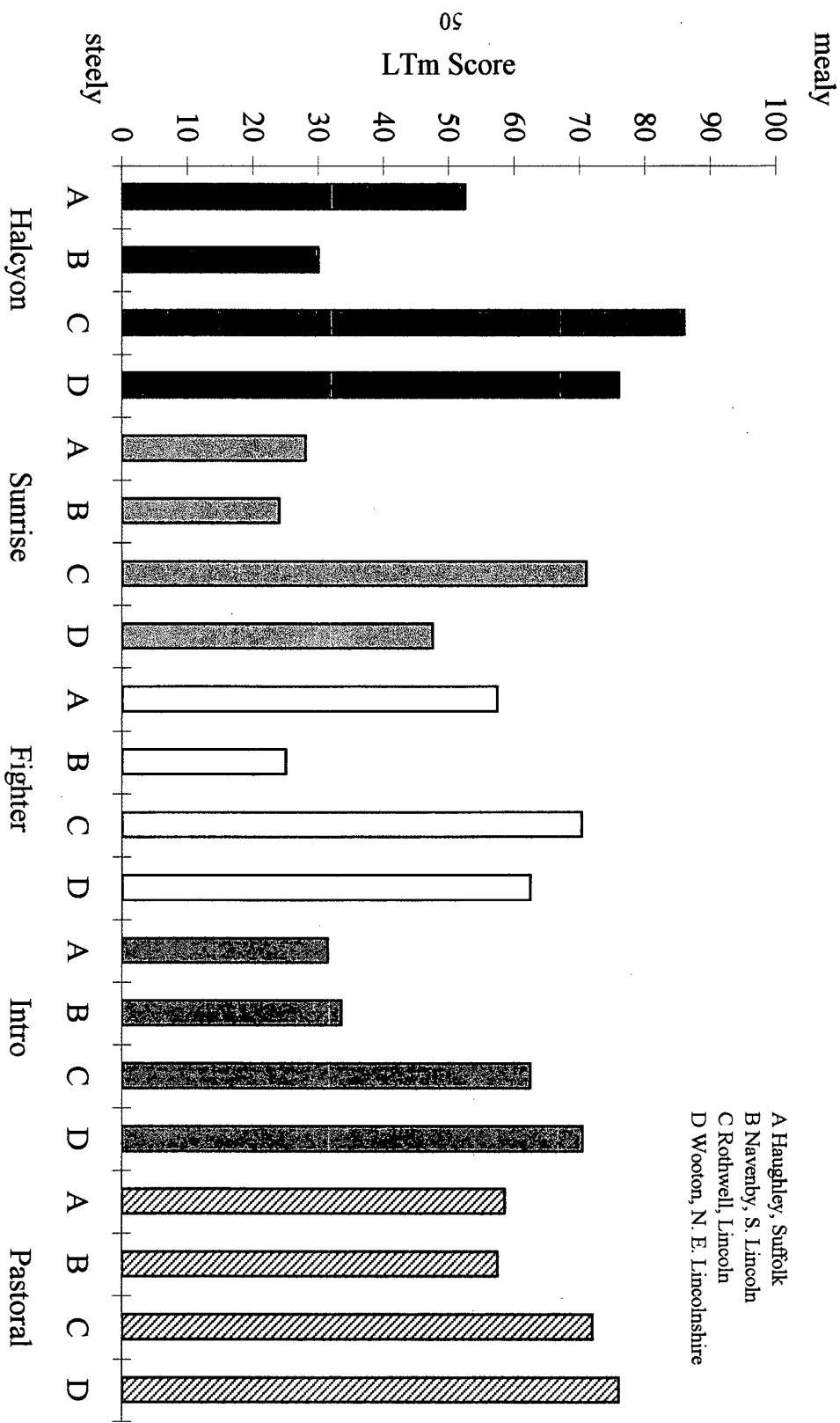




Figure 12 : LTm scores non-malting grade barley varieties



A Haughley, Suffolk  
 B Navenby, S. Lincoln  
 C Rothwell, Lincoln  
 D Wooton, N. E. Lincolnshire

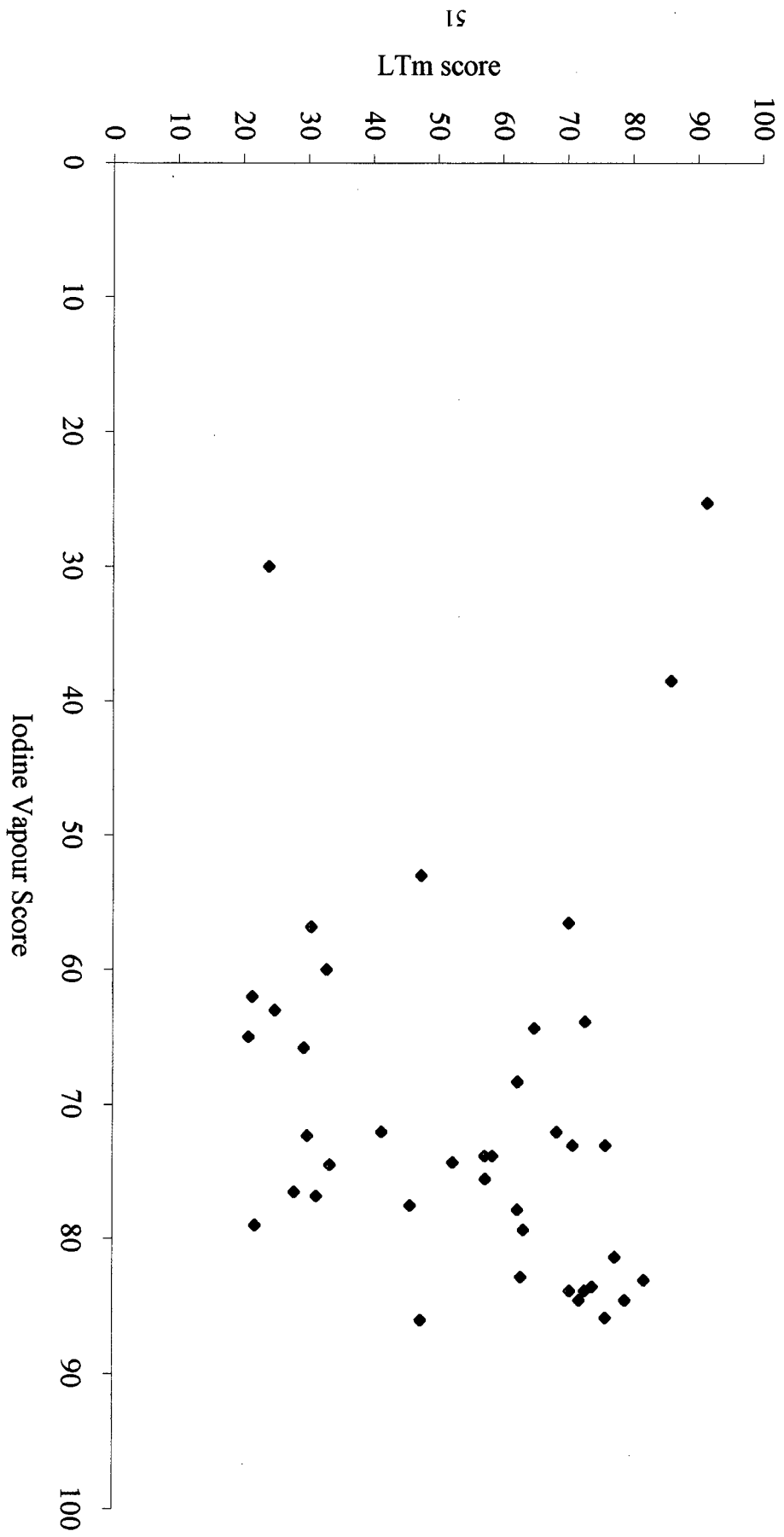


Figure 13 : Relationship between LTrm and Iodine vapour scores of barley varieties

Figure 14 : Relationship between LTm scores and Total  $\beta$ -Glucan levels of barley varieties

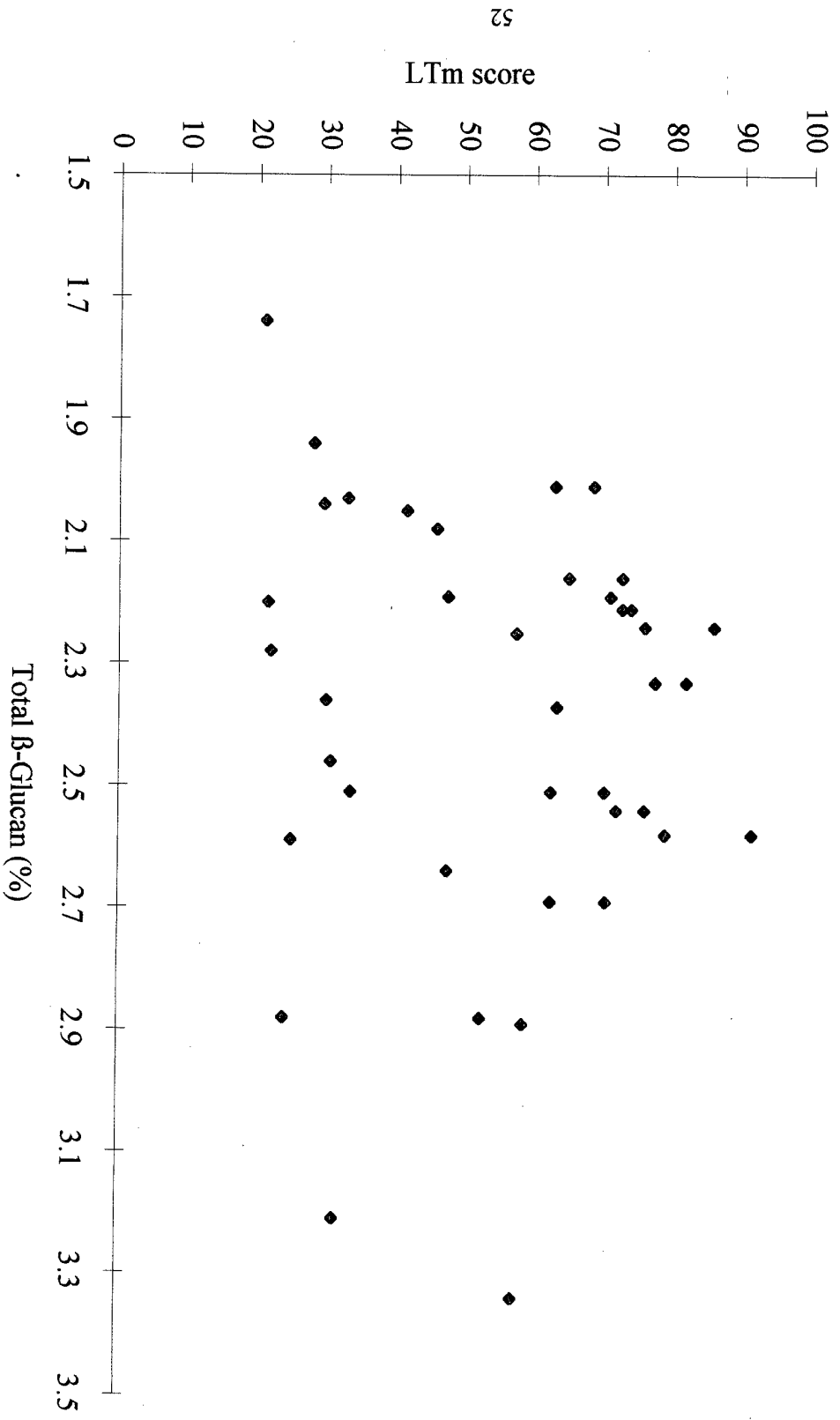


Figure 15 : Distribution of L<sub>Tm</sub> groups of Fanfare barley dehusked by sulphuric acid

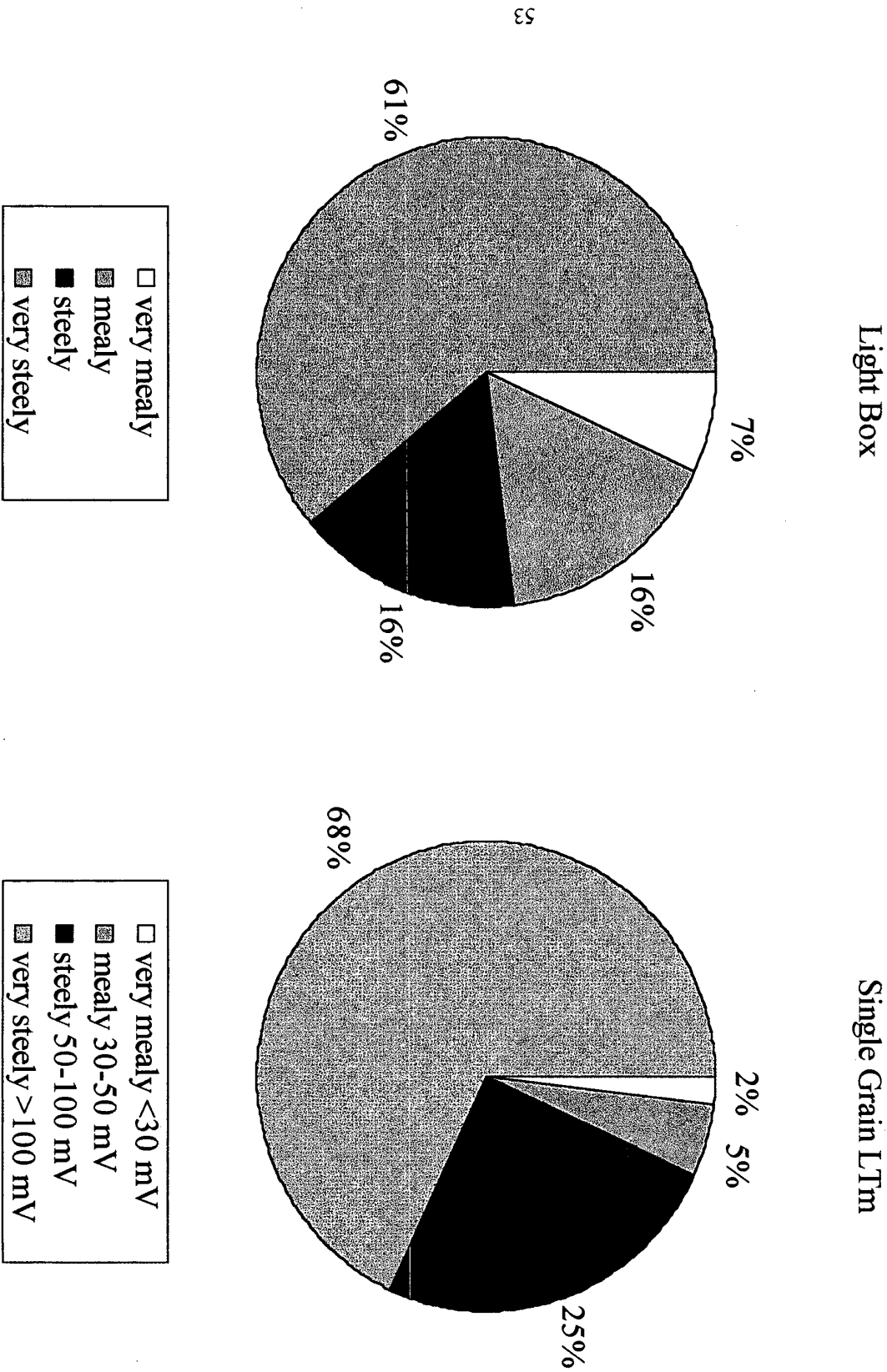


Figure 16 : Distribution of L<sub>Tm</sub> groups of Fanfare barley dehusked by pearling

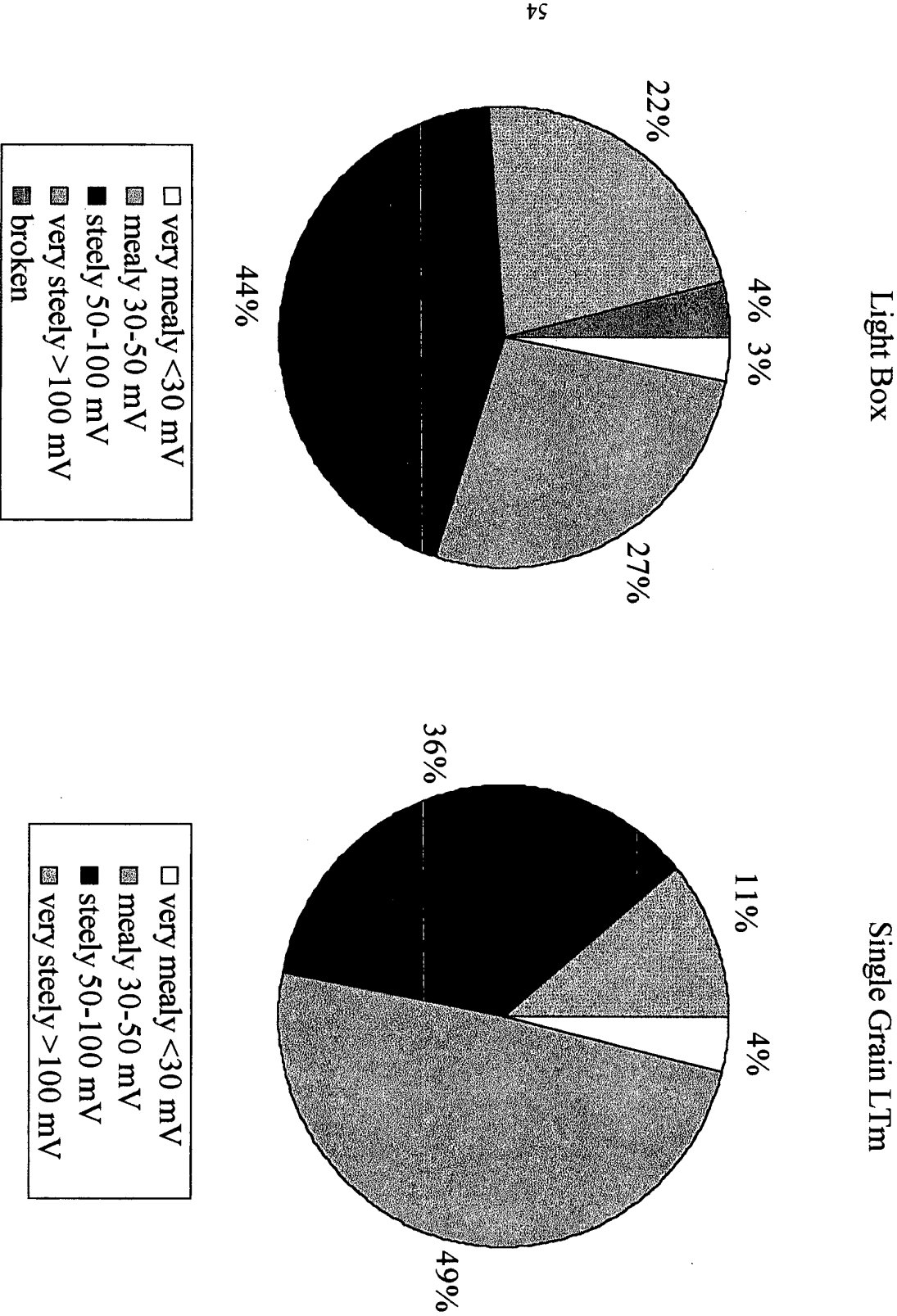


Figure 17 : Distribution of L<sub>Tm</sub> groups of Fanfare barley (husked vs dehusked)

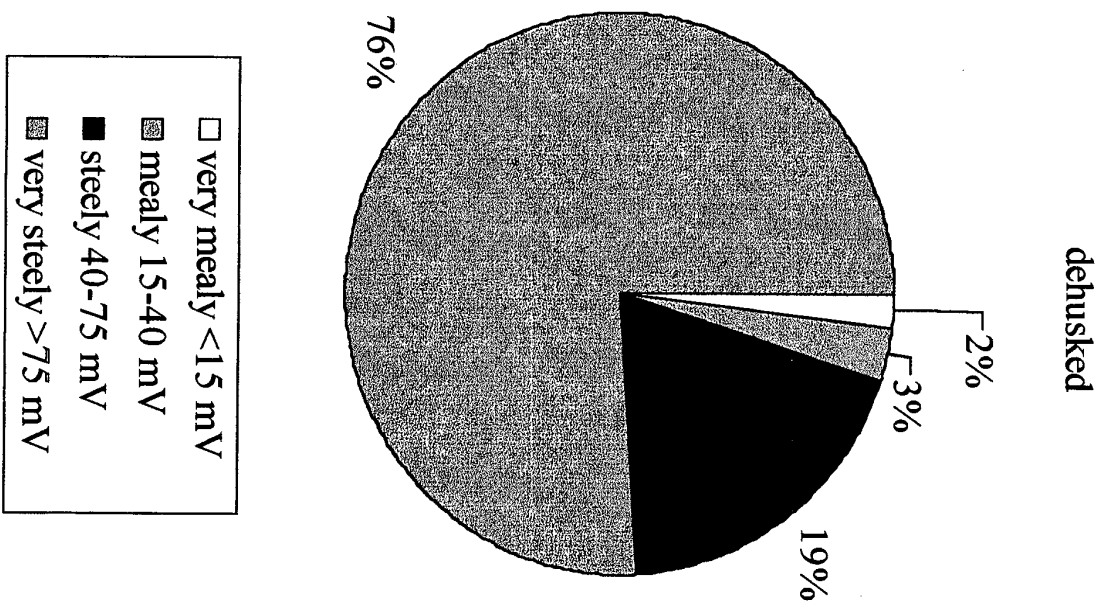
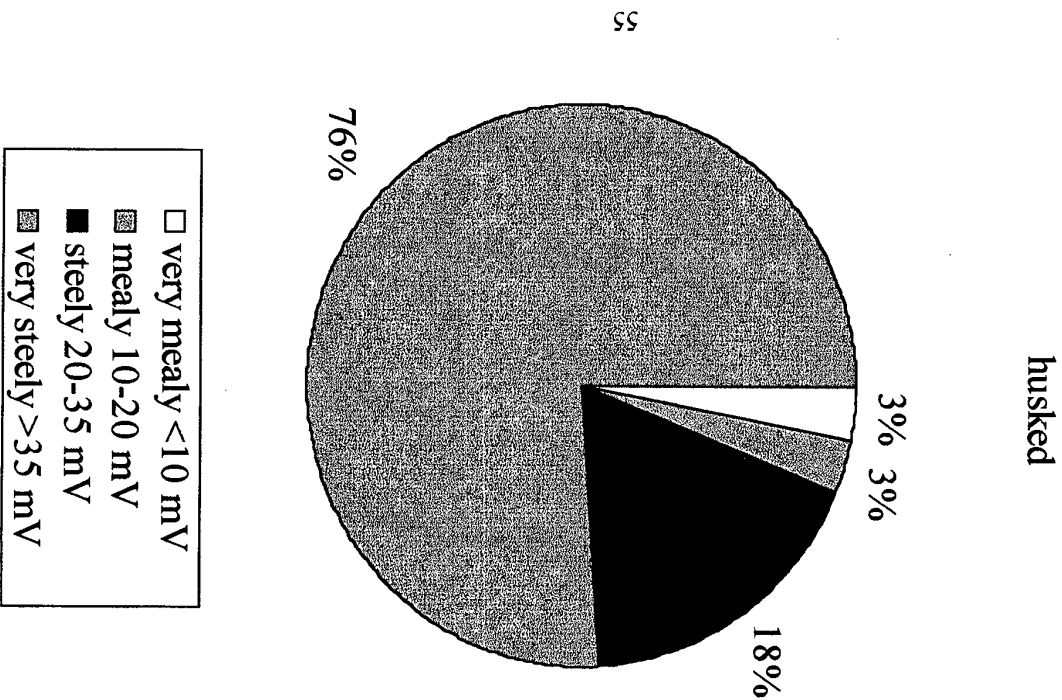
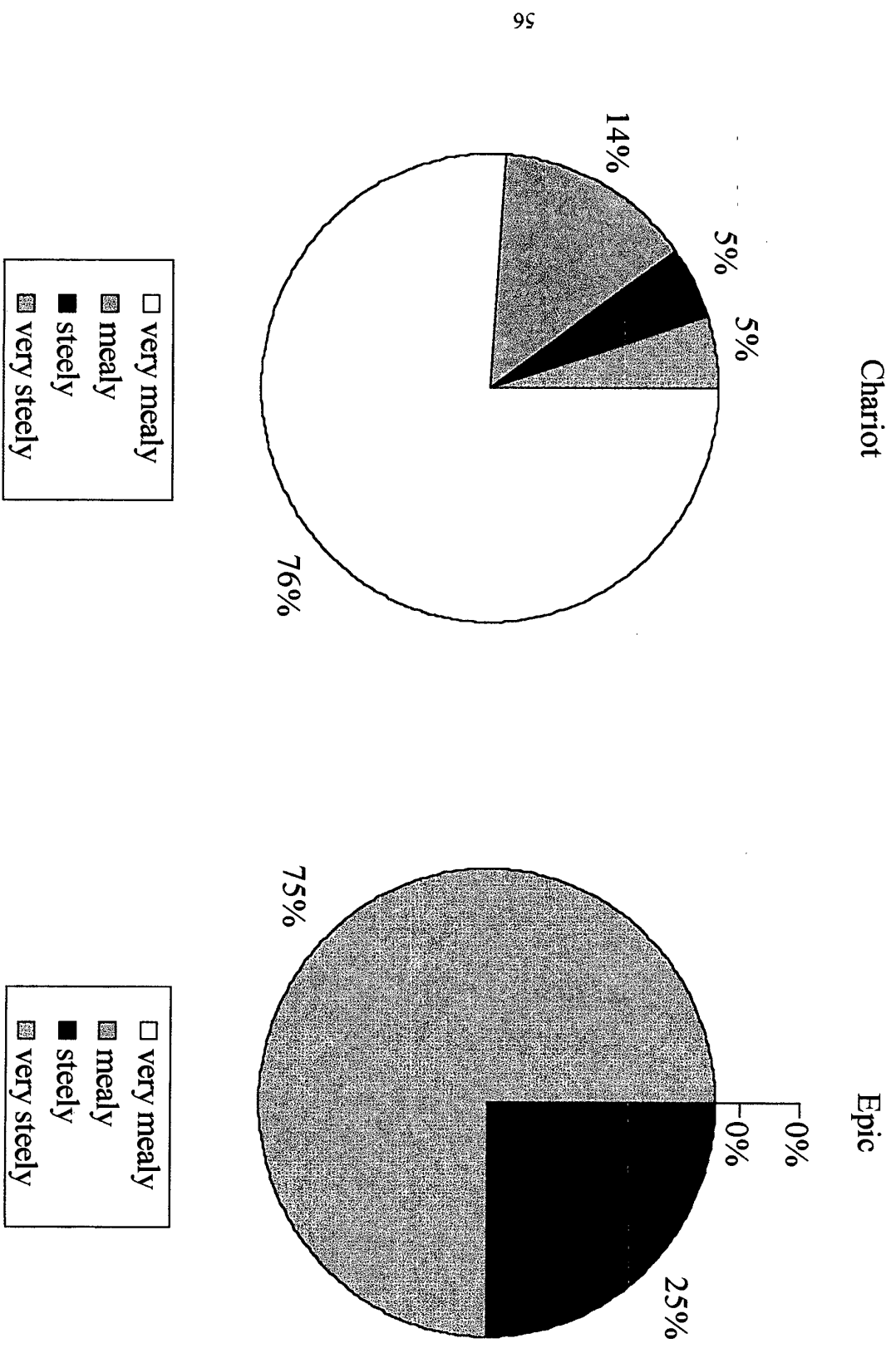


Figure 18 : Distribution of L Tm groups of Chariot and Epic barleyes



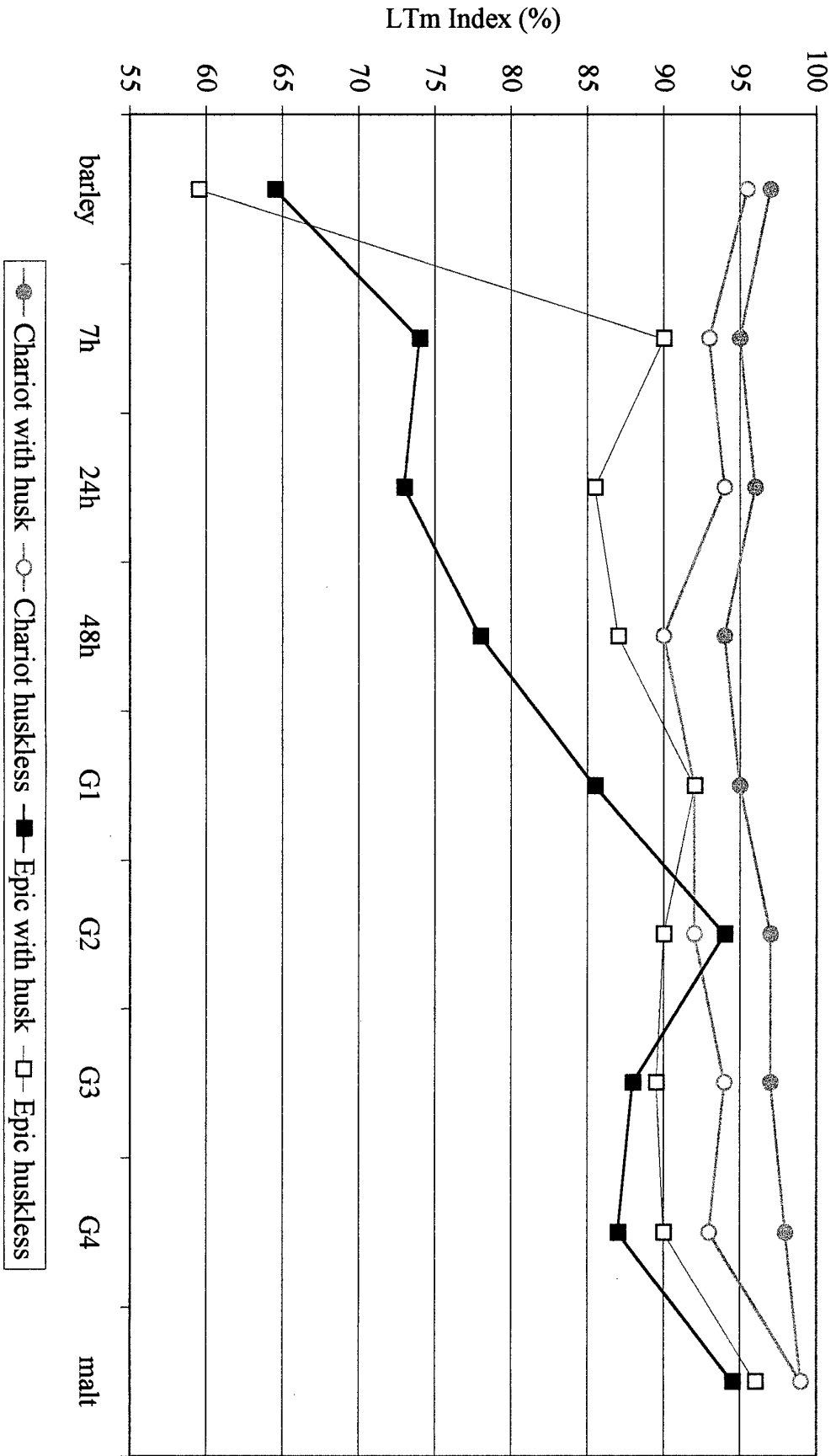


Figure 19 : LTm Index of Chariot and Epic during malting (husked vs dehusked)



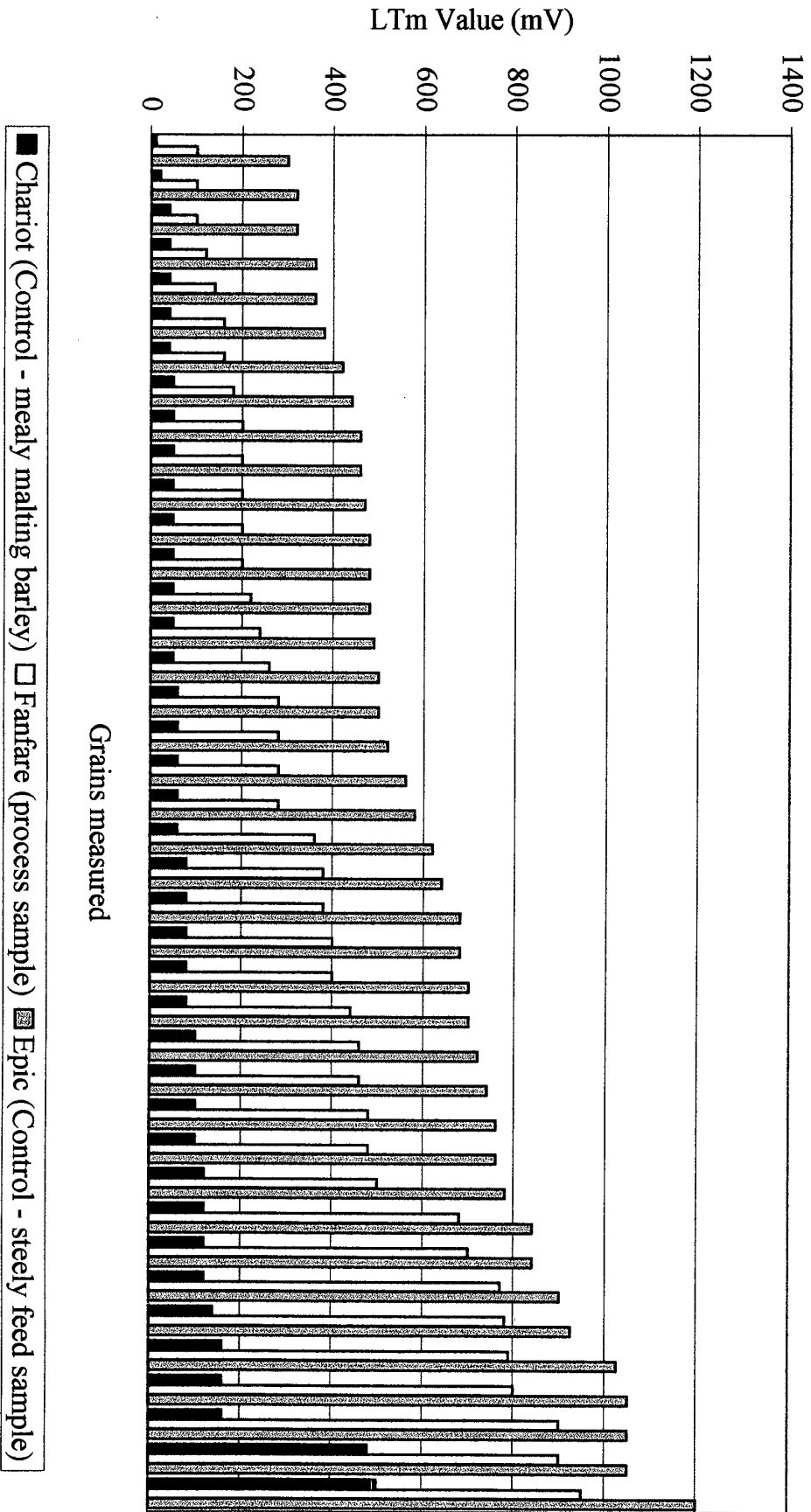


Figure 20 : LTM measurement of Chariot, Fanfare and Epic barley varieties

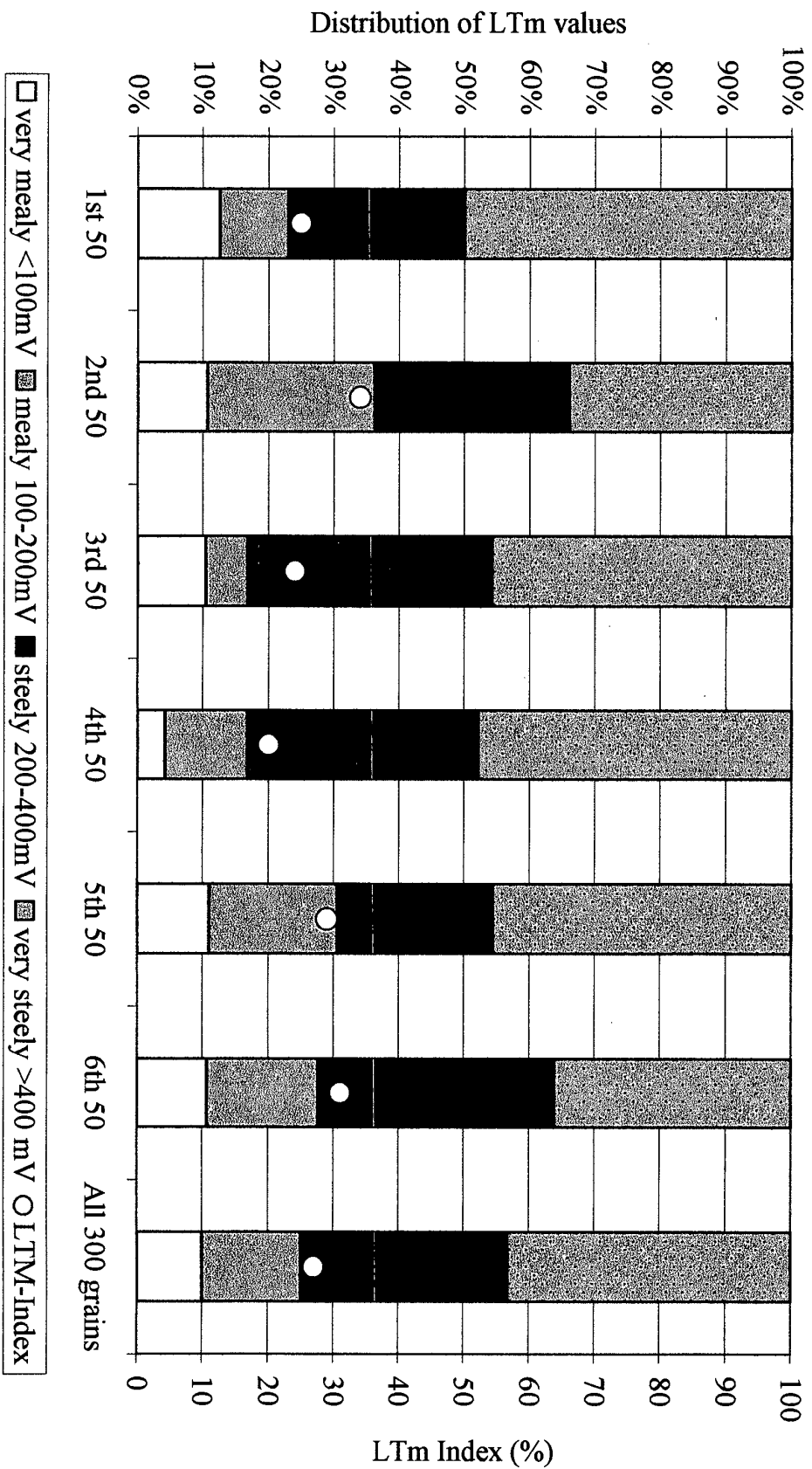


Figure 21 : Variation in LTm measurement of a single sample

Figure 22 : Minimum numbers of grains required to determine endosperm structure

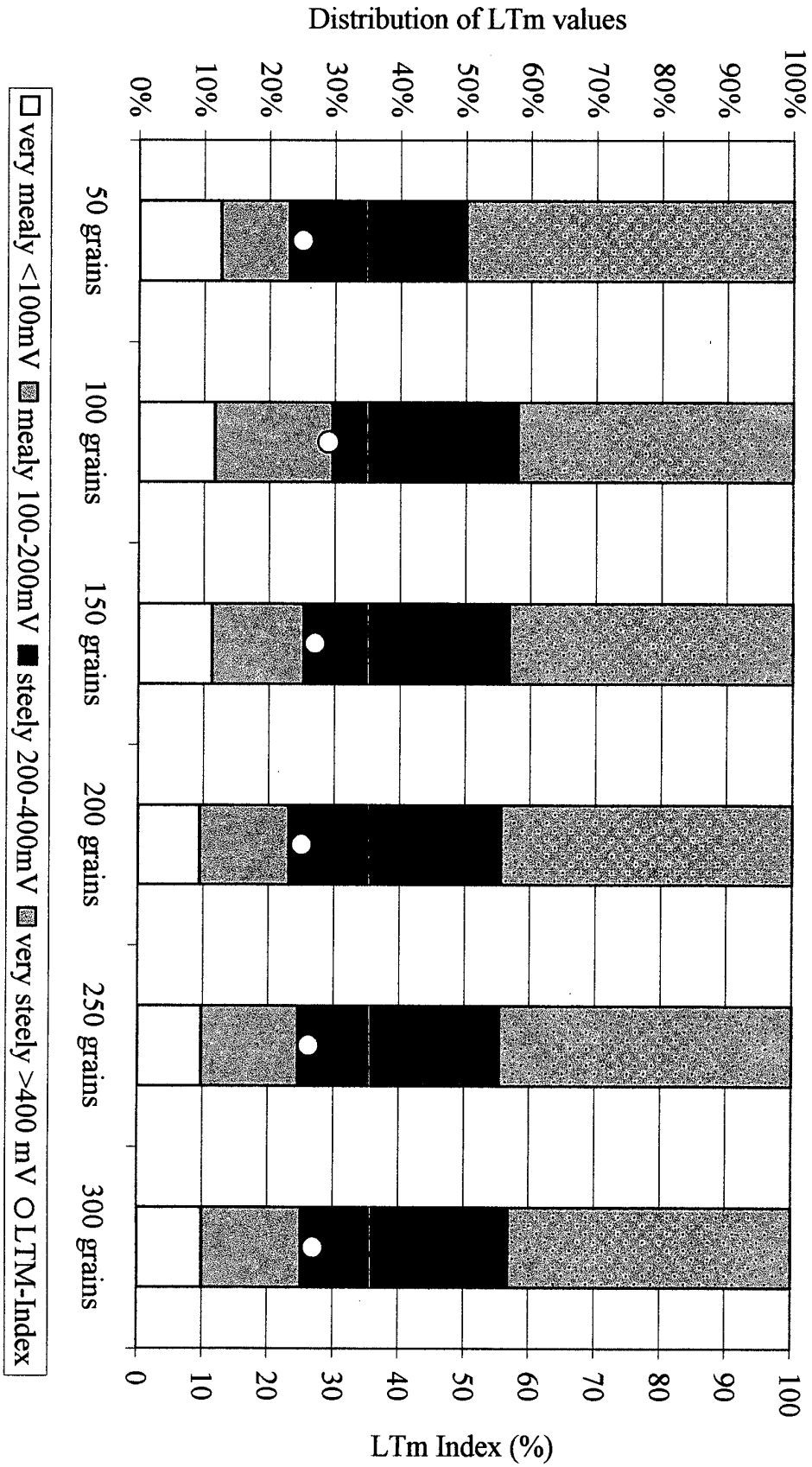


Figure 23 : Repeatability of LTm measurement

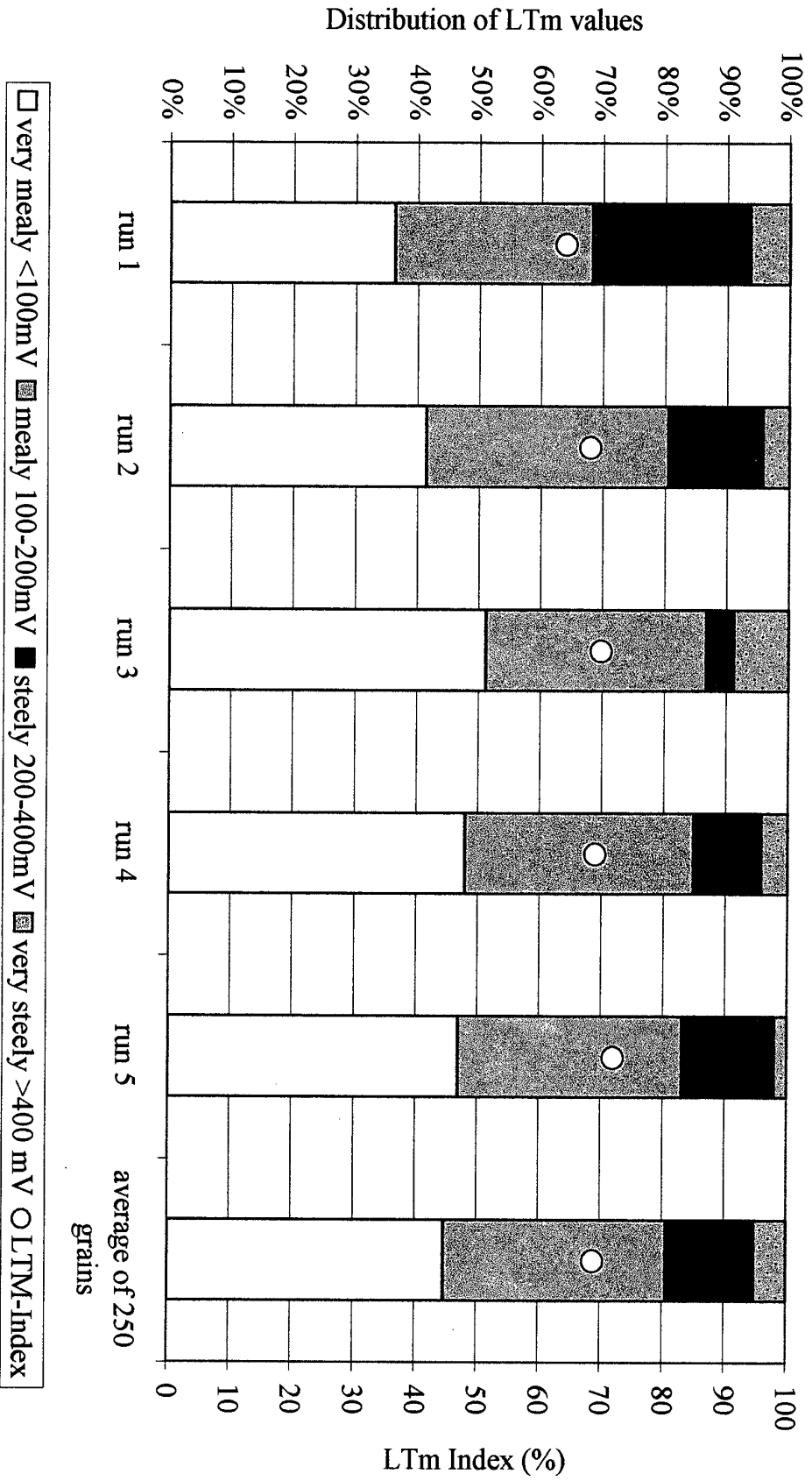


Figure 24 : LTm Index and moisture content during drying trial

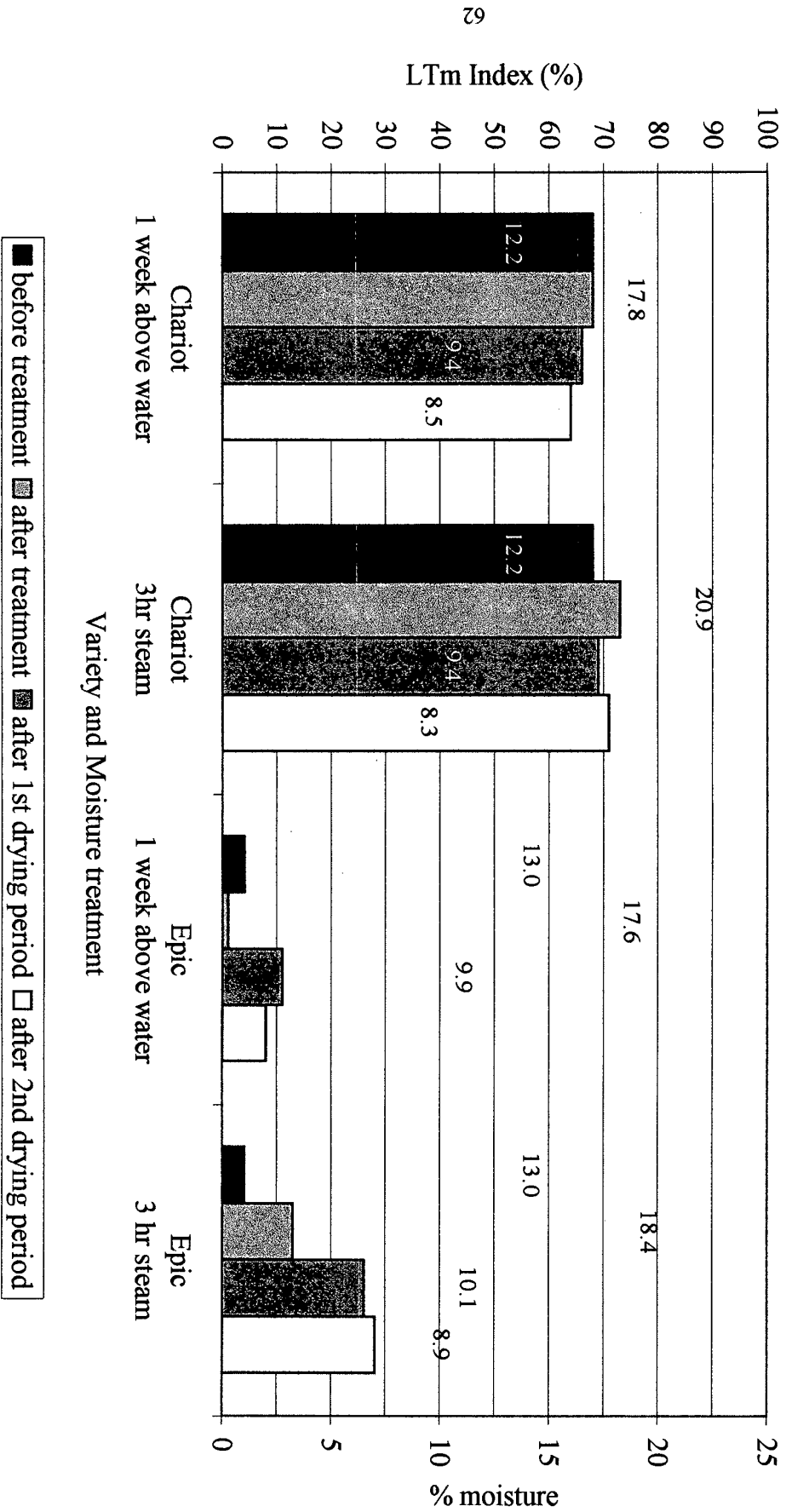
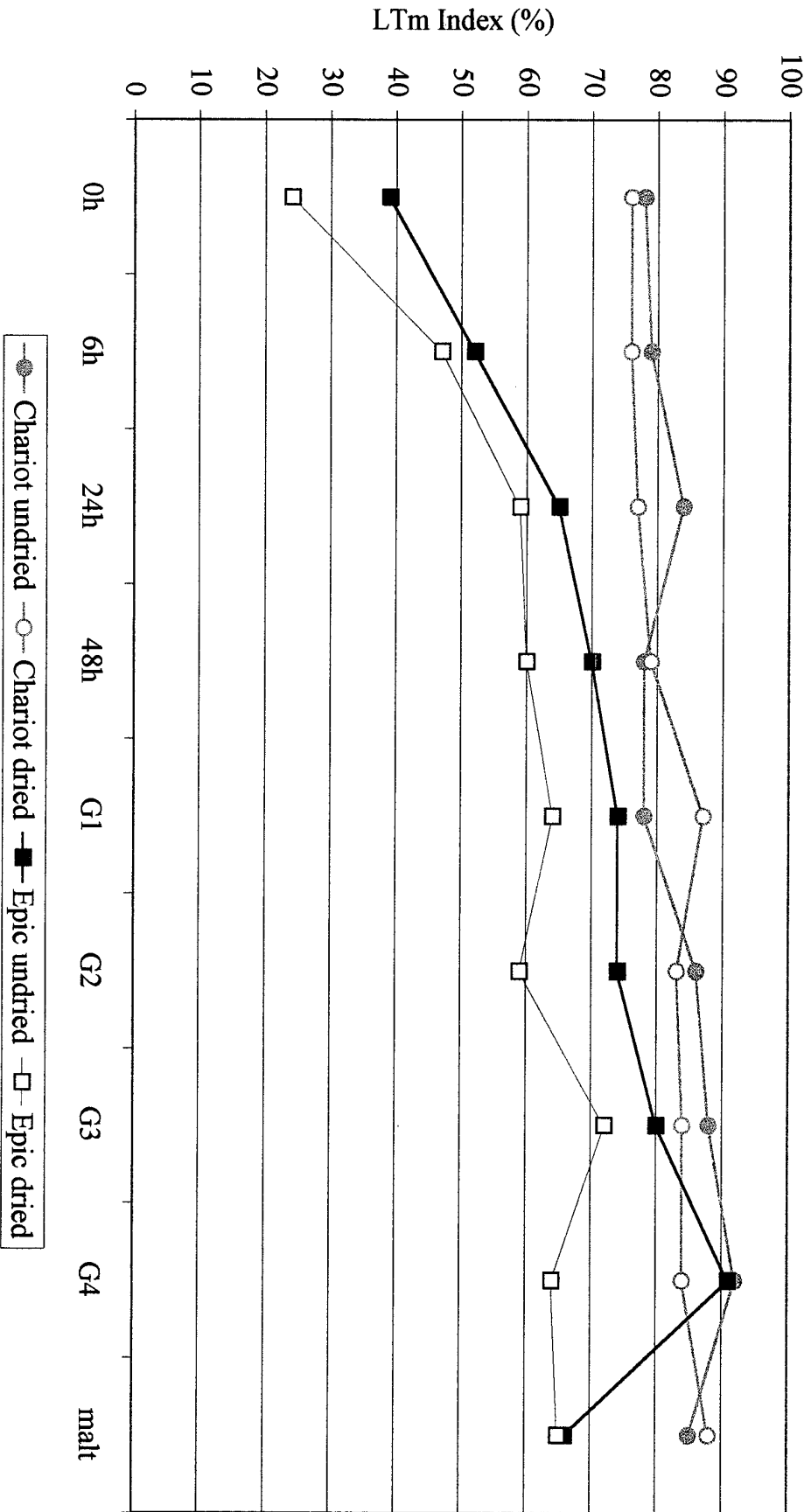


Figure 25 : LTm Index of Chariot and Epic during malting (undried vs dried)



Distribution of LTm values

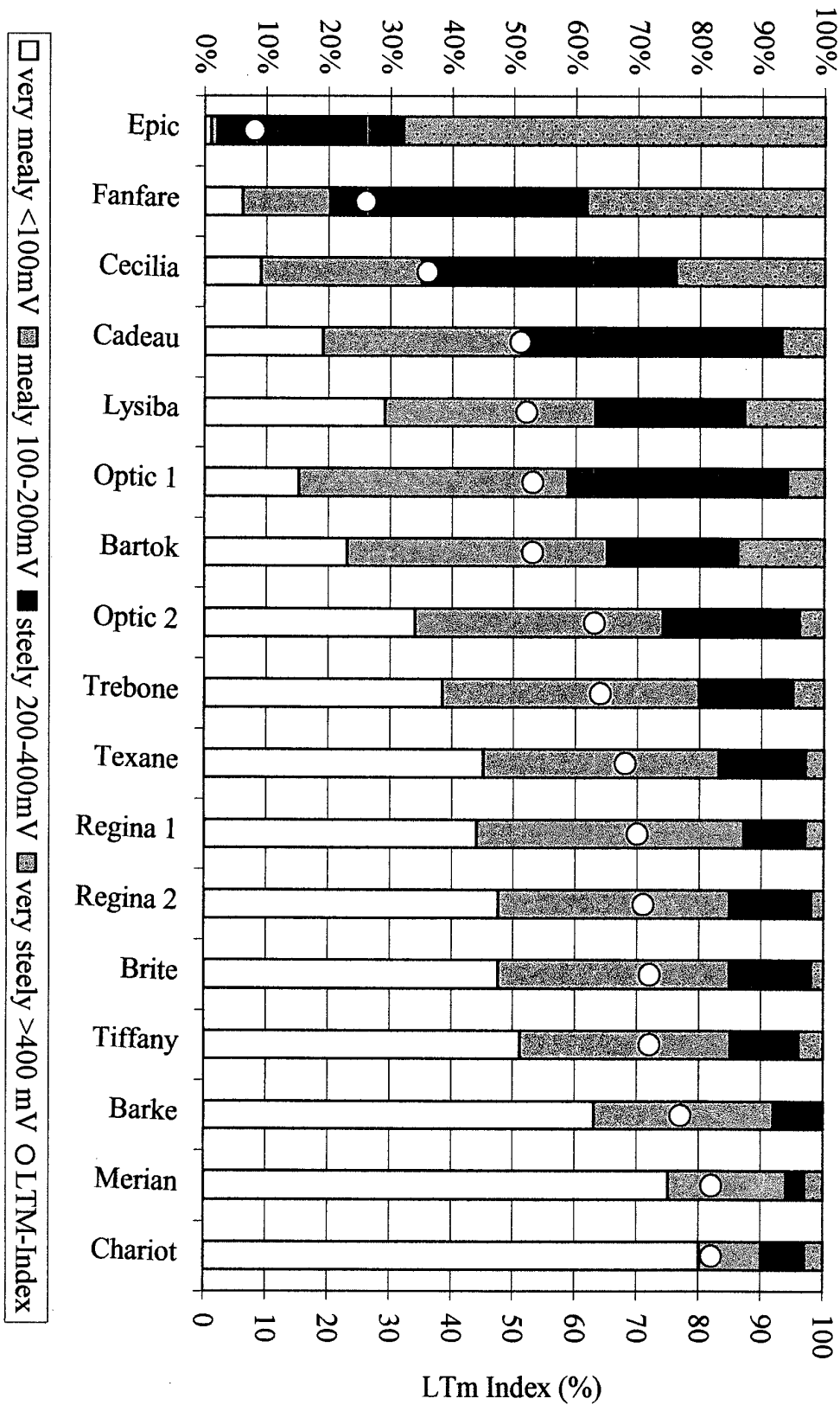


Figure 26 : LTm measurement of different barley varieties

Figure 27 : Relationship between LTm Index and Total Nitrogen of barley varieties

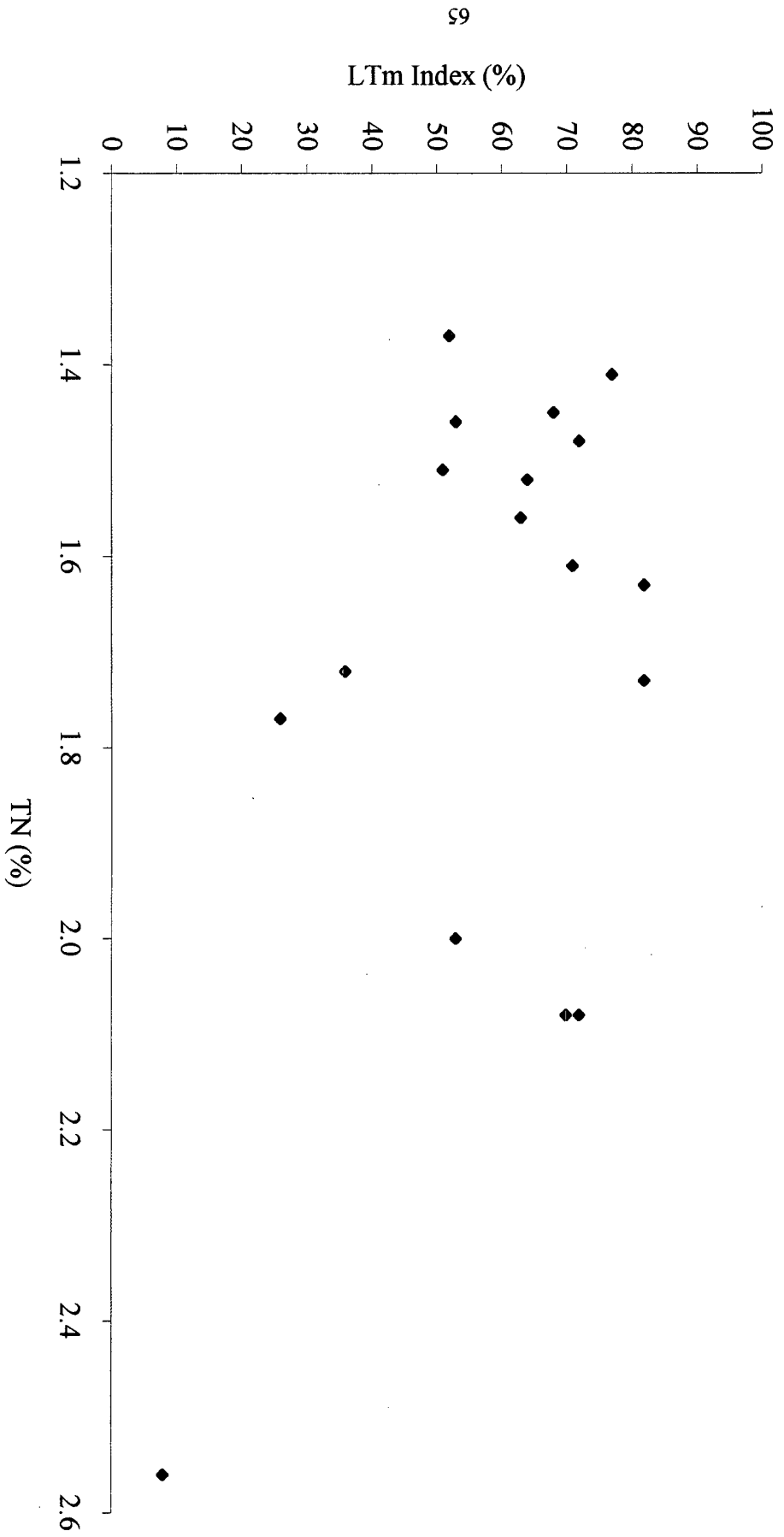




Figure 28 : Distribution of LTM groups of barley varieties from Haughley site

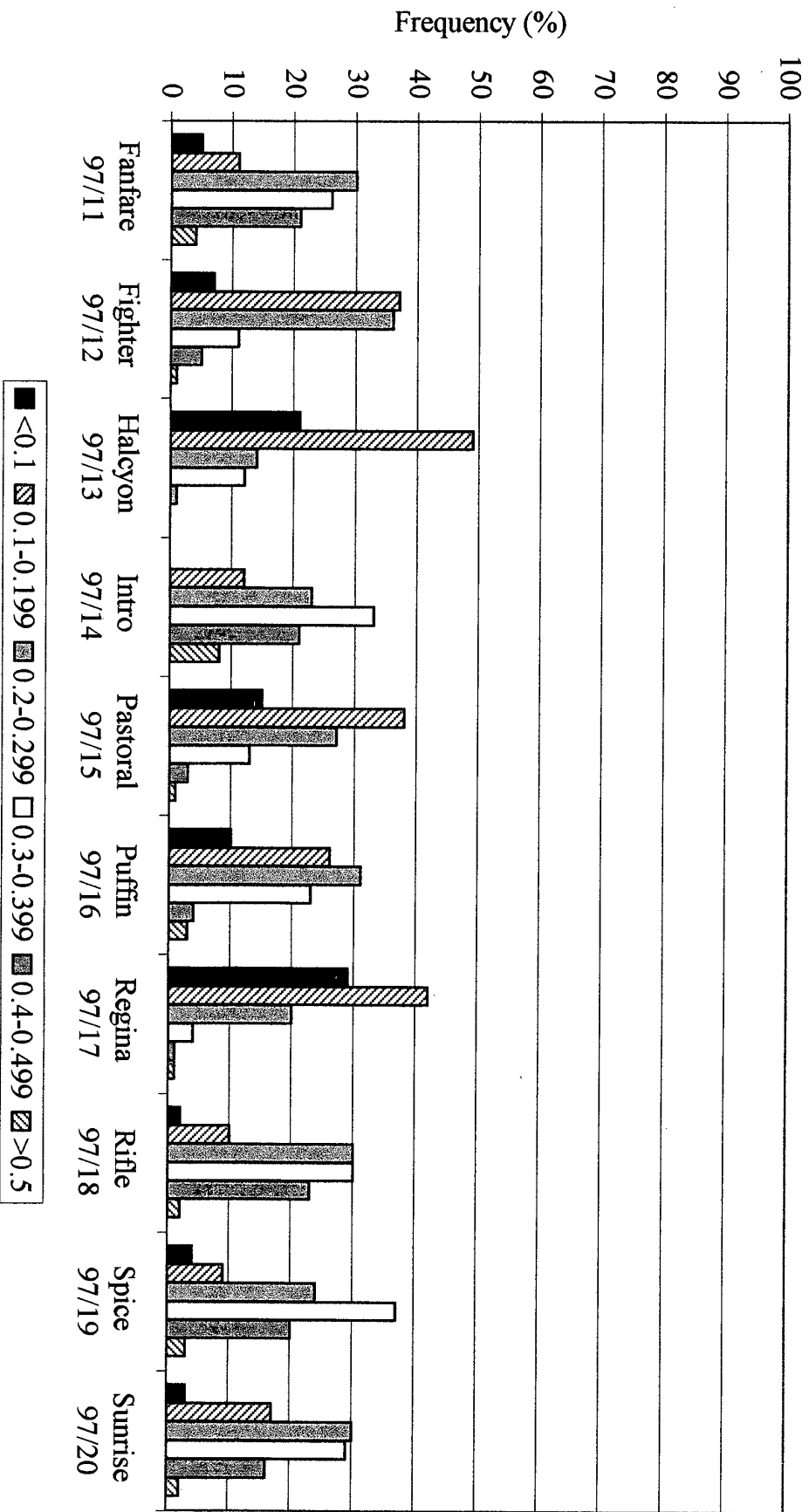


Figure 29 : Distribution of L<sub>Tm</sub> groups of barley varieties from Navenby site

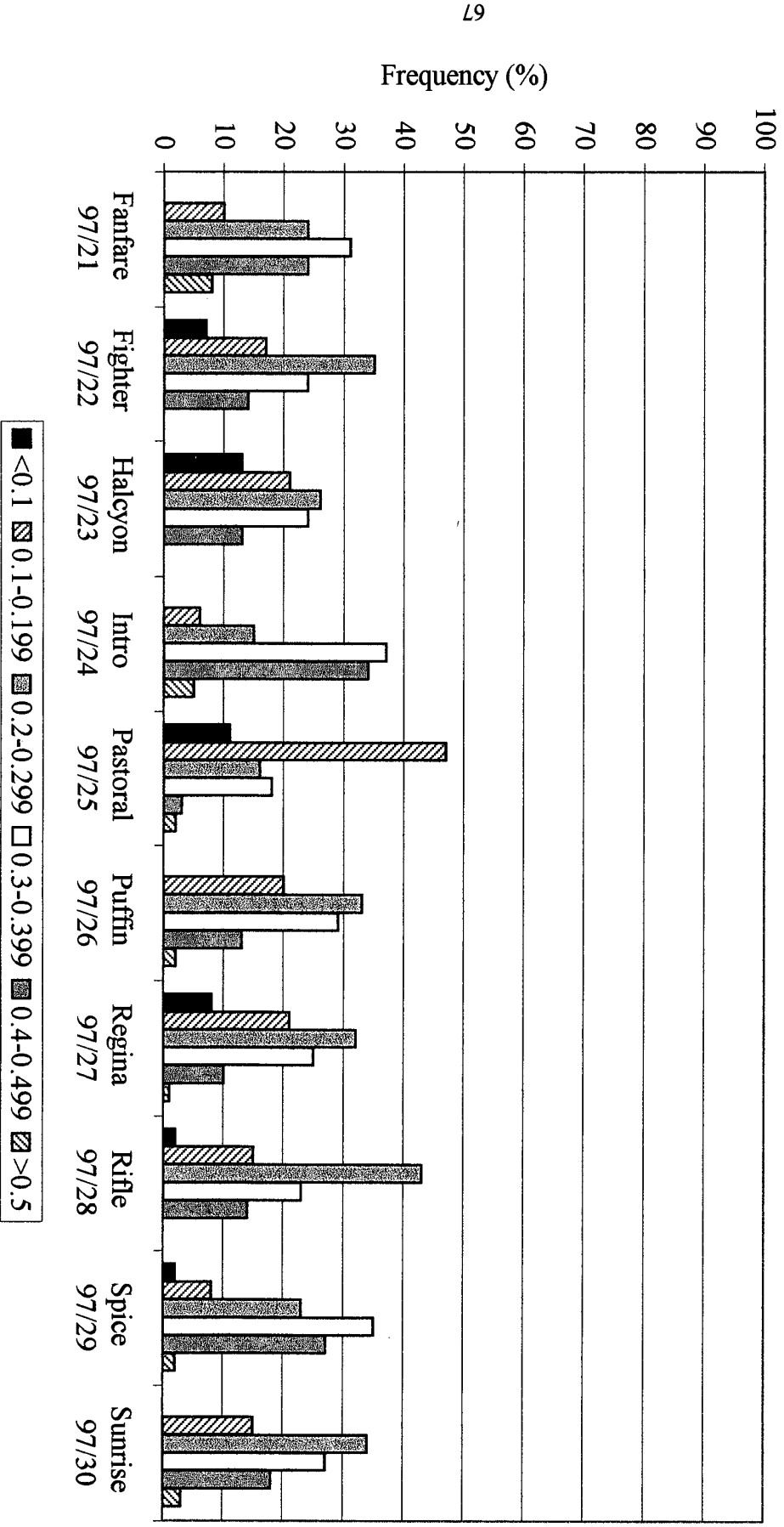


Figure 30 : Distribution of L<sub>Tm</sub> groups of barley varieties from Rothwell site

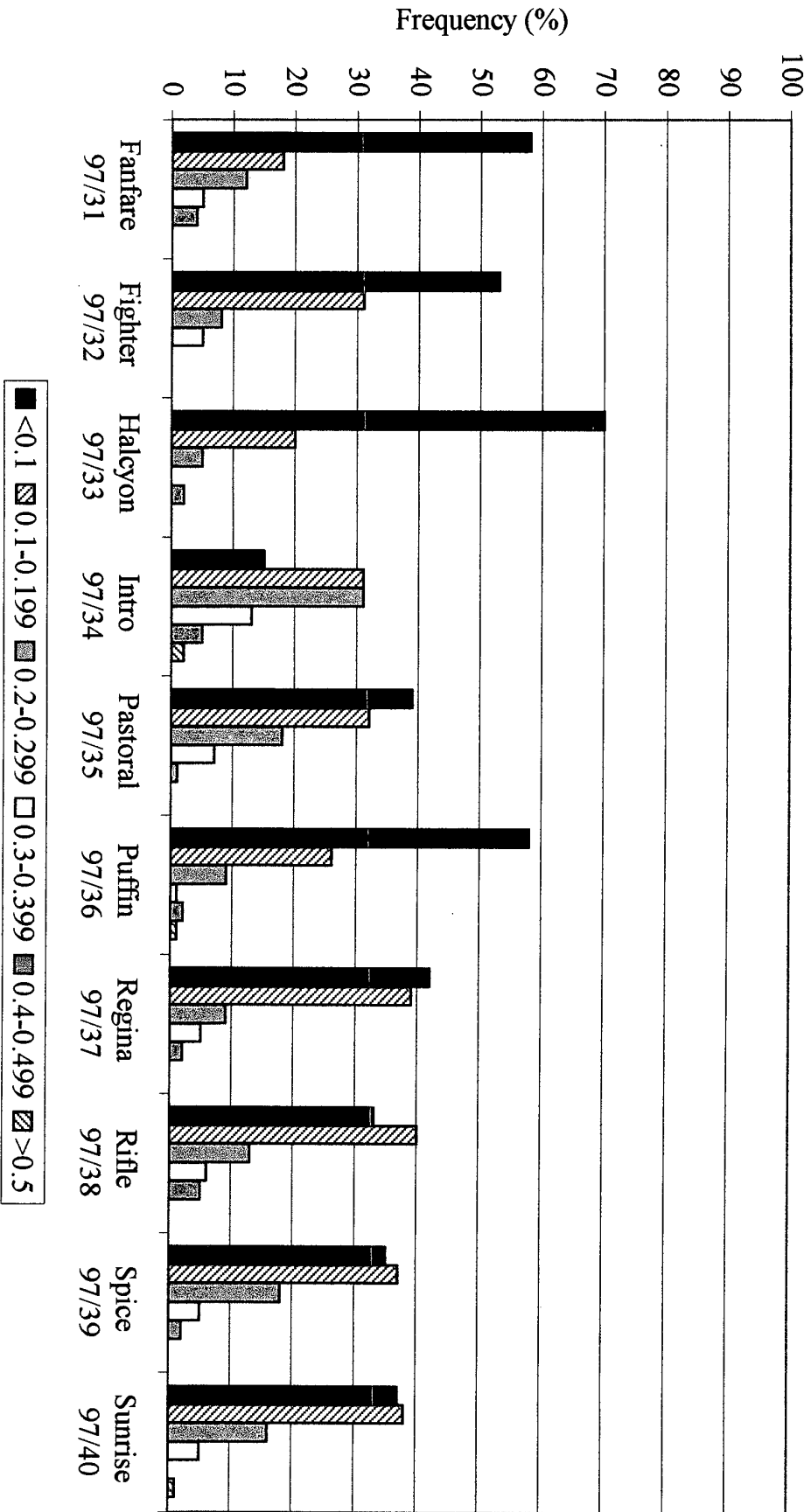


Figure 31 : Distribution of LTM groups of barley varieties from Woolpit site

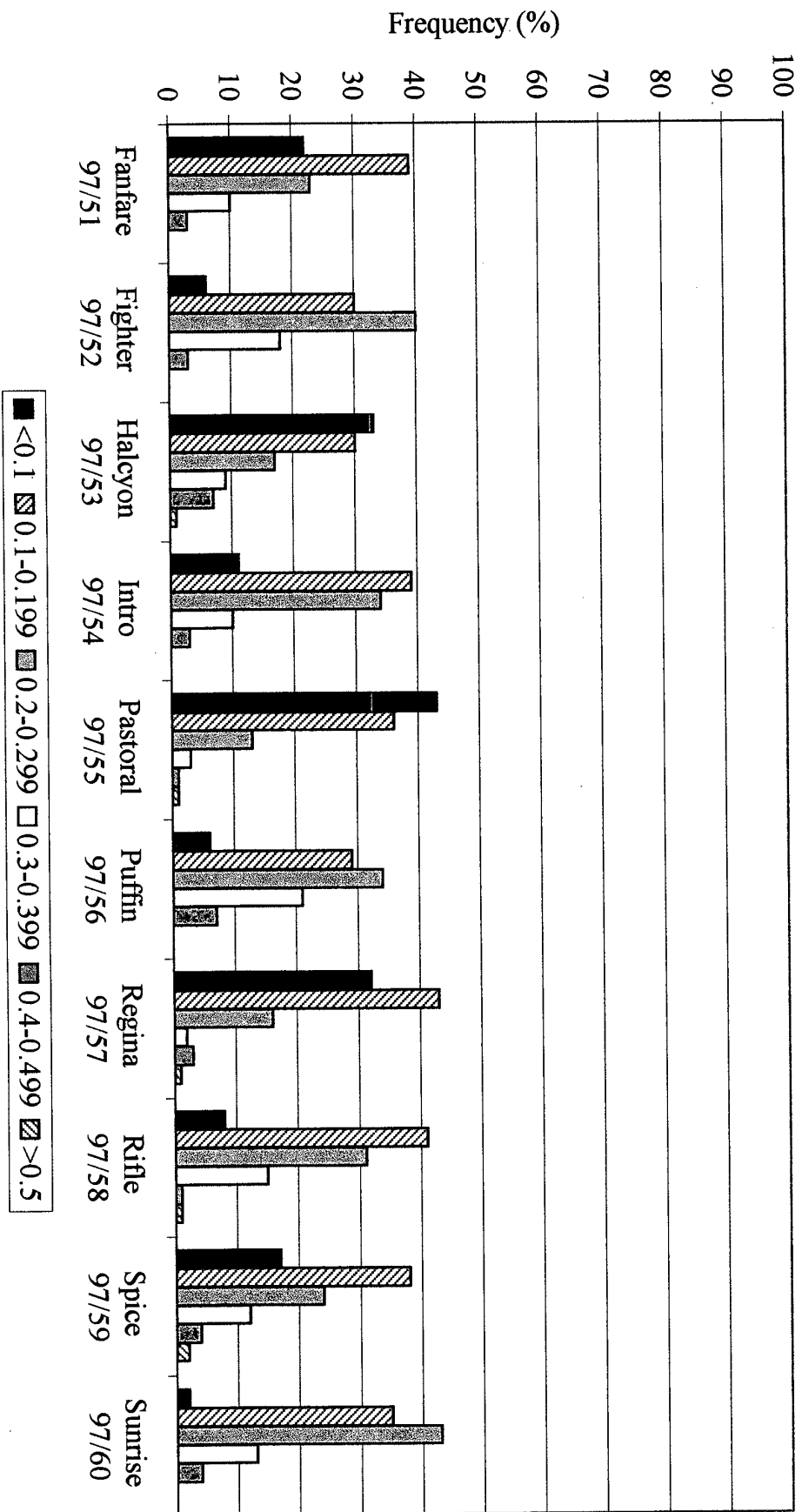


Figure 32 : Distribution of L<sub>Tm</sub> groups of barley varieties from Wootton site

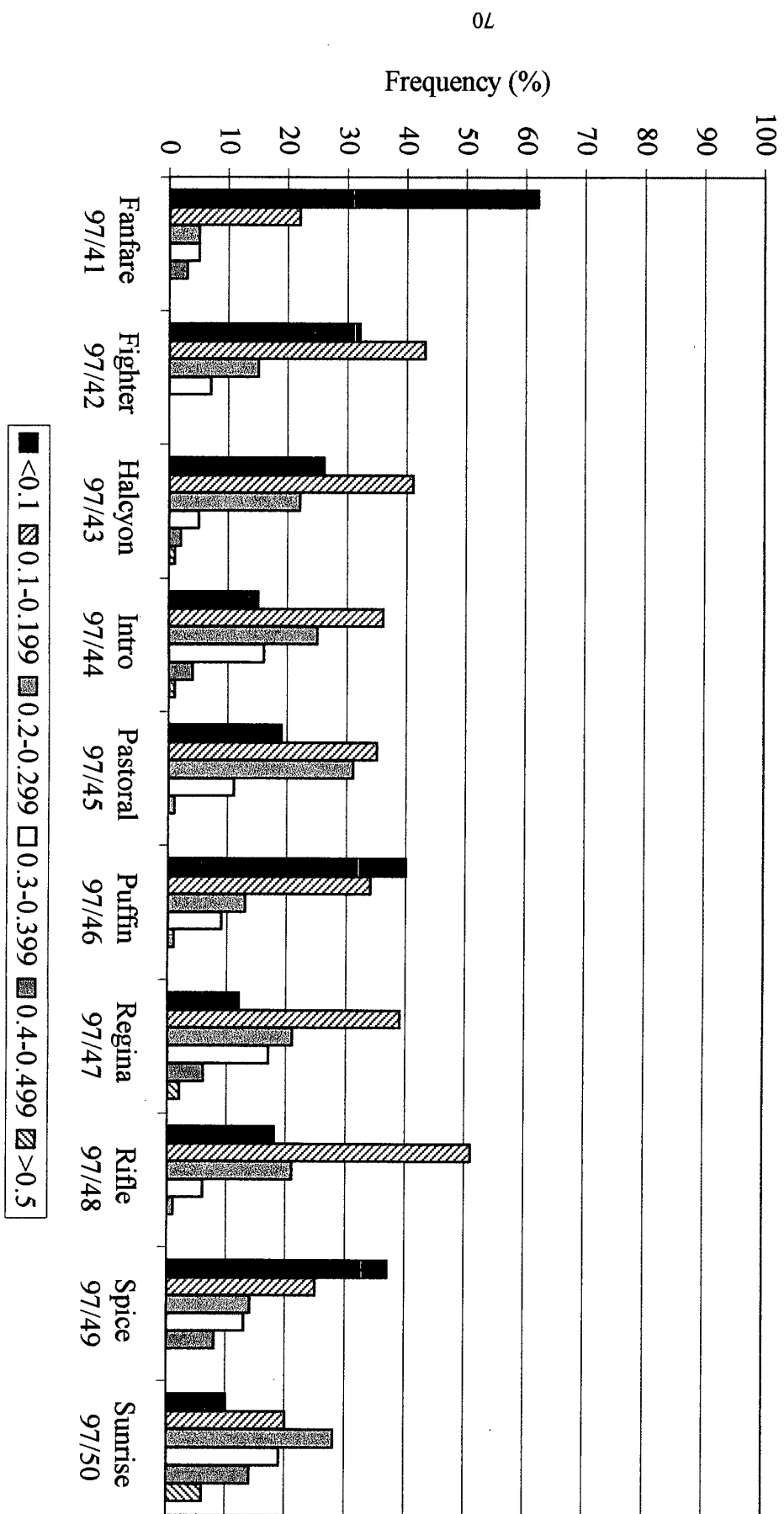


Figure 33 : Relationship between % mealy and Total Nitrogen of barley varieties

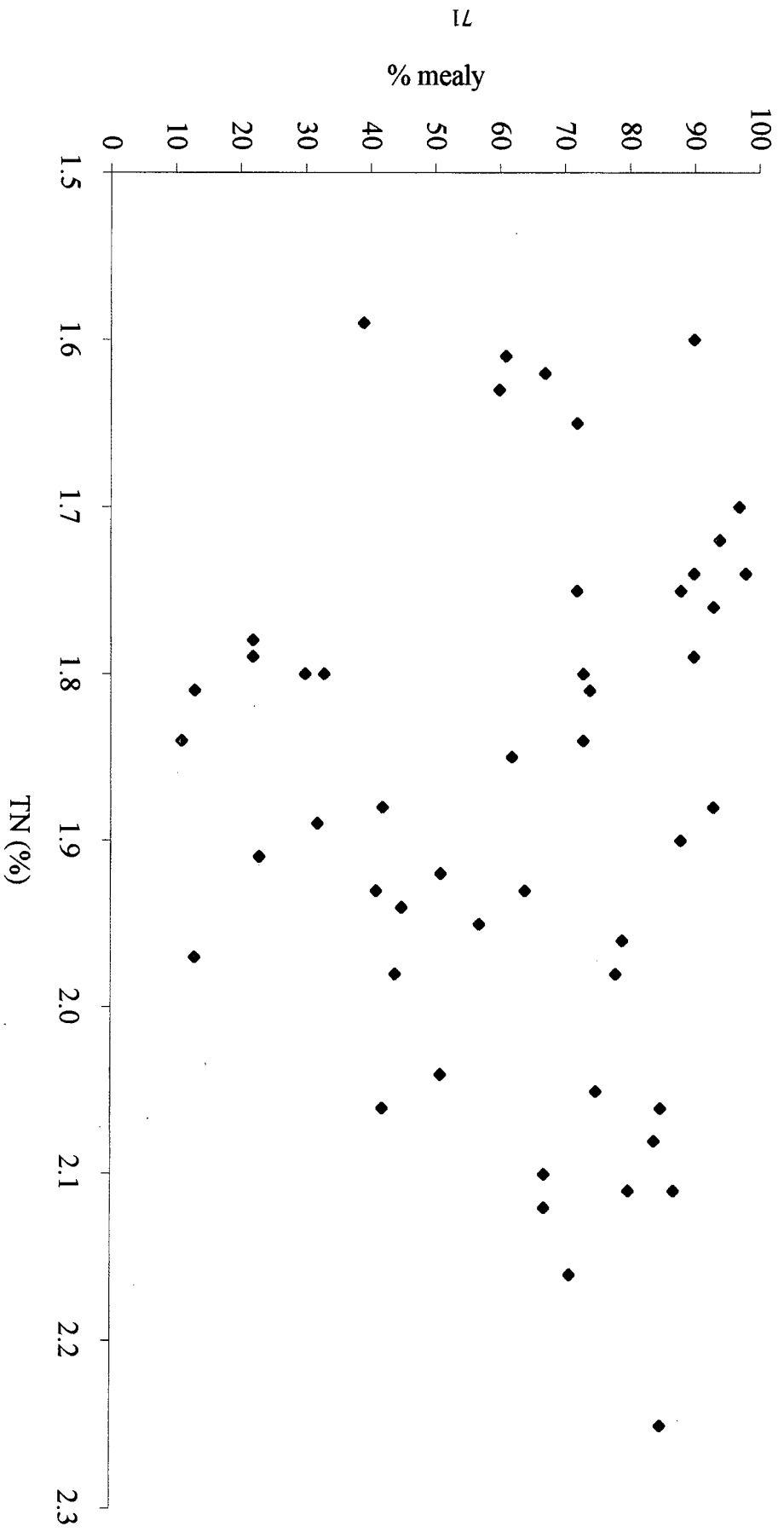


Figure 34 : Relationship between % mealy and Total  $\beta$ -Glucan of barley varieties

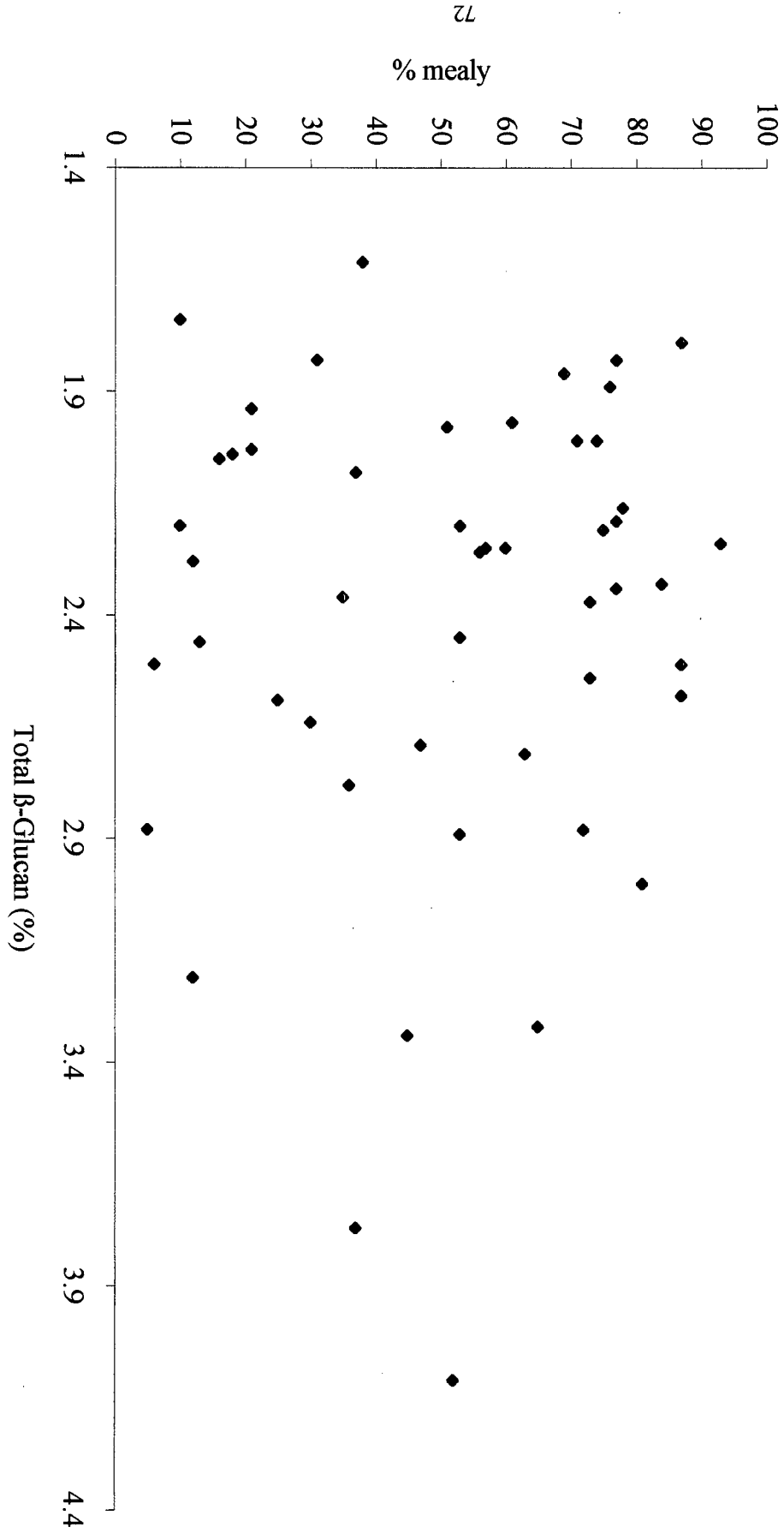


Figure 35 : Effect of mixing mealy and steely barley varieties on the LTm Index

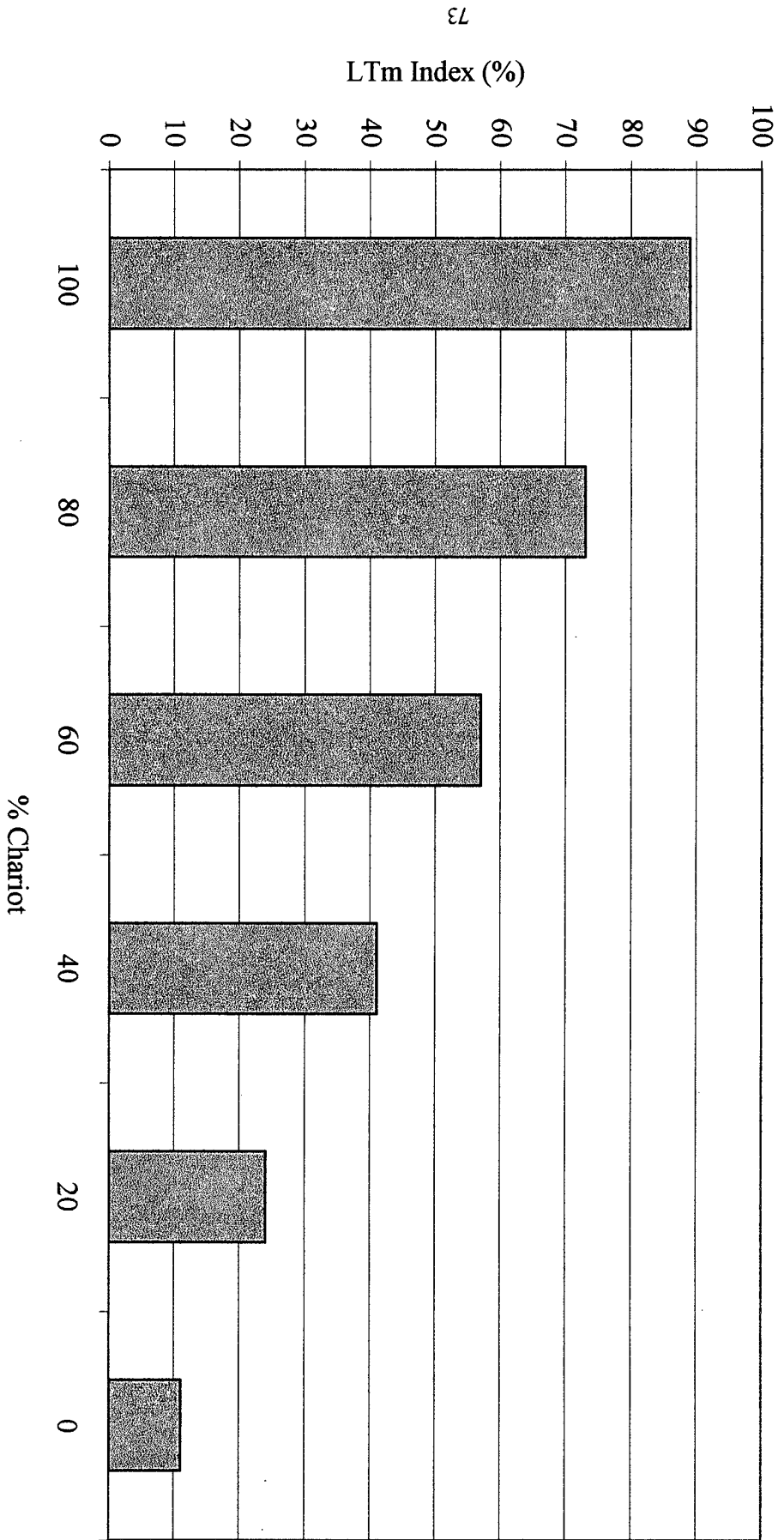




Figure 36 : Comparison of barley LTm values and malt calcofluor on 100% Chariot

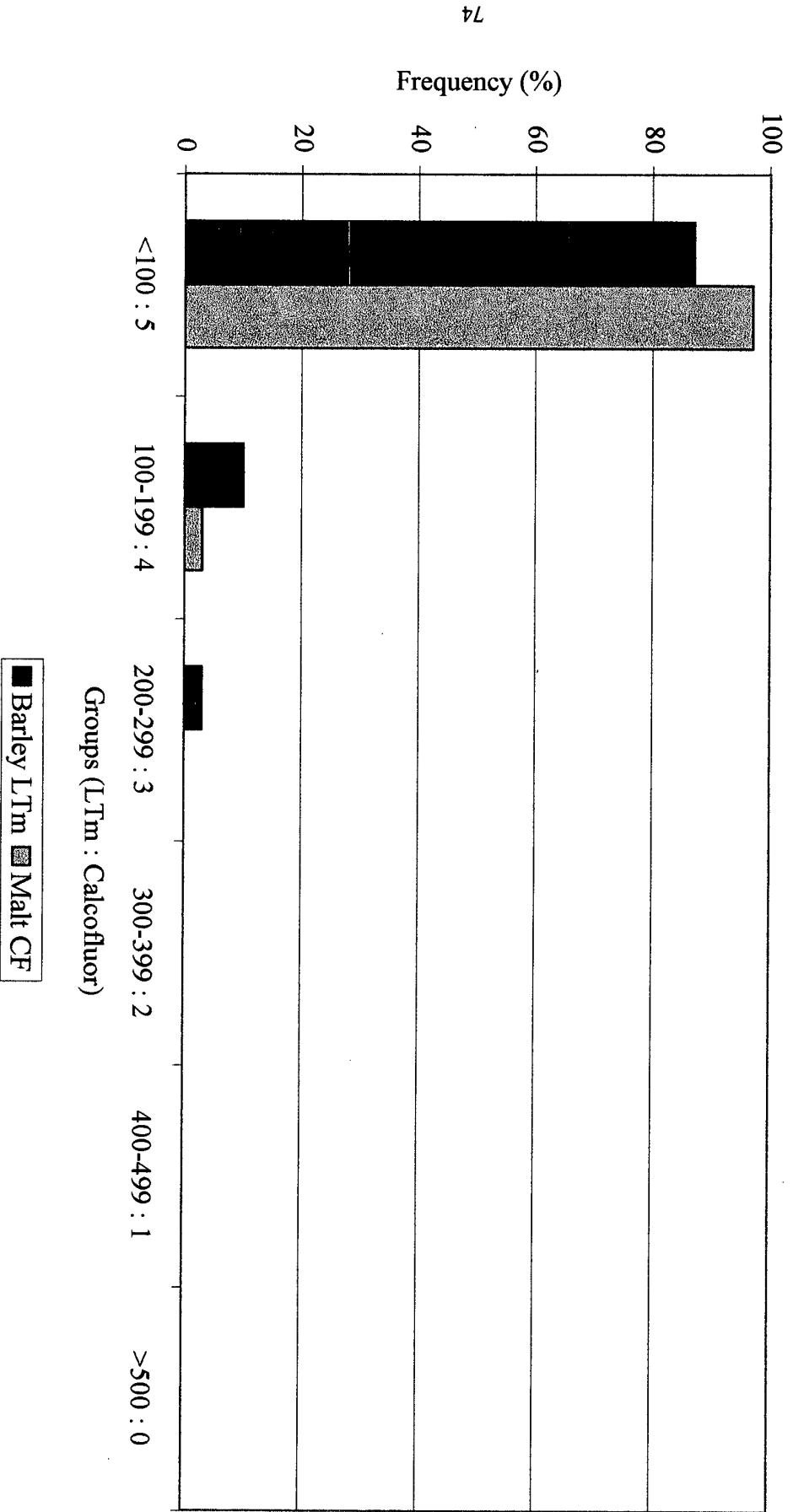


Figure 37 : Comparison of barley L<sub>Tm</sub> values and malt calcofluor on a mixture of 80% Chariot : 20% Epic

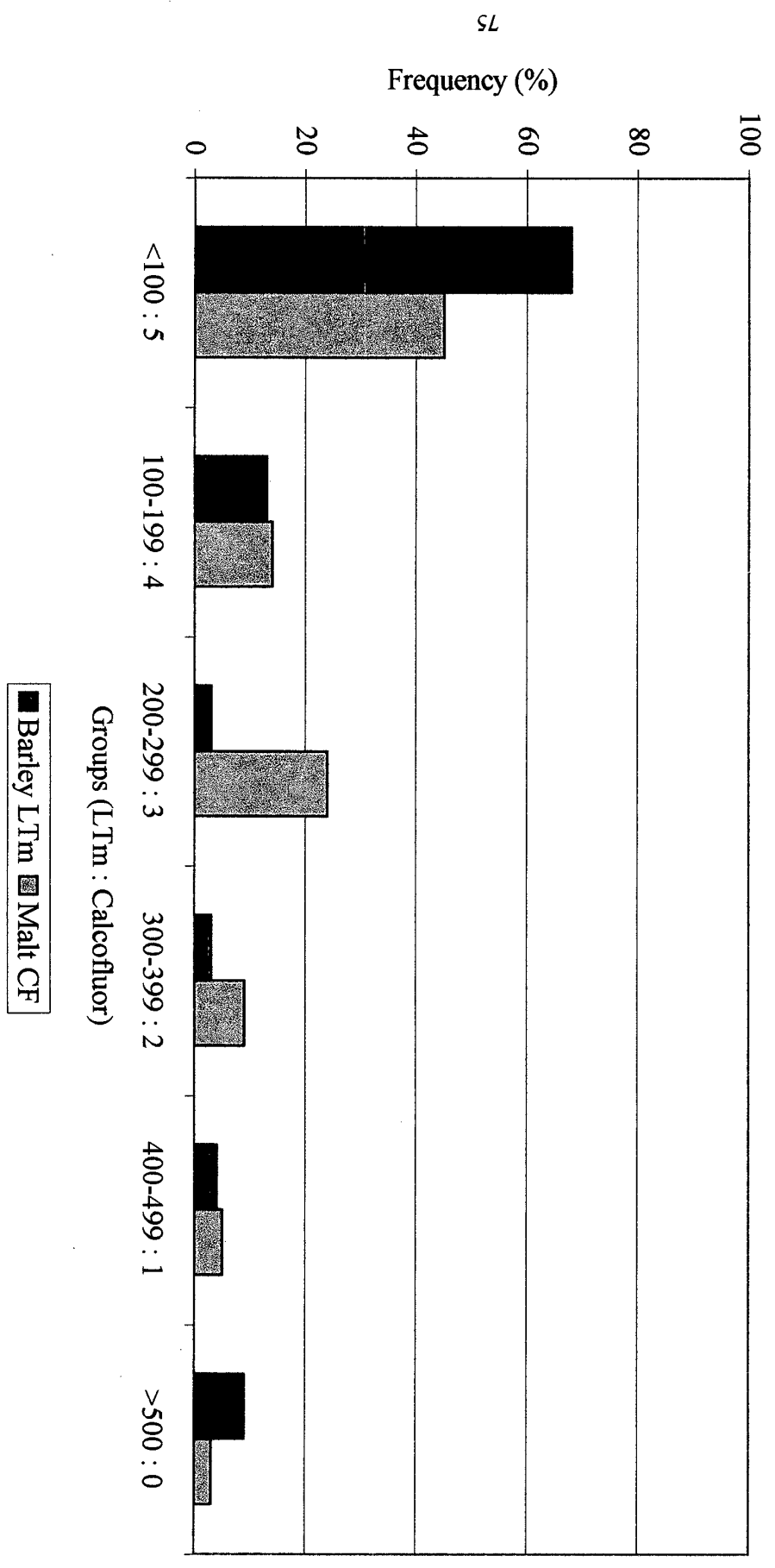


Figure 38 : Comparison of barley LTm values and malt calcofluor on a mixture of 60% Chariot : 40% Epic

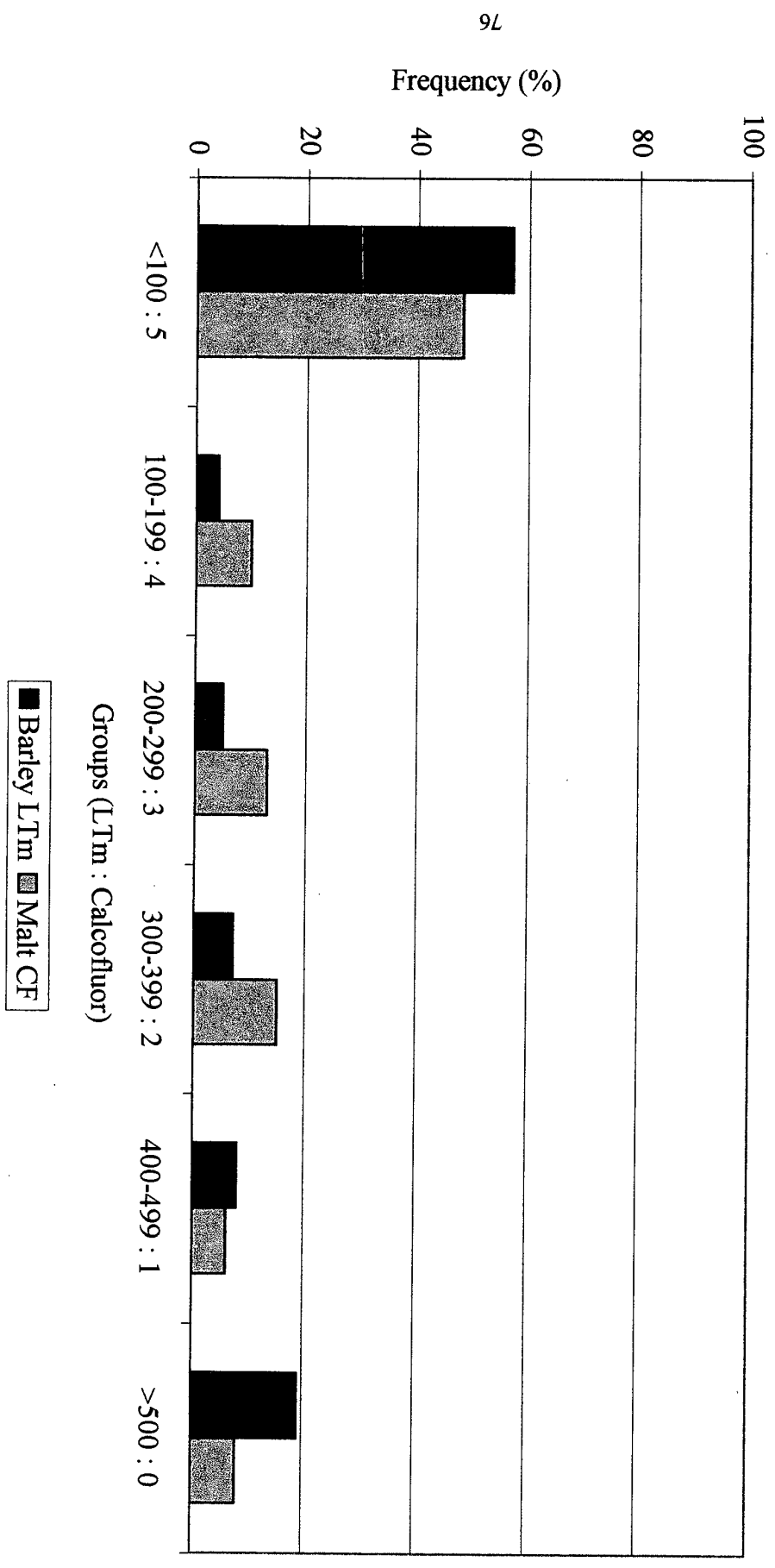


Figure 39 : Comparison of barley L<sub>Tm</sub> values and malt calcofluor on a mixture of 40% Chariot : 60% Epic

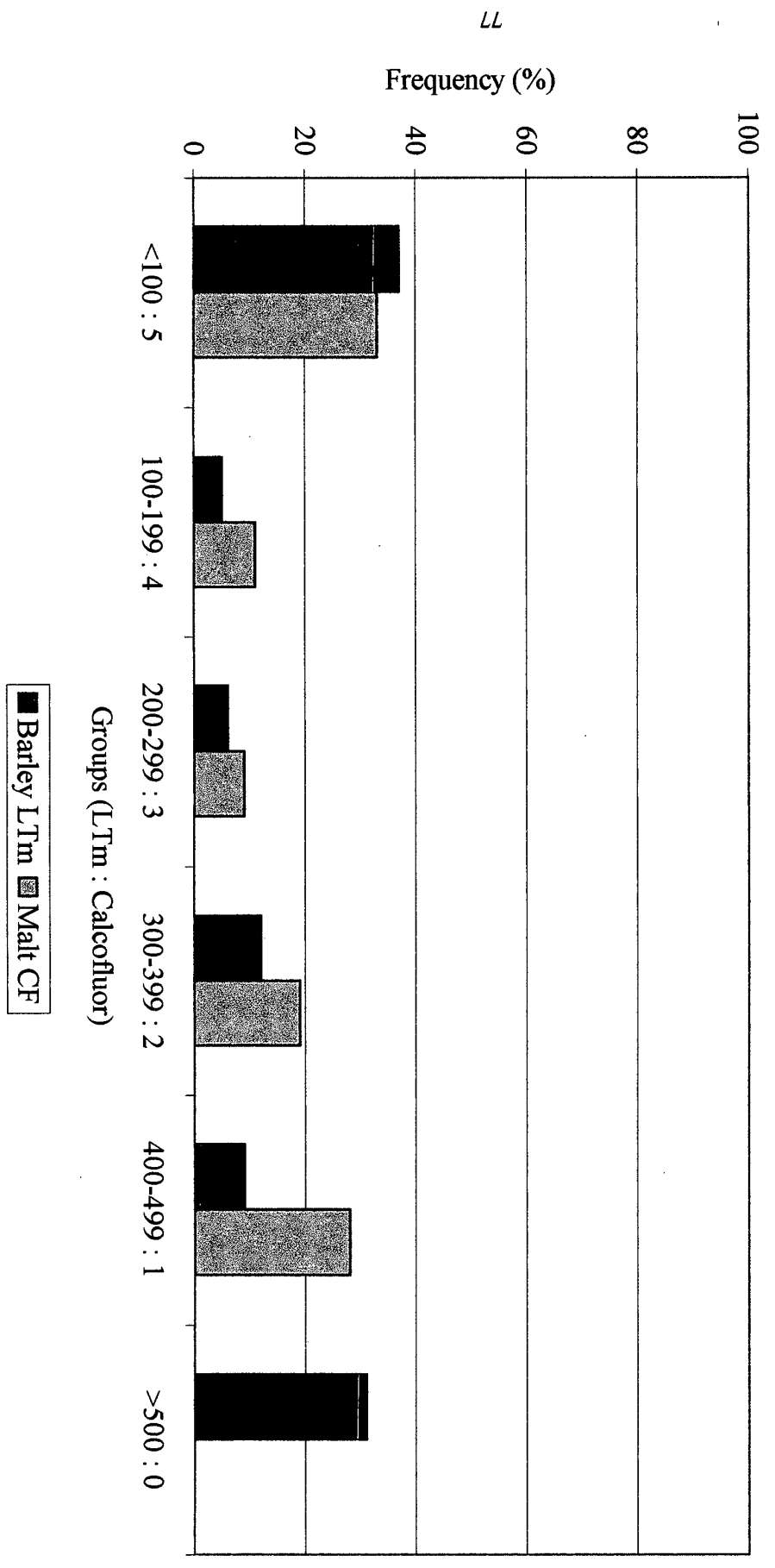


Figure 40 : Comparison of barley LTm values and malt calcofluor on a mixture of 20% Chariot : 80% Epic

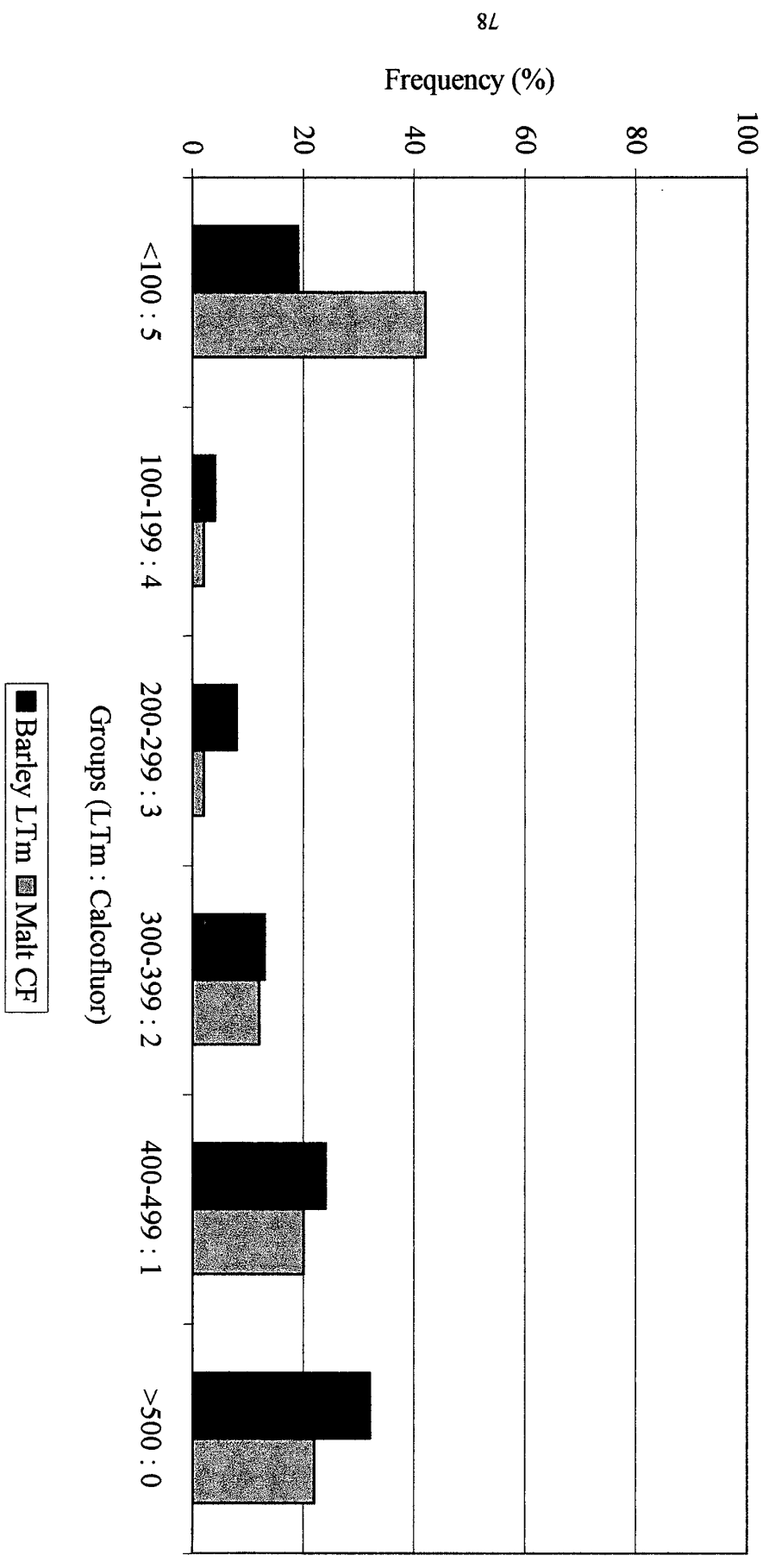
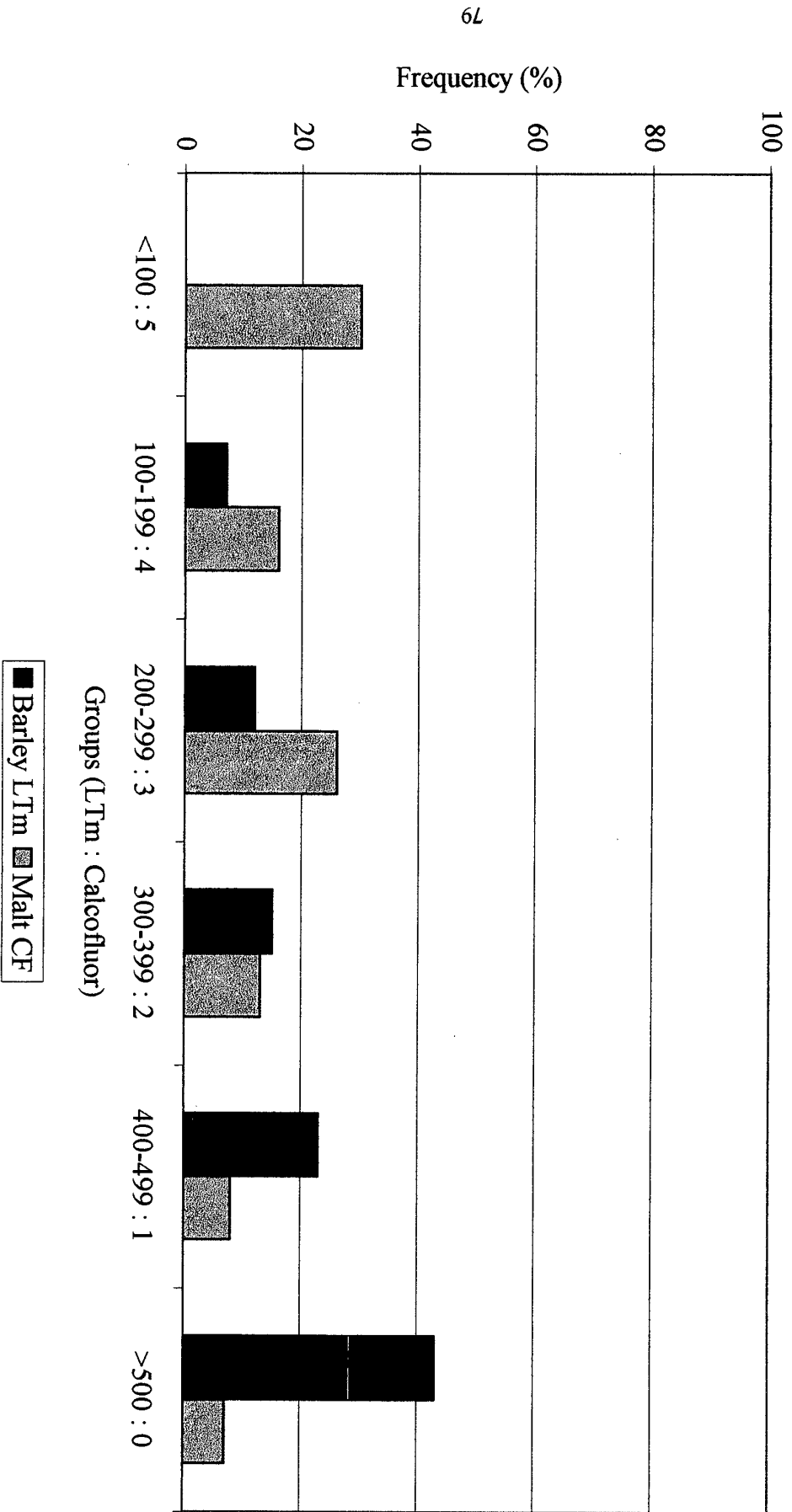


Figure 41 : Comparison of barley L<sub>Tm</sub> values and malt calcofluor on 100% Epic



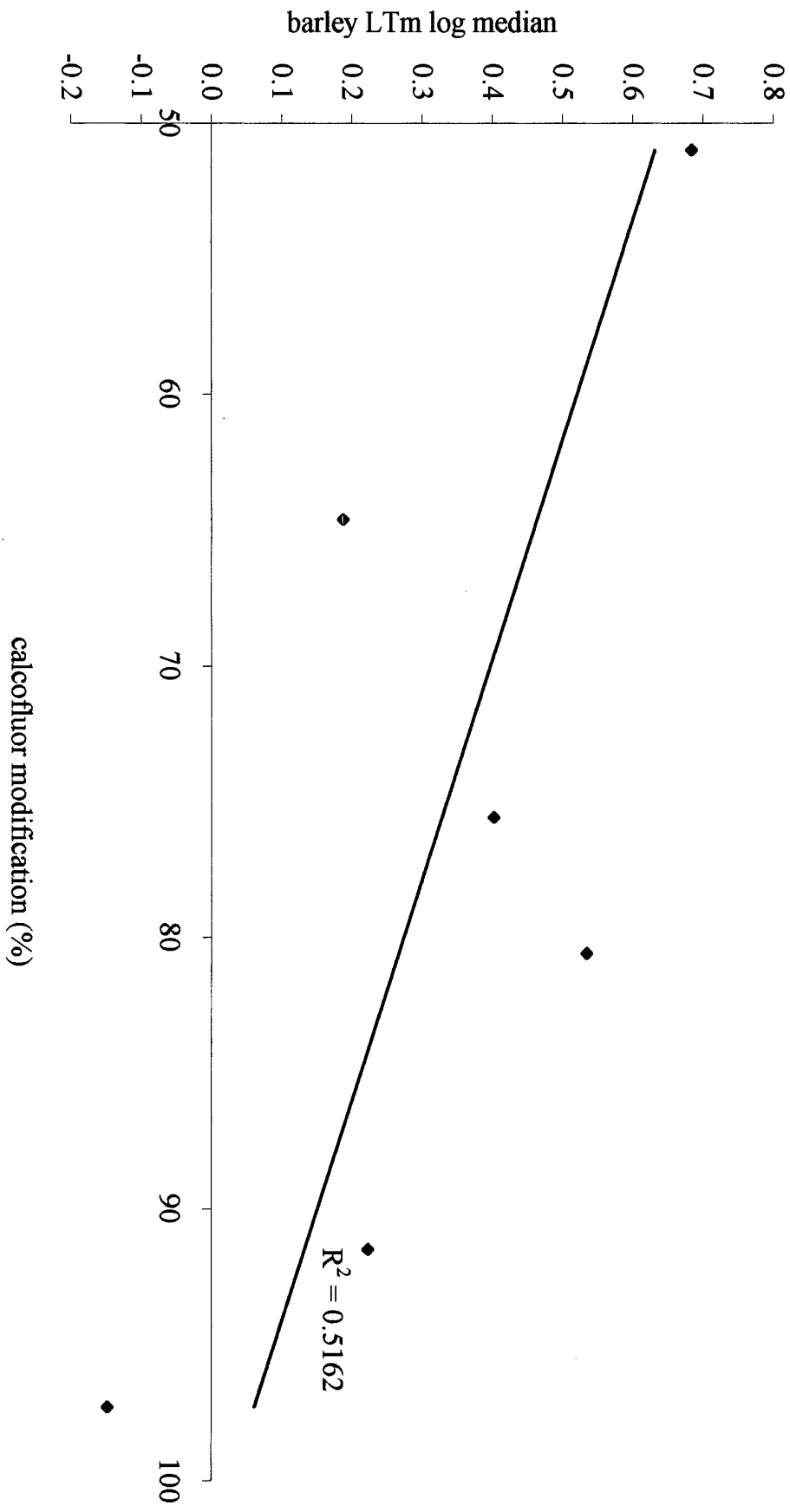


Figure 42 : Relationship between barley LTM and calcofluor modification

Figure 43 : Detection of water uptake (Chariot)

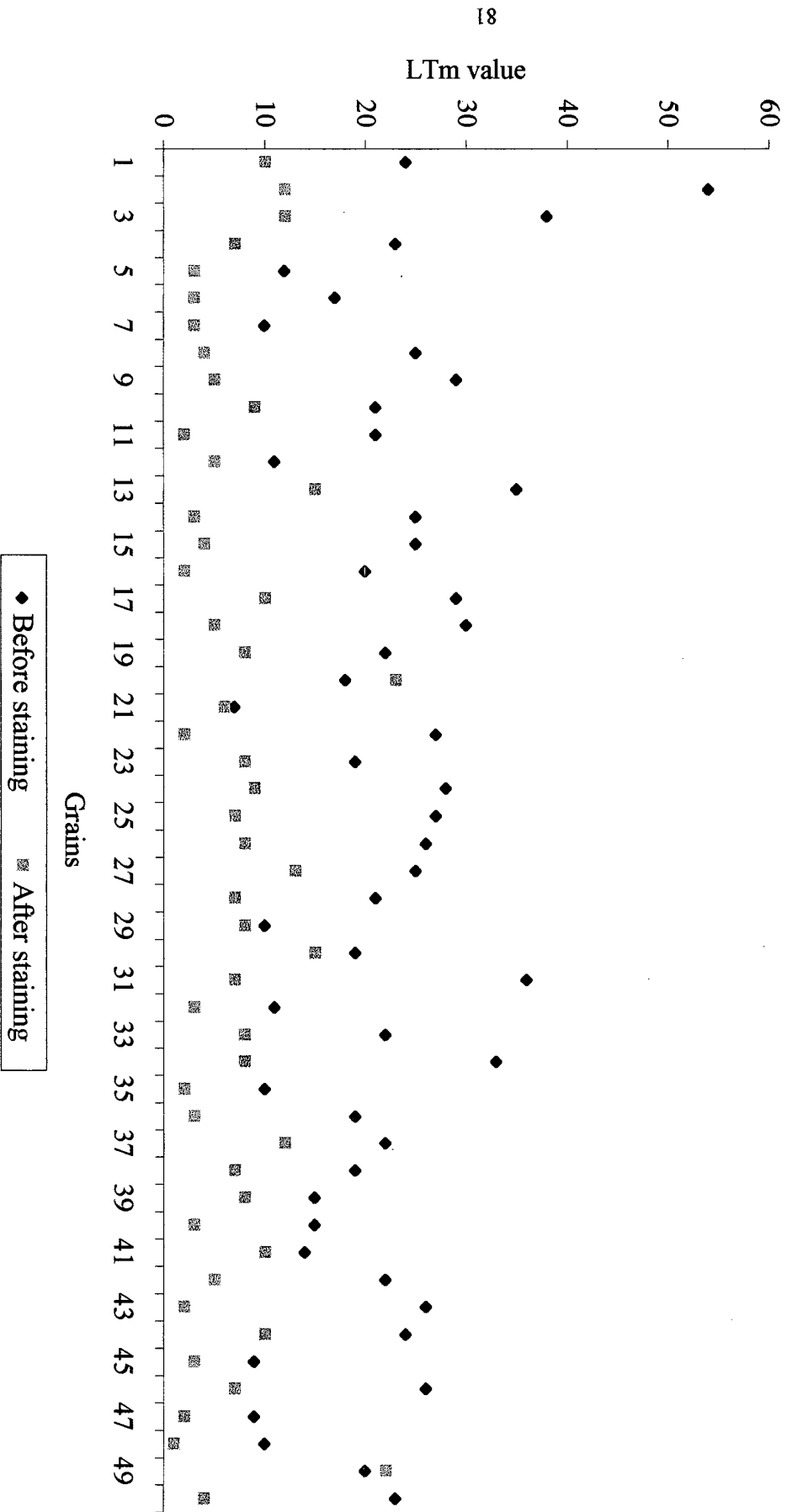




Figure 44 : Detection of water uptake during malting (Chariot)

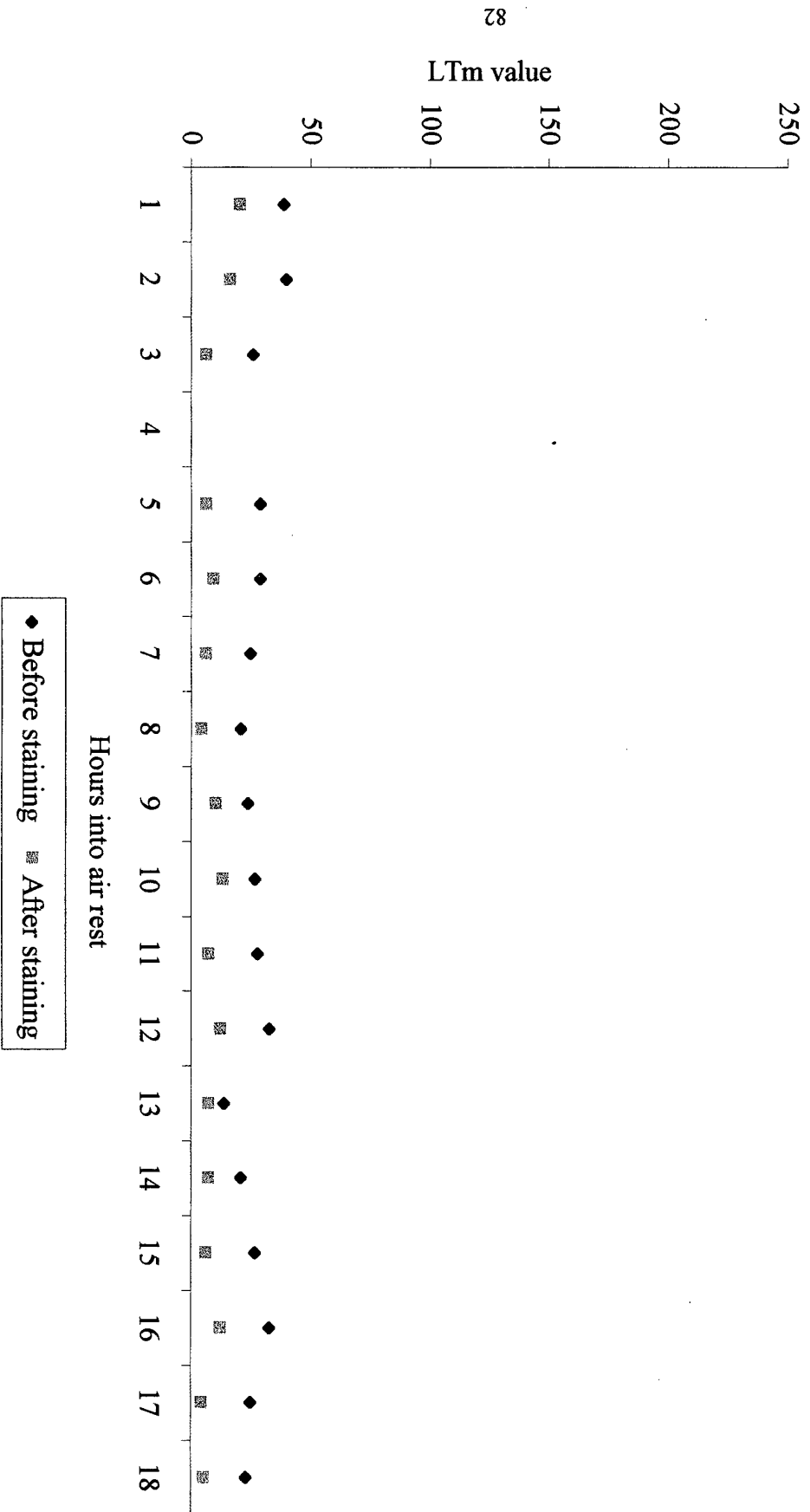
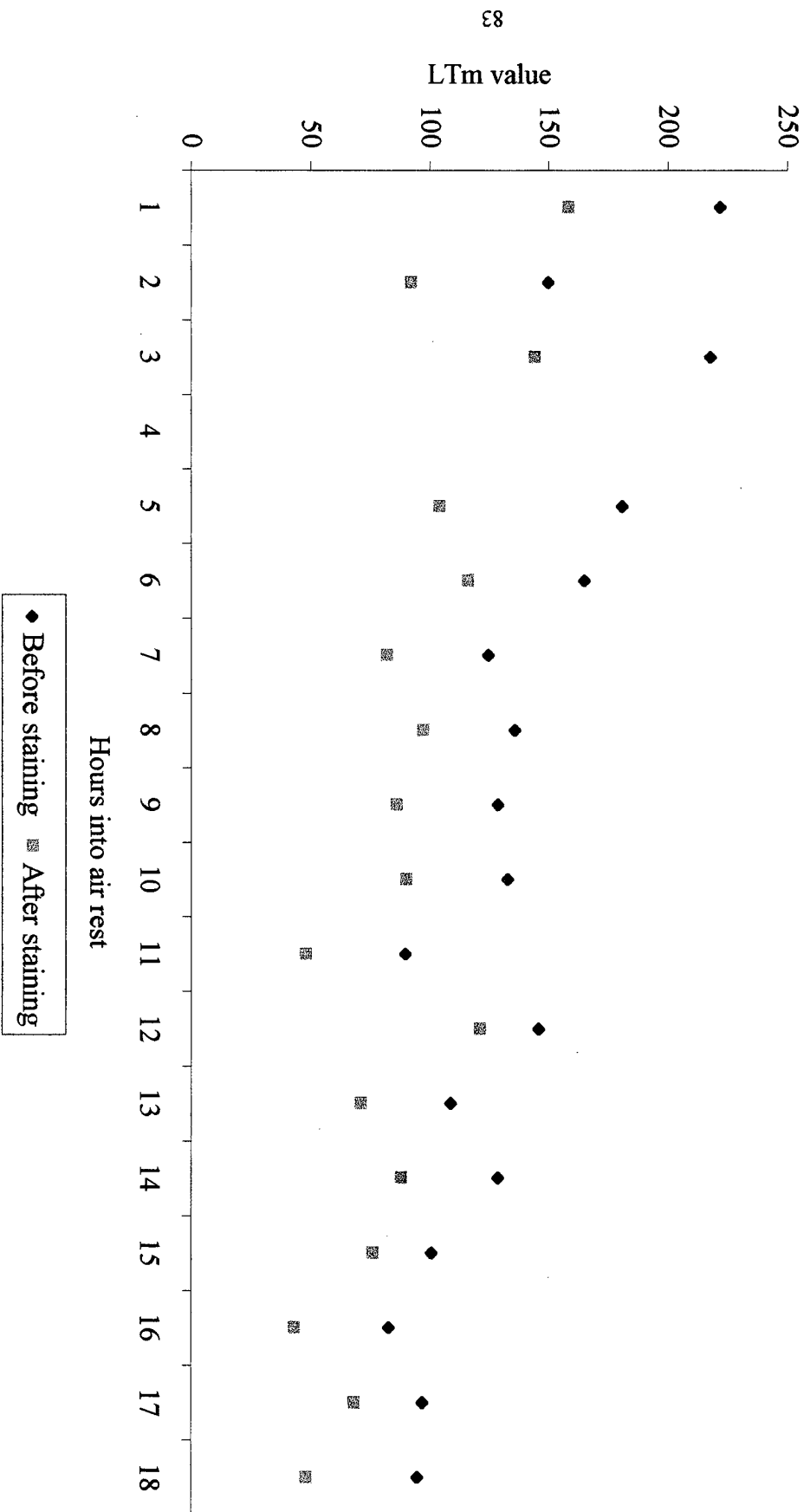
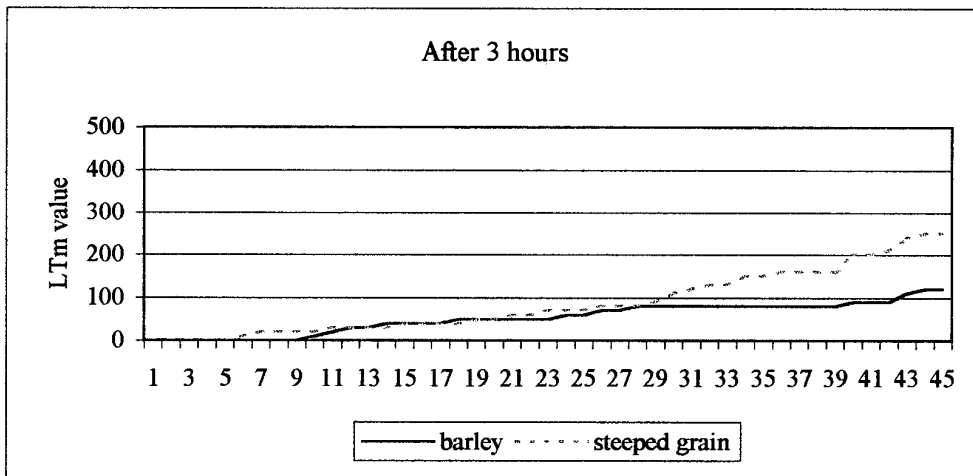
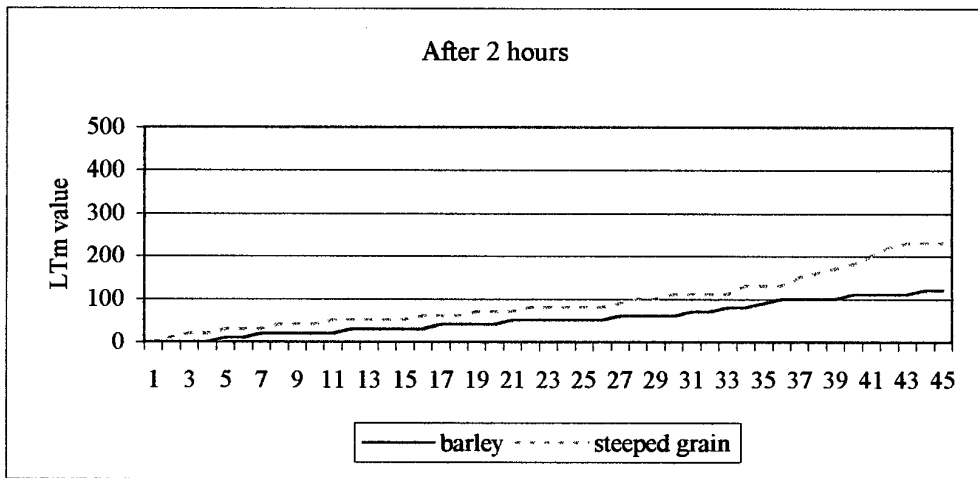
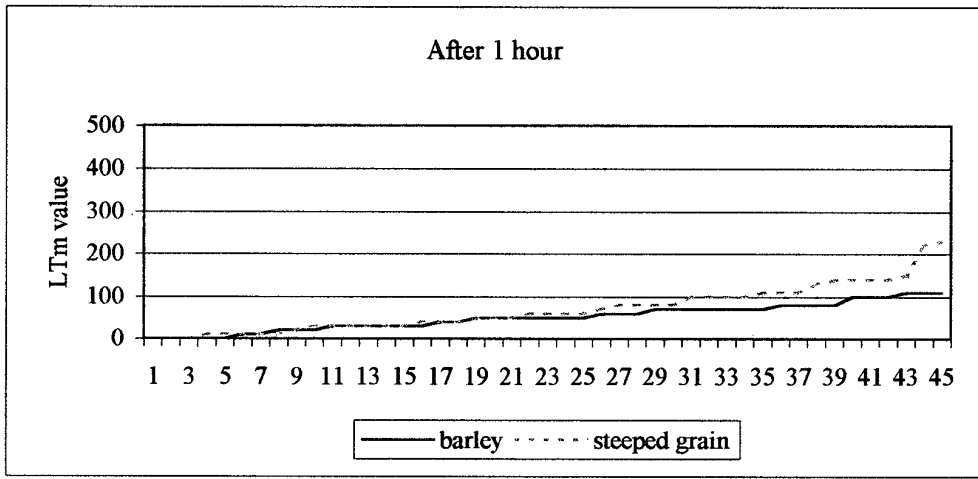


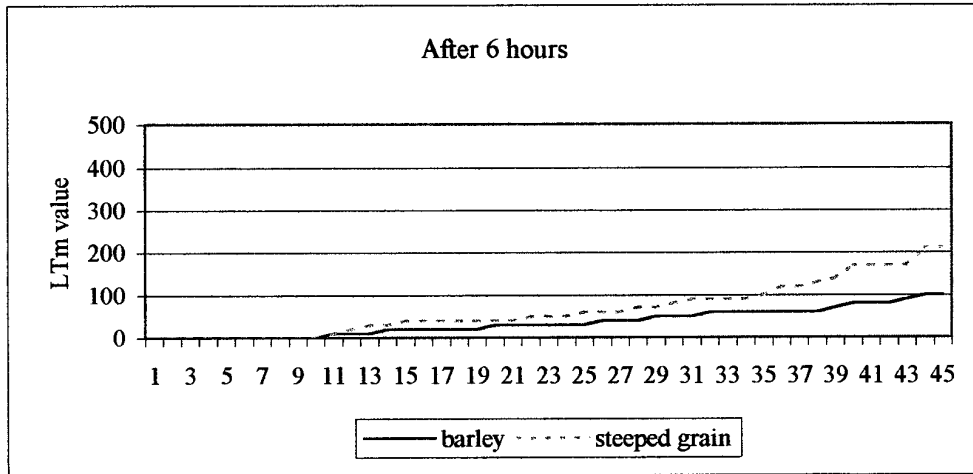
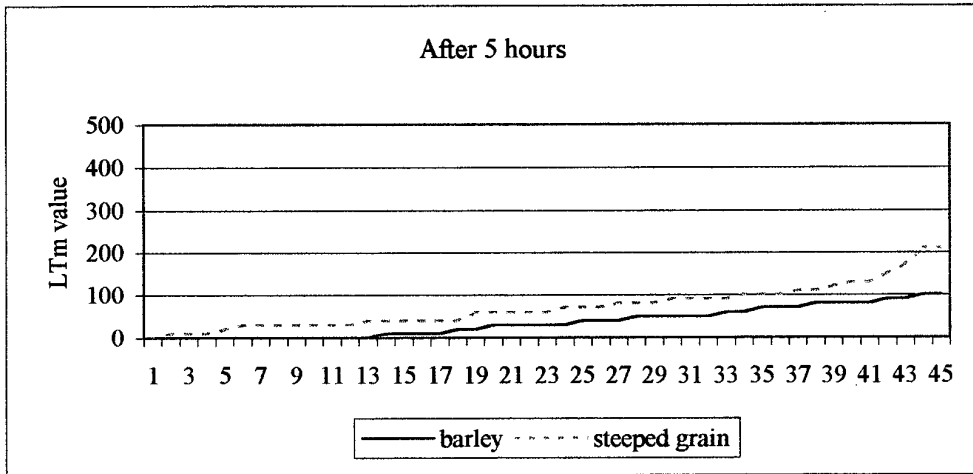
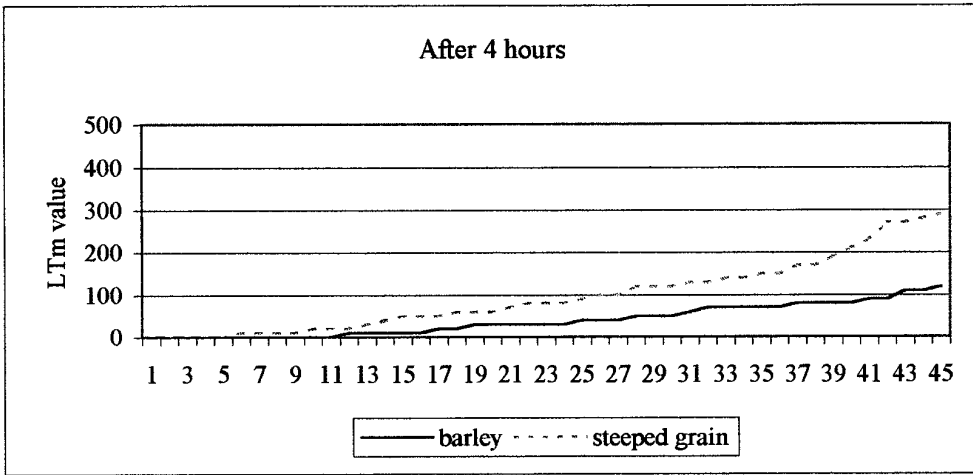
Figure 45 : Detection of water uptake during maling (Epic)



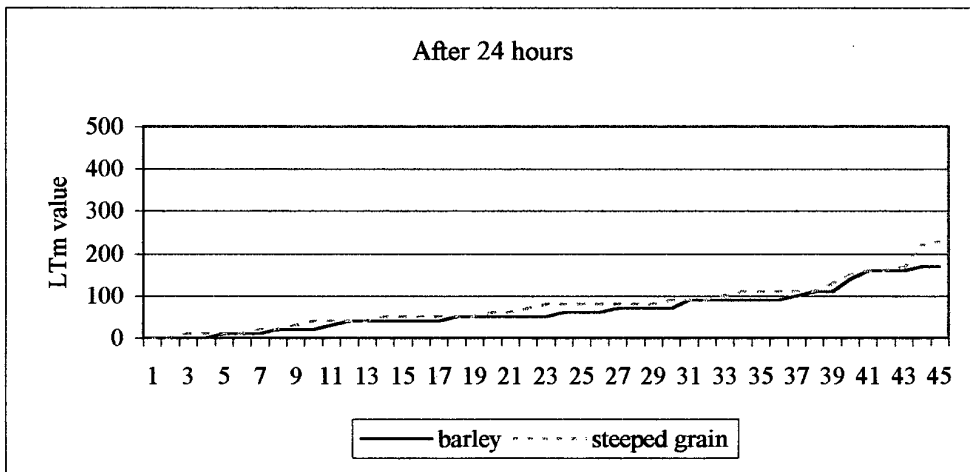
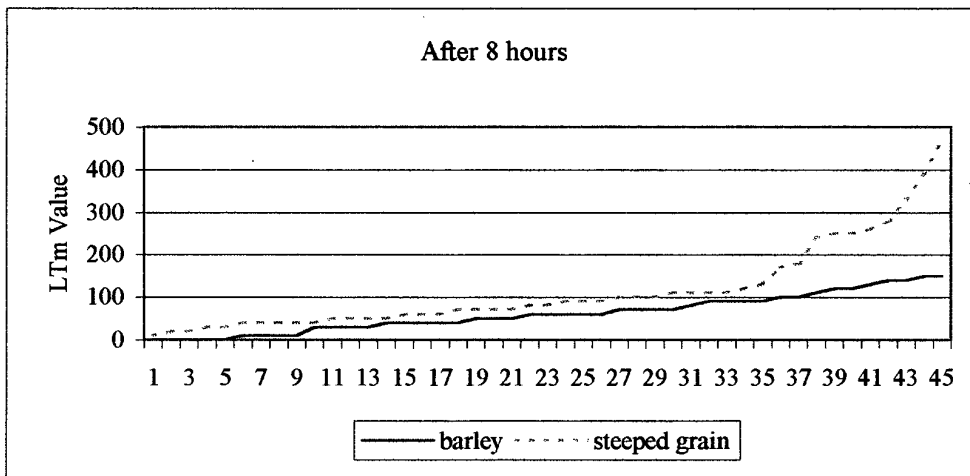
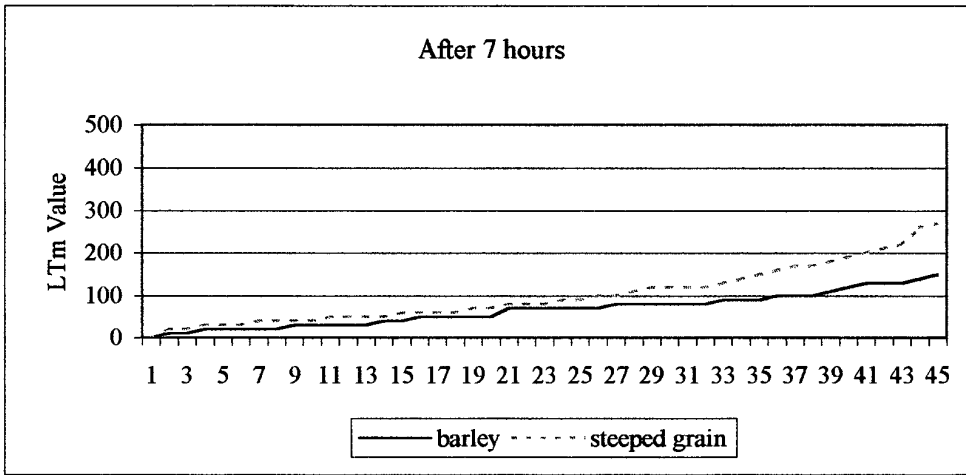
Figures 46-48 : LTm measurement during steeping (Chariot)



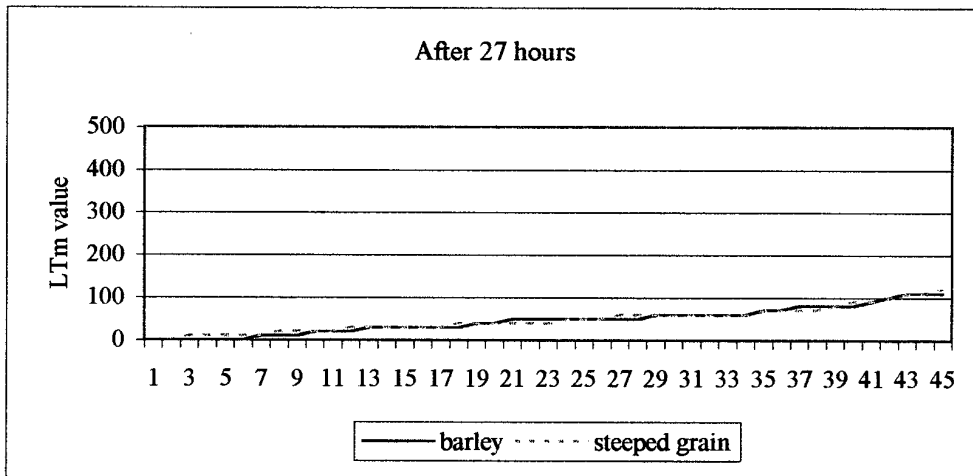
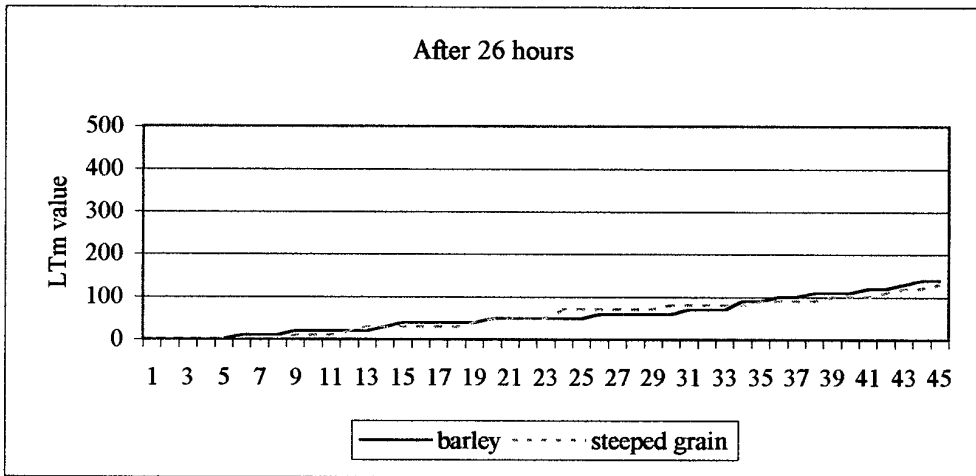
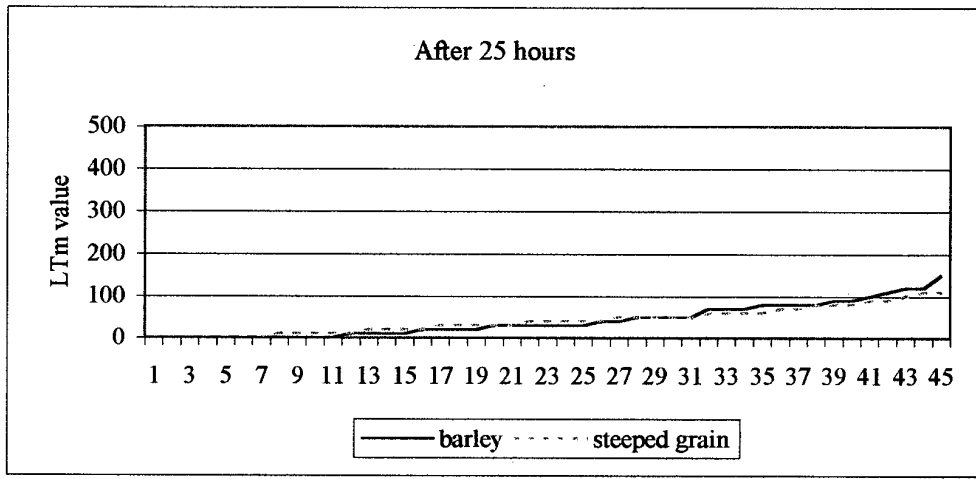
Figures 49-51 : LTm measurement during steeping (Chariot)



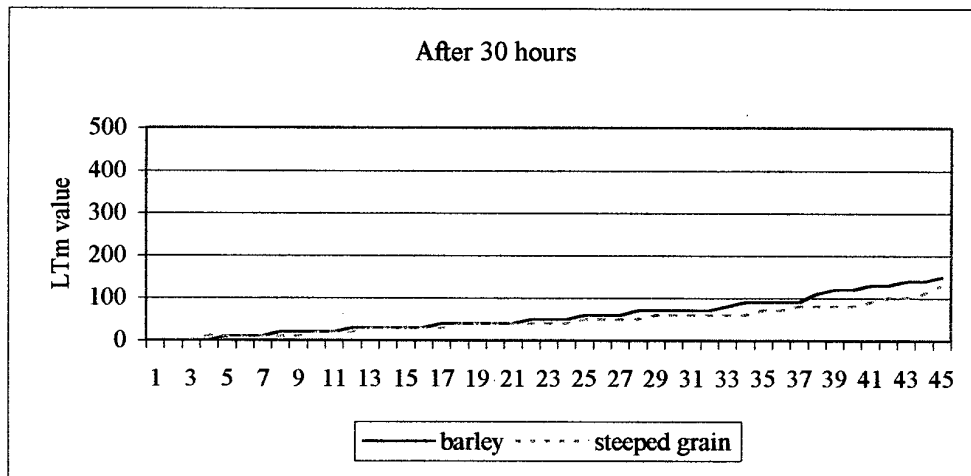
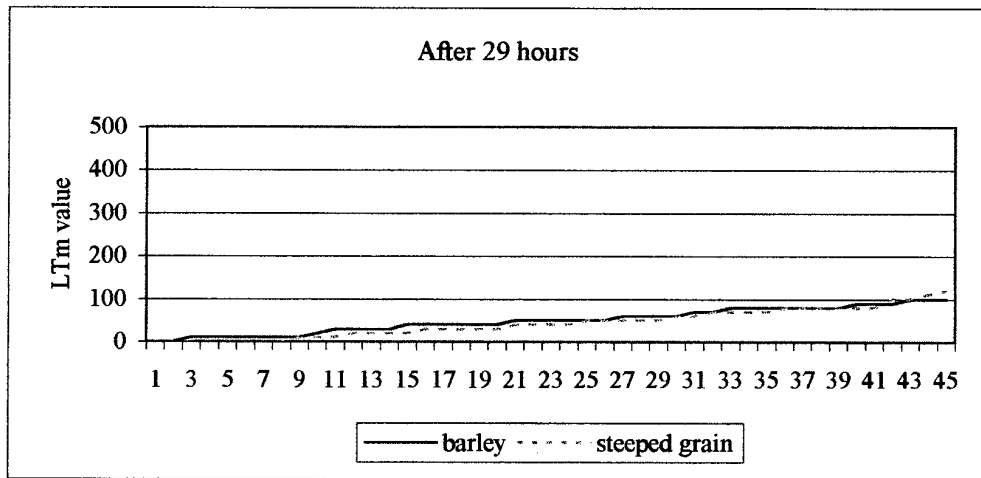
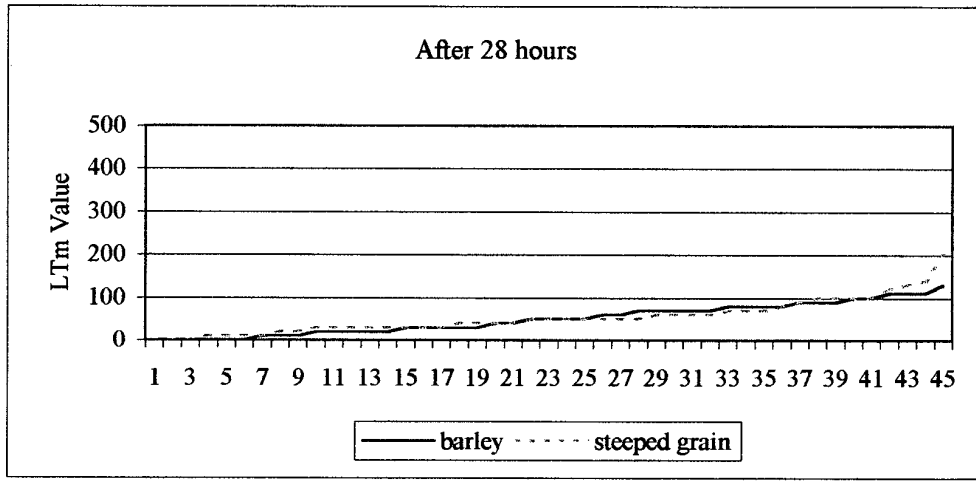
Figures 52-54 : LTm measurement during steeping (Chariot)



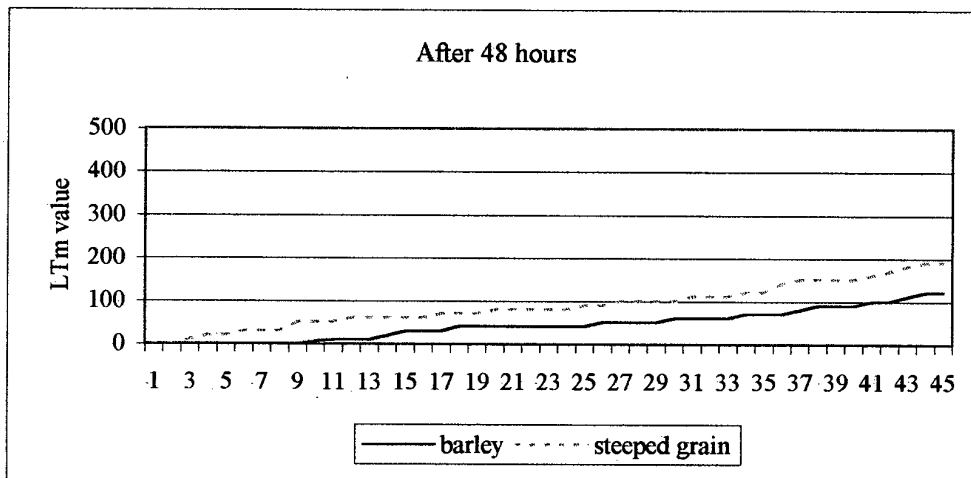
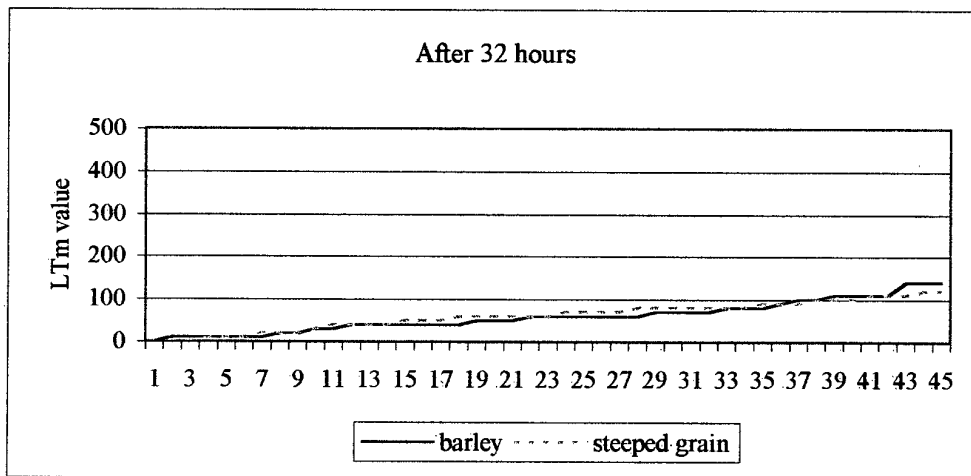
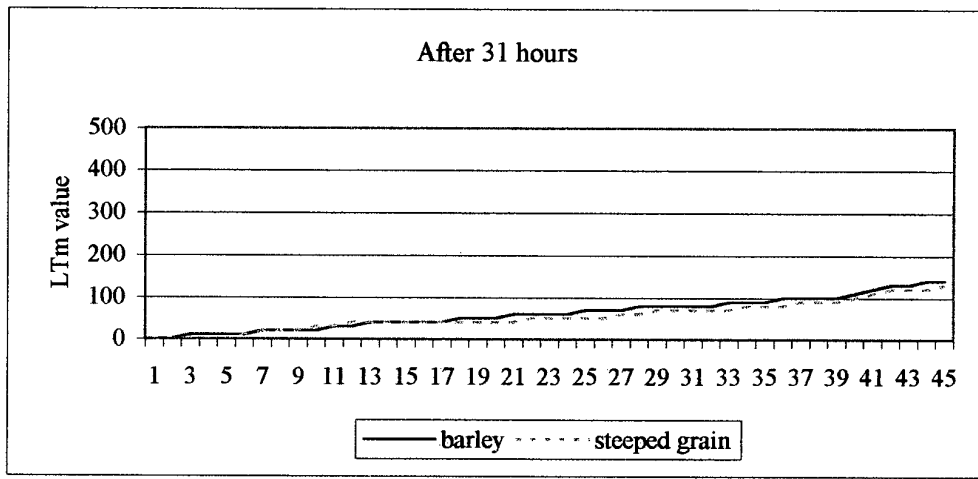
Figures 55-57 : LTm measurement during steeping (Chariot).



Figures 58-60 : LTm measurement during steeping (Chariot)

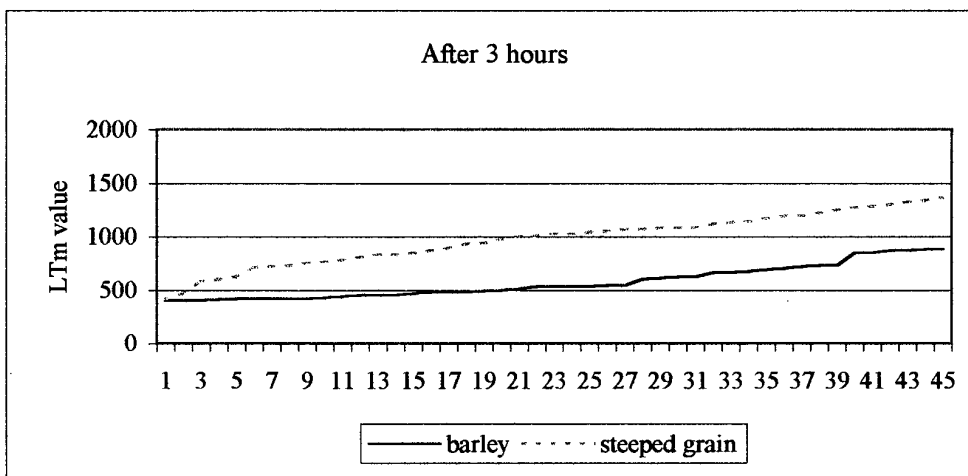
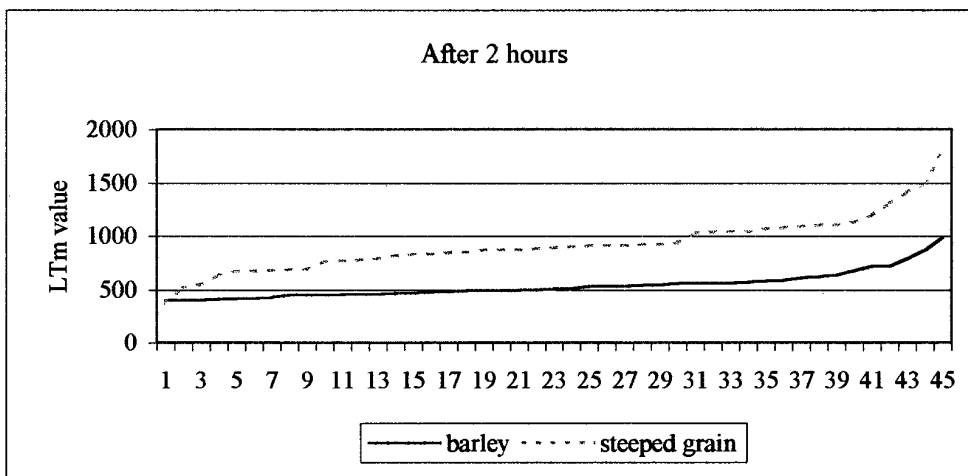
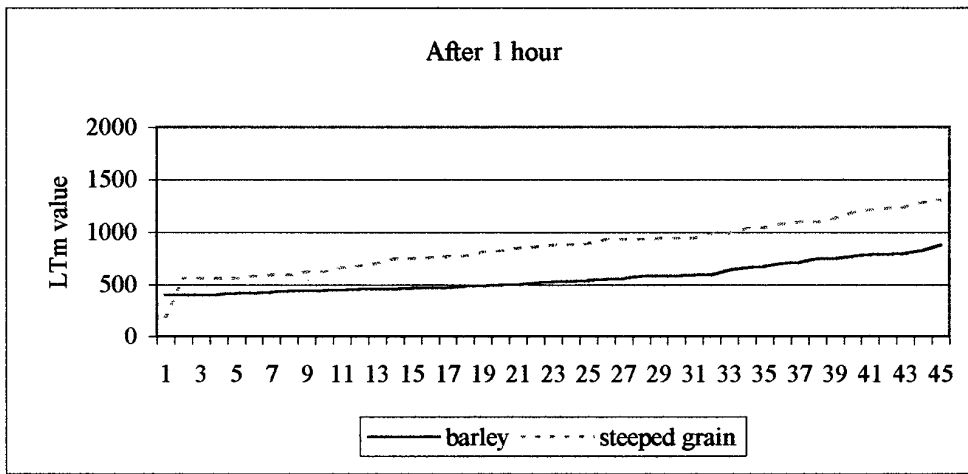


Figures 61-63 : LTm measurement during steeping (Chariot)

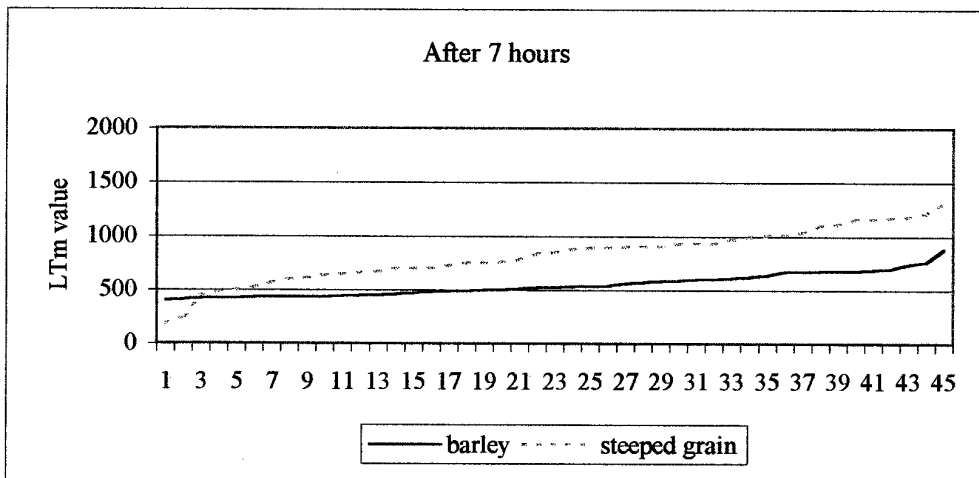
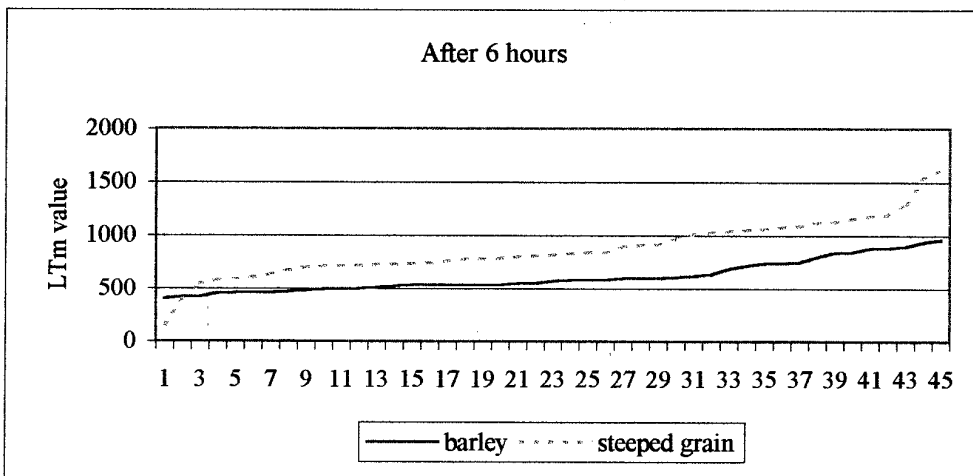
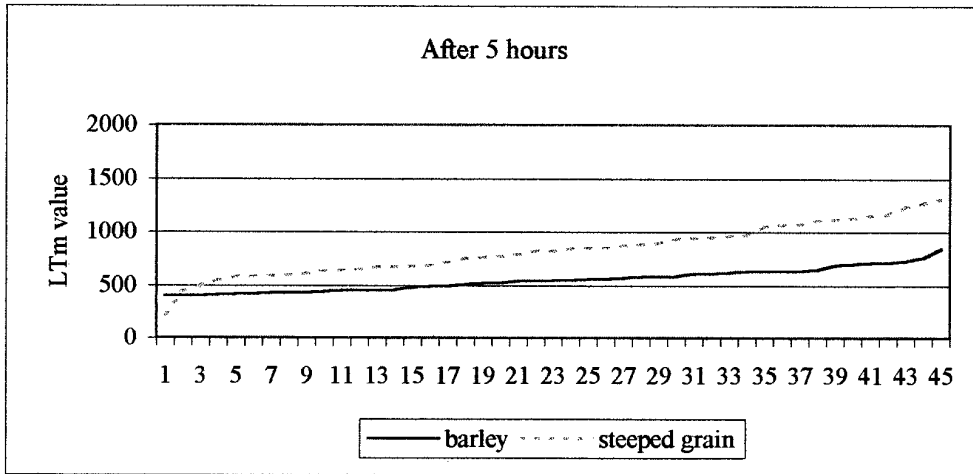




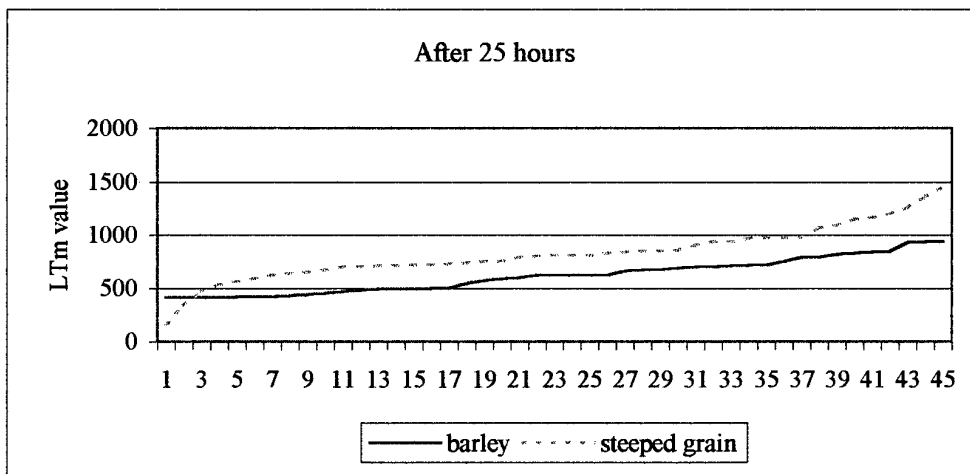
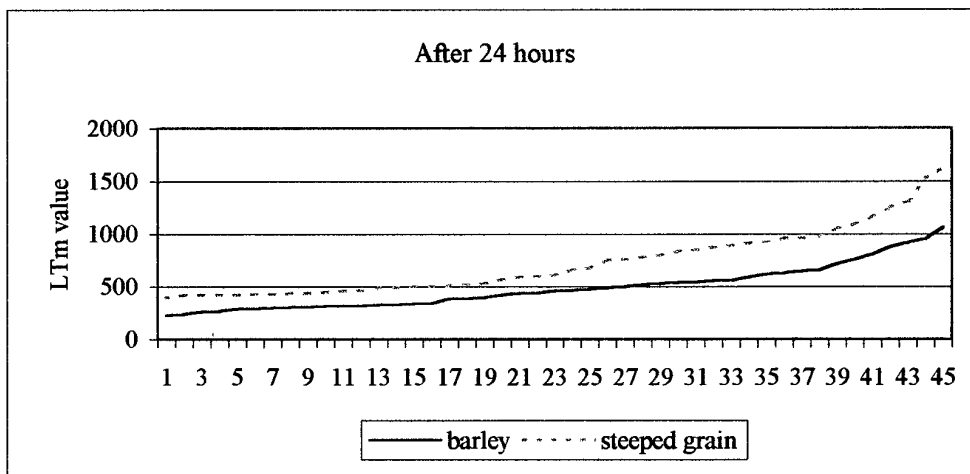
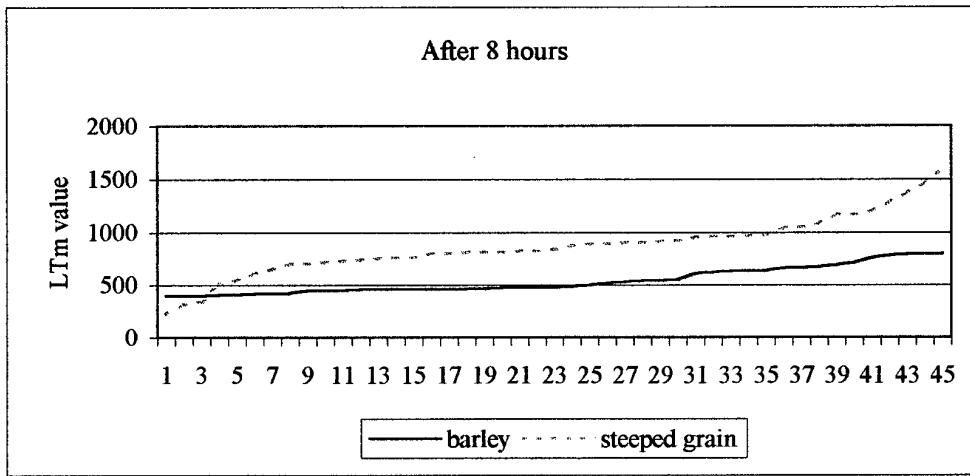
Figures 64-66 : LTm measurement during steeping (Epic)



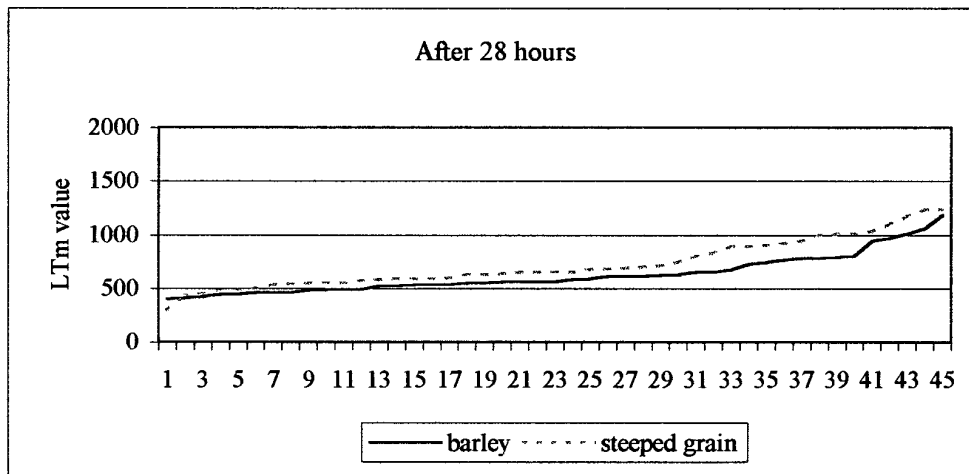
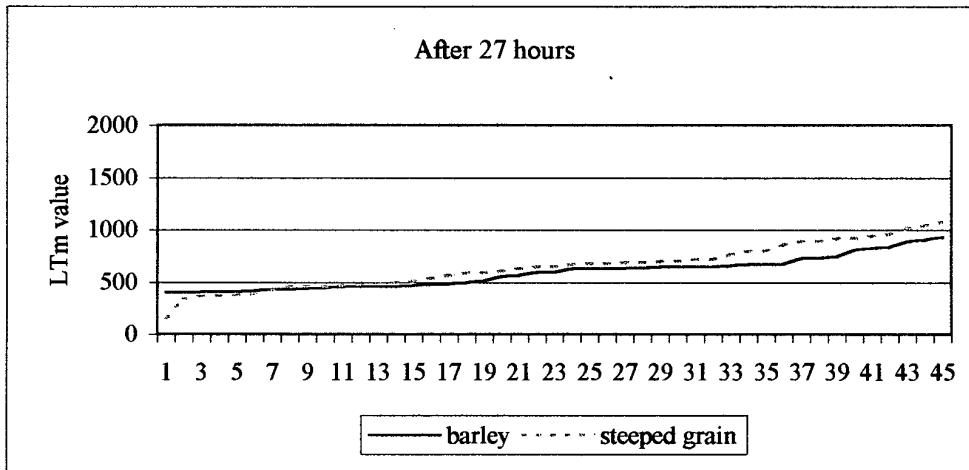
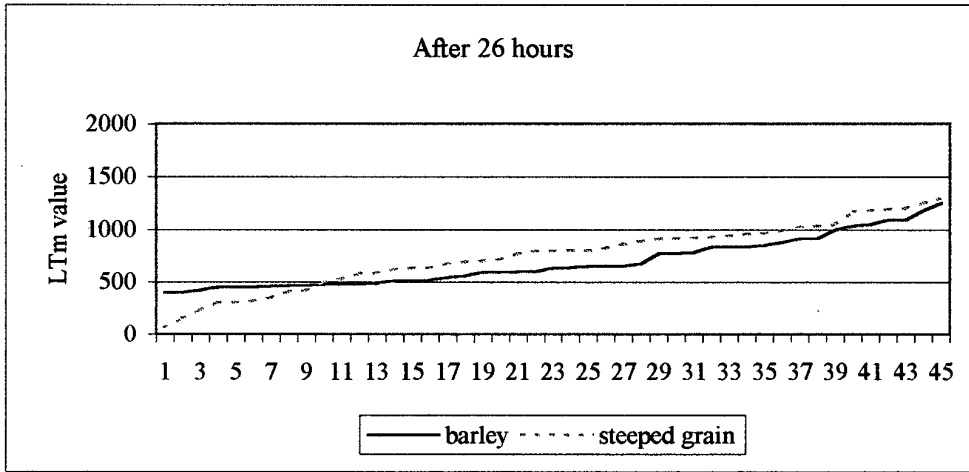
Figures 67-69 : LTm measurement during steeping (Epic)



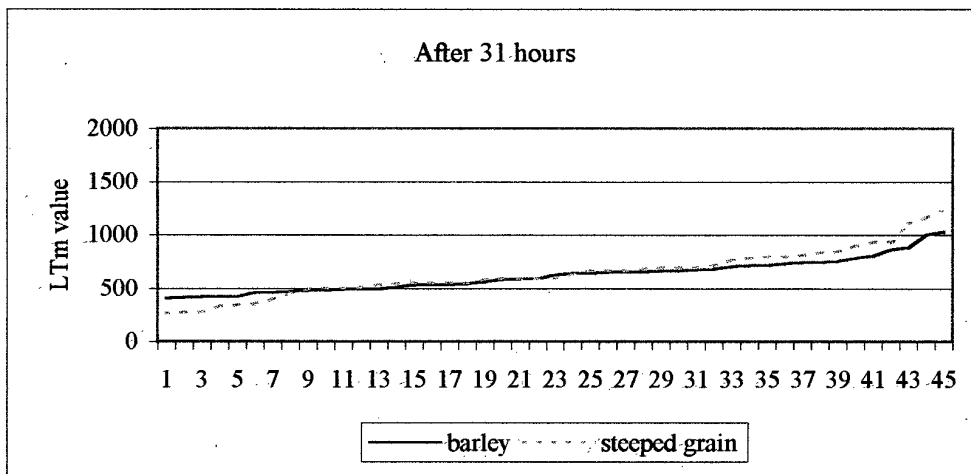
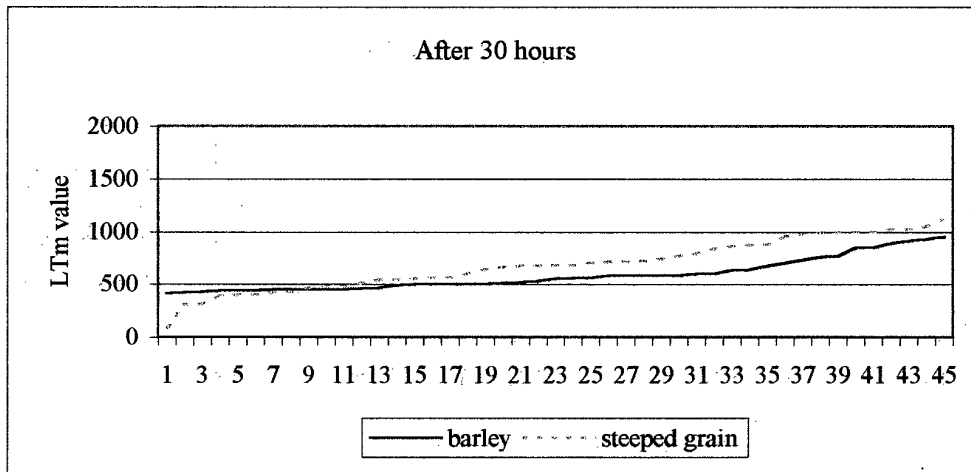
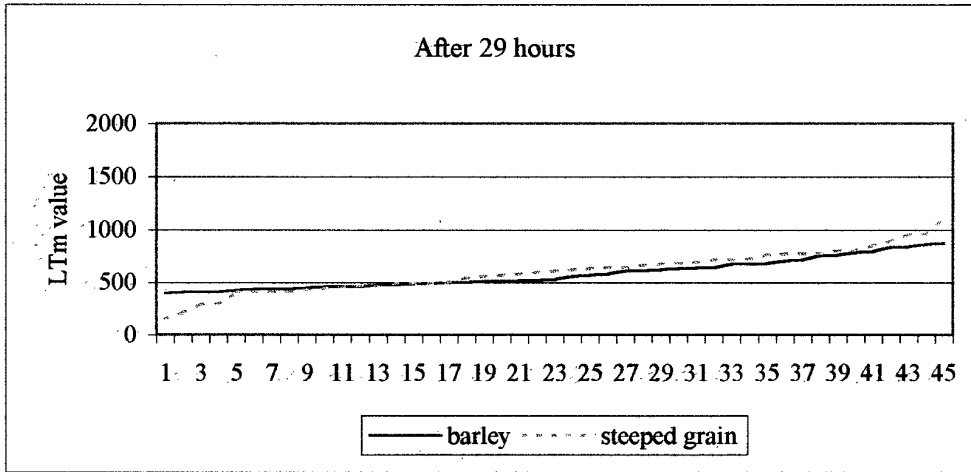
Figures 70-72 : LTm measurement during steeping (Epic)



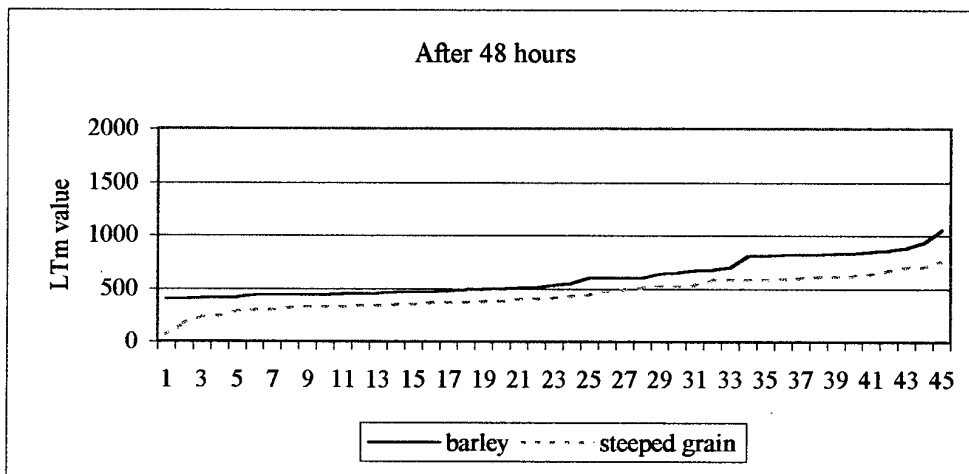
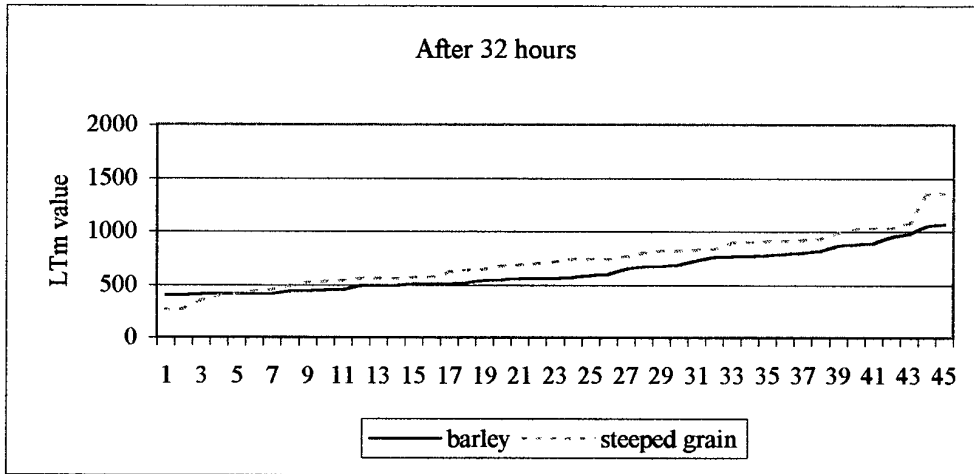
Figures 73-75 : LTm measurement during steeping (Epic)



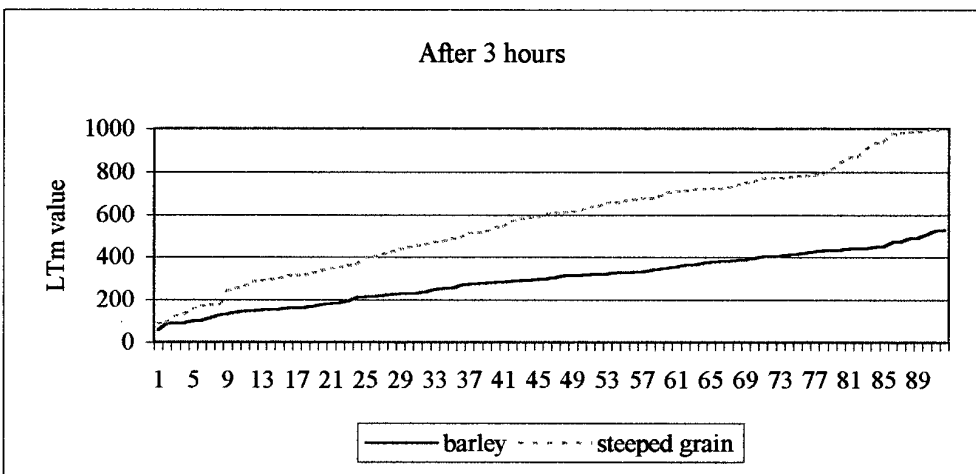
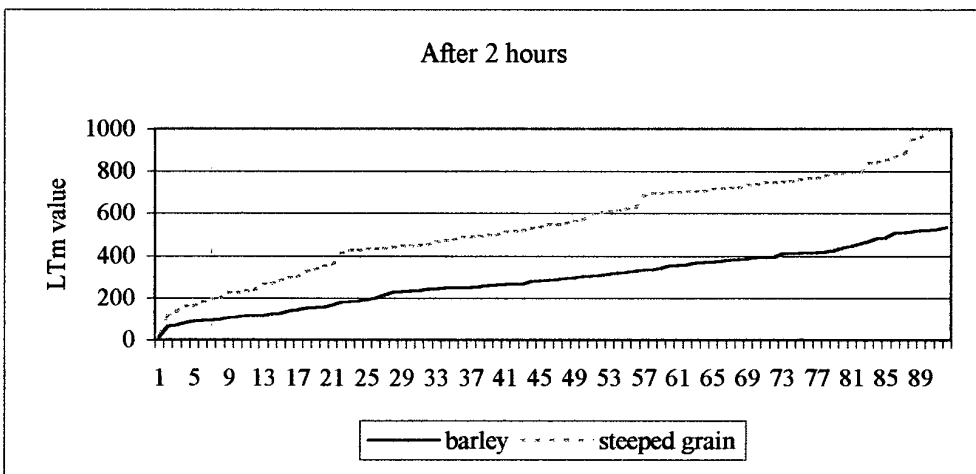
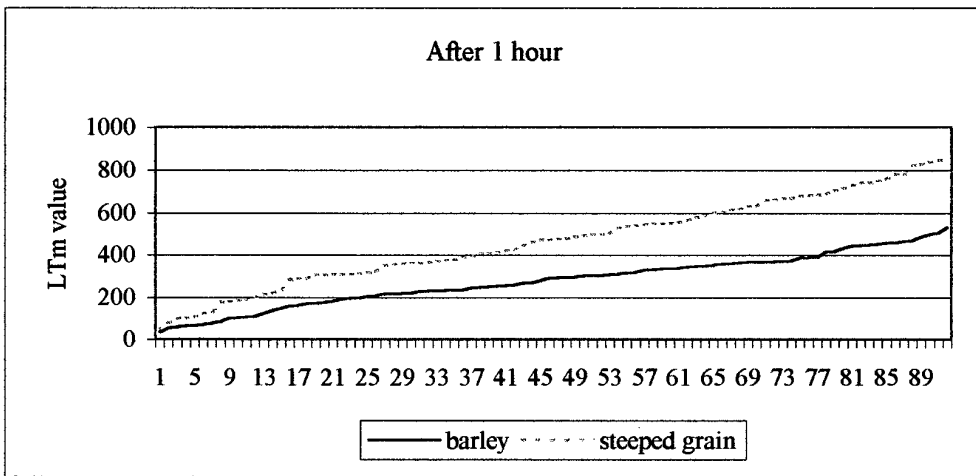
Figures 76-78 : LTm measurement during steeping (Epic)



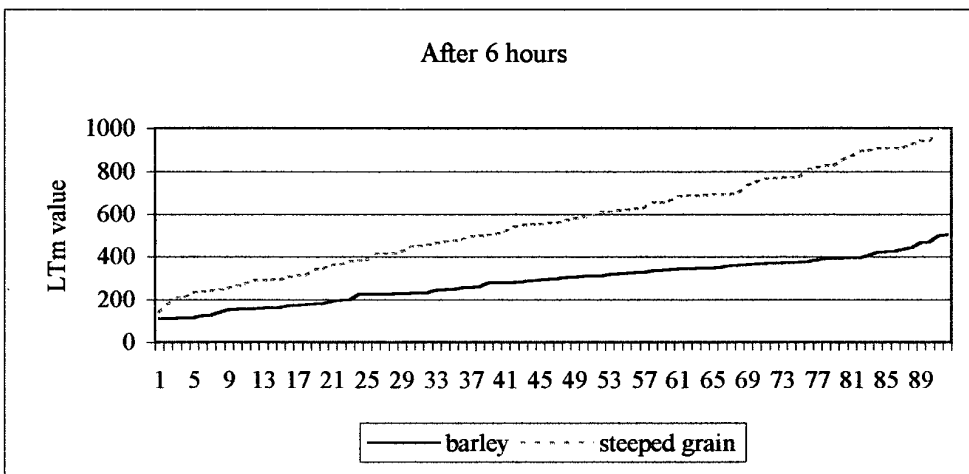
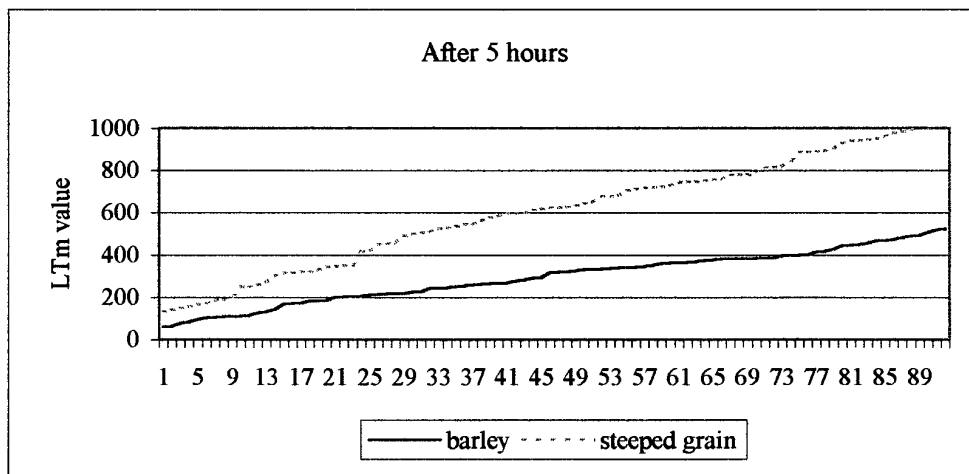
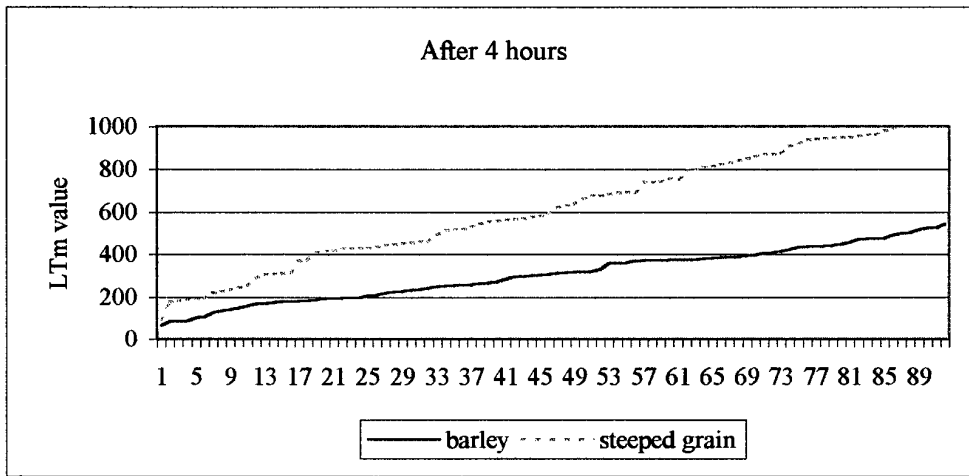
Figures 79 and 80 : LTm measurement during steeping (Epic)



Figures 81-83 : LTm measurement during steeping (Fanfare)

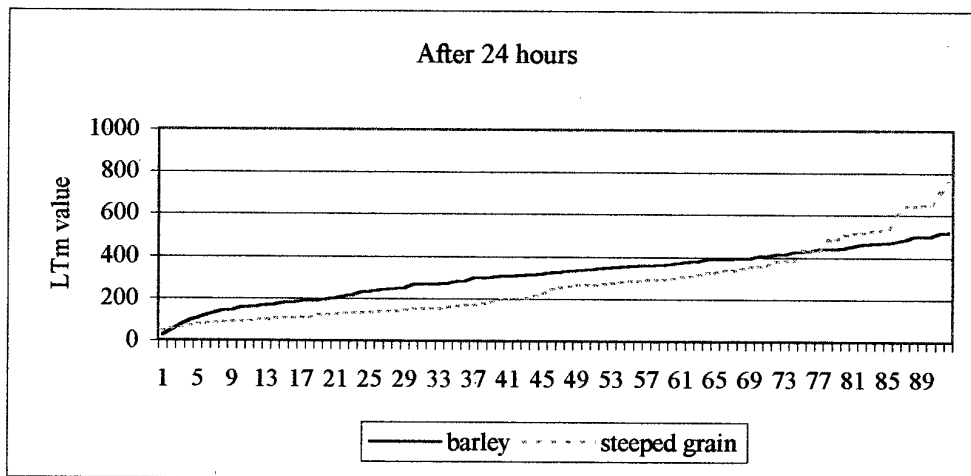
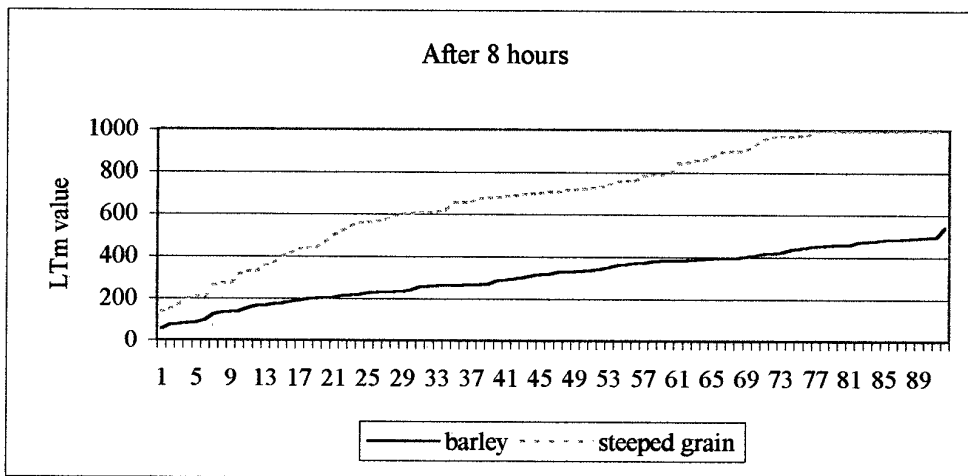
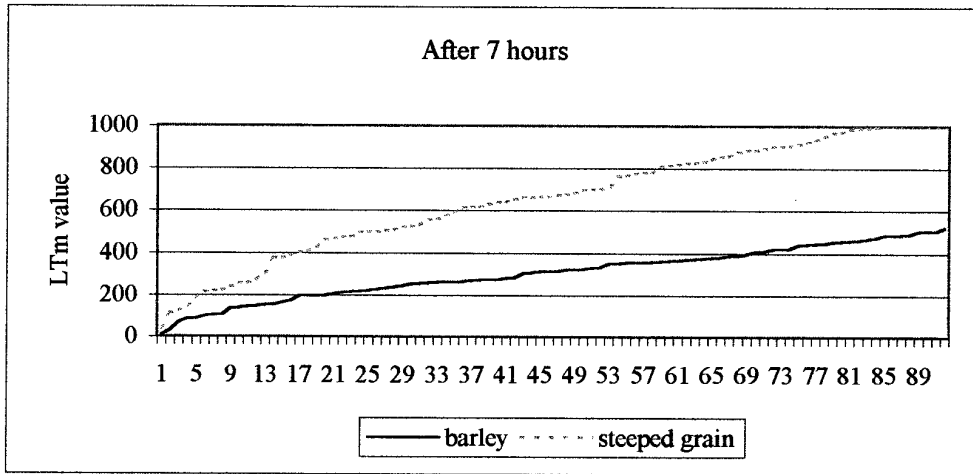


Figures 84-86 : LTm measurement during steeping (Fanfare)

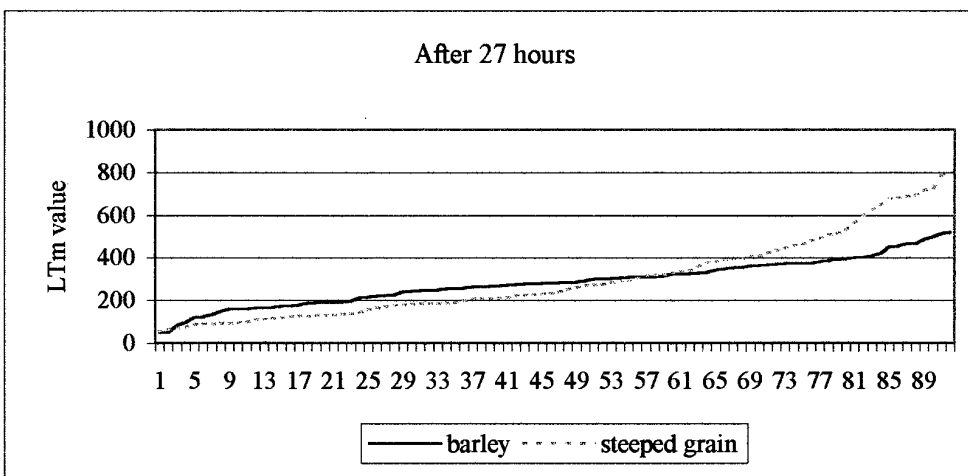
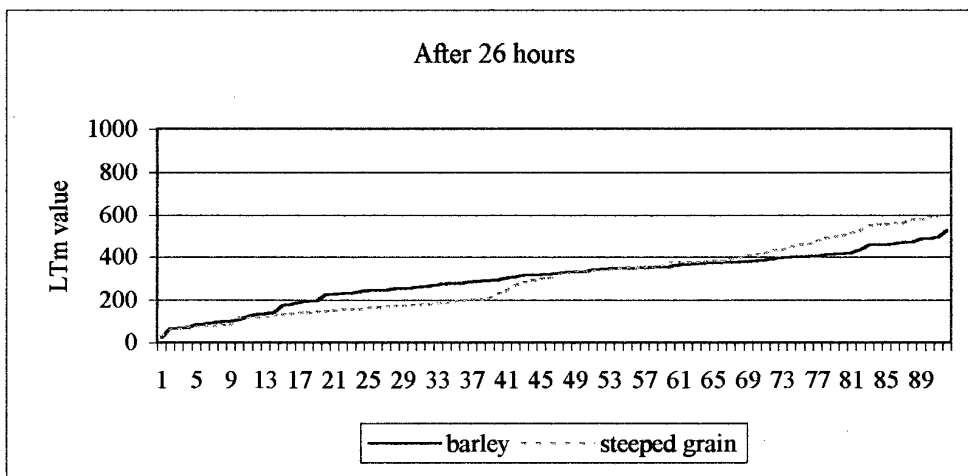
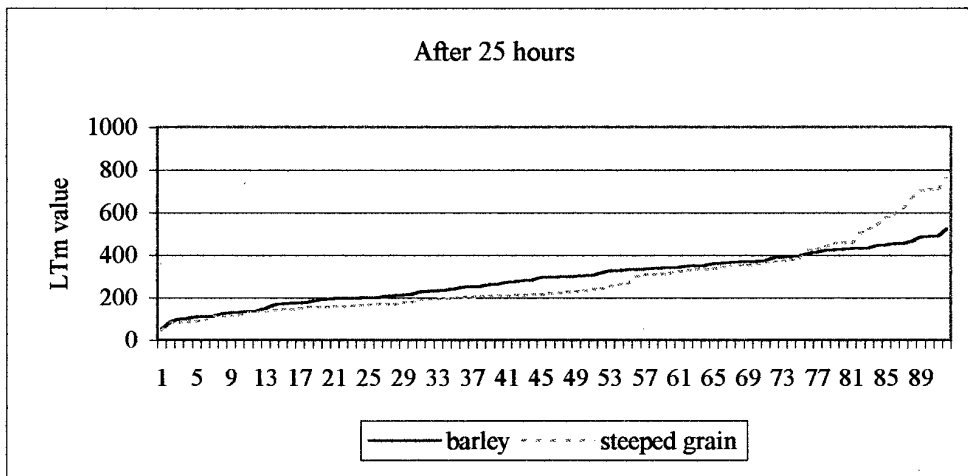




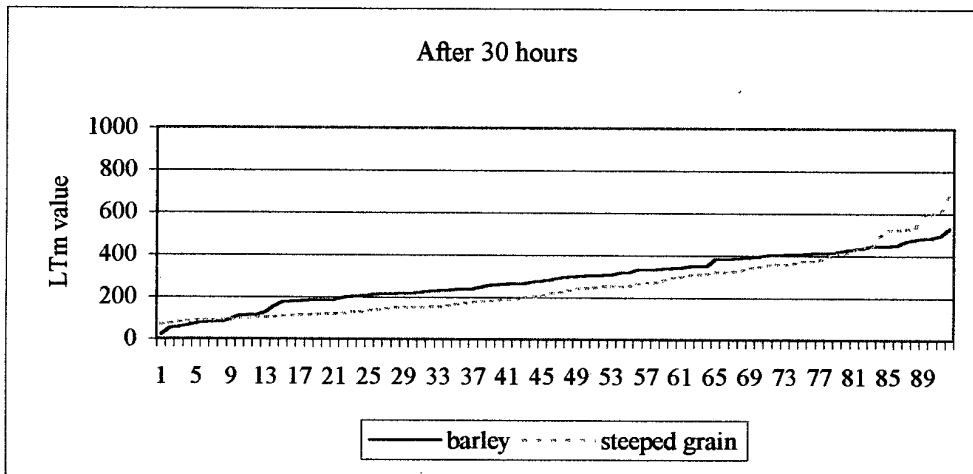
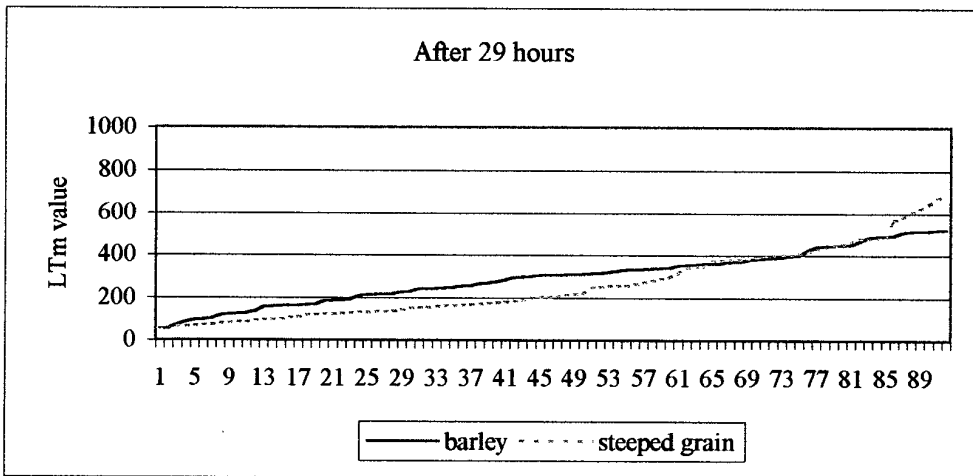
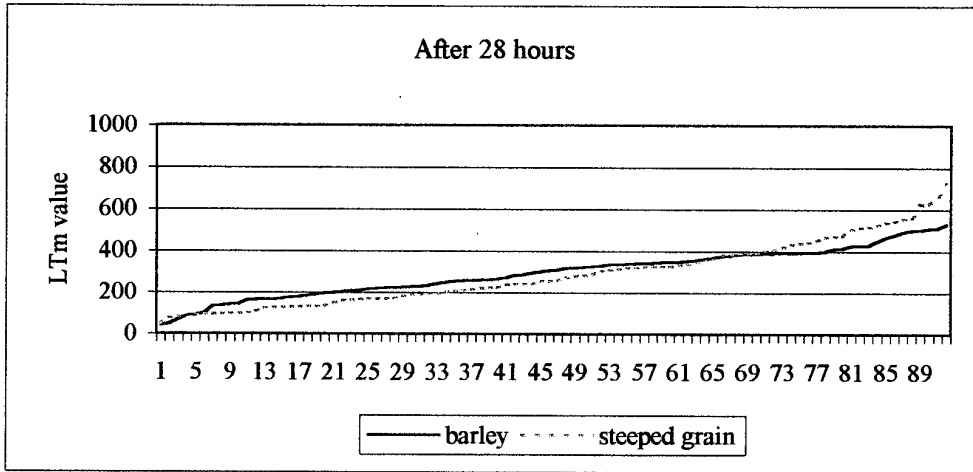
Figures 87-89 : LTm measurement during steeping (Fanfare)



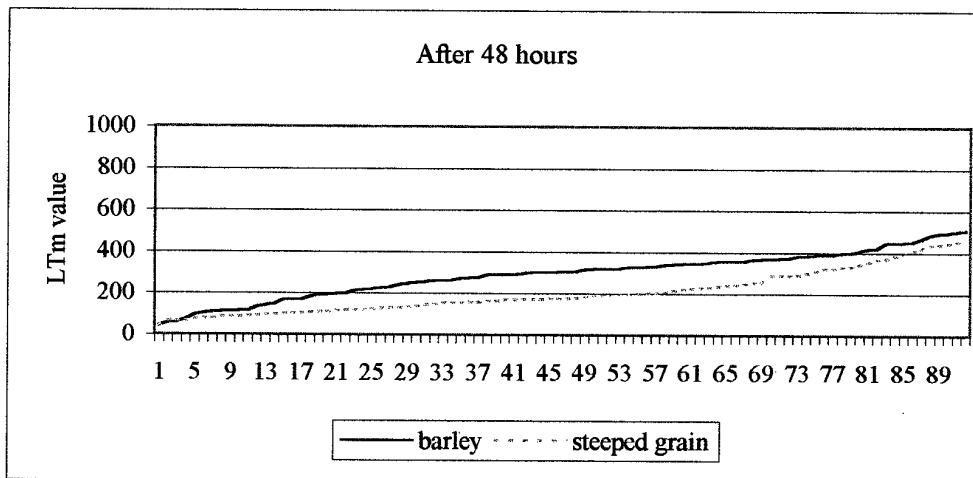
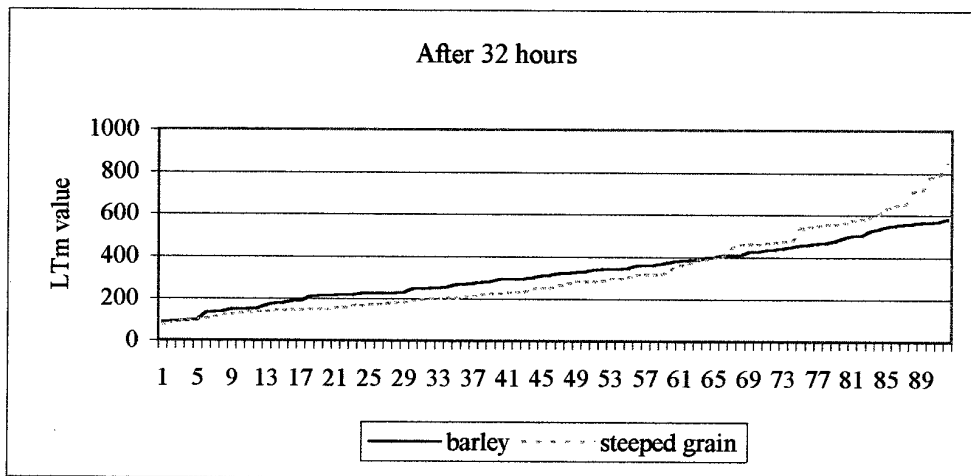
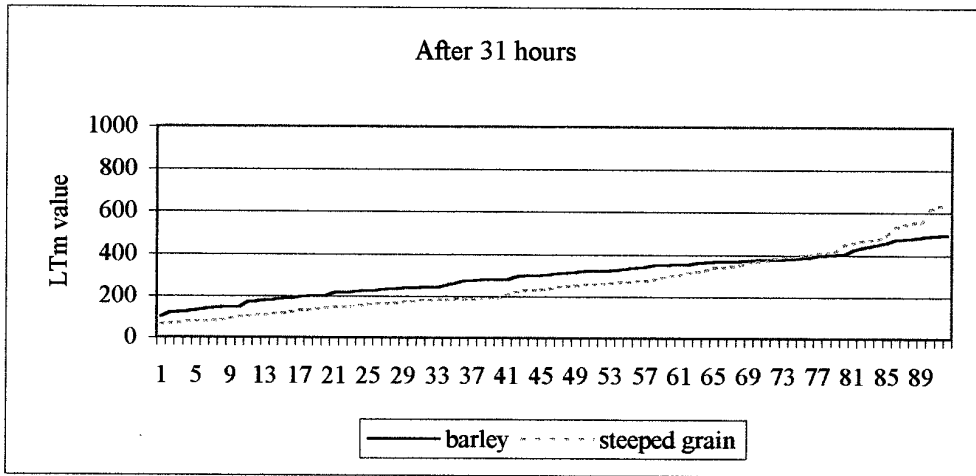
Figures 90-92 : LTm measurement during steeping (Fanfare)



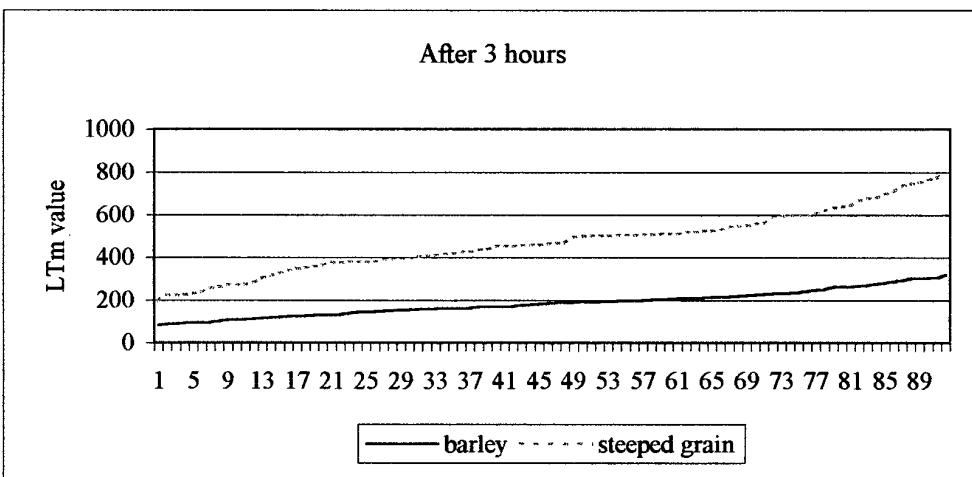
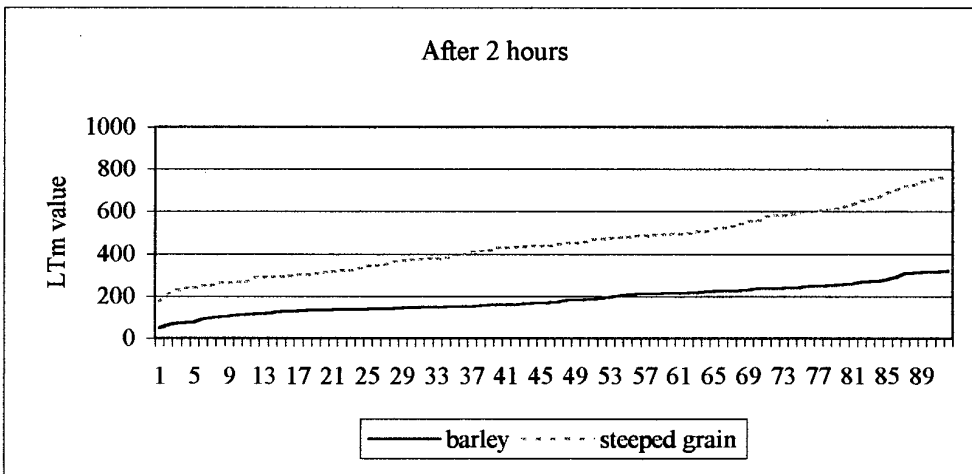
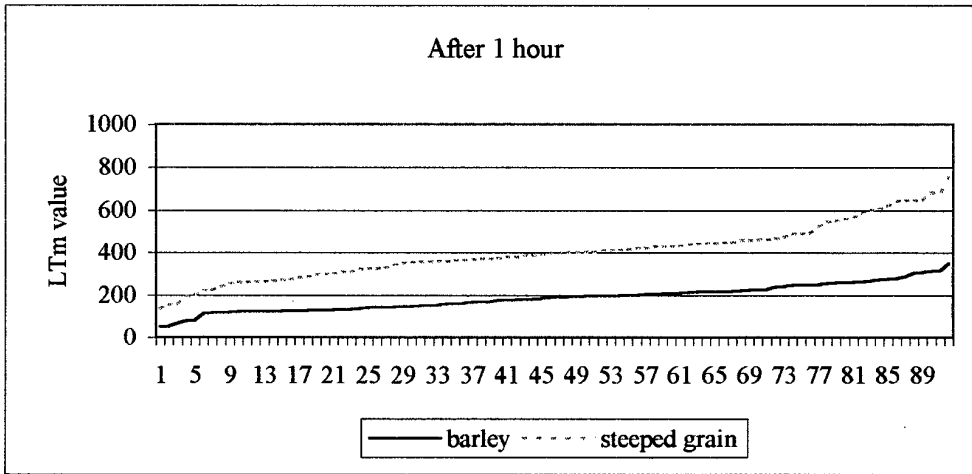
Figures 93-95 : LTm measurement during steeping (Fanfare)



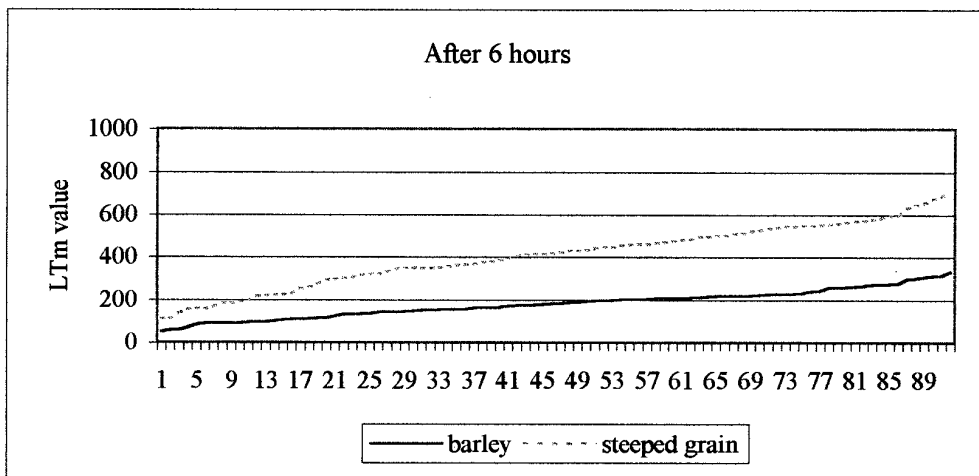
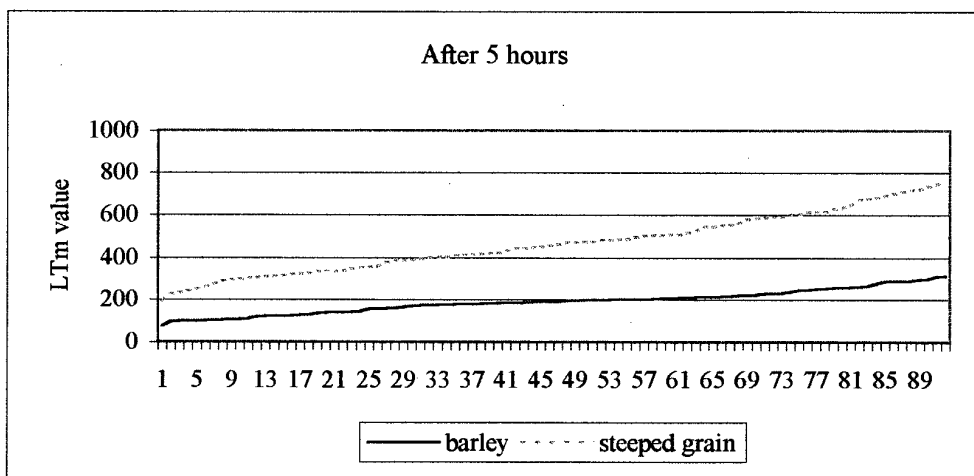
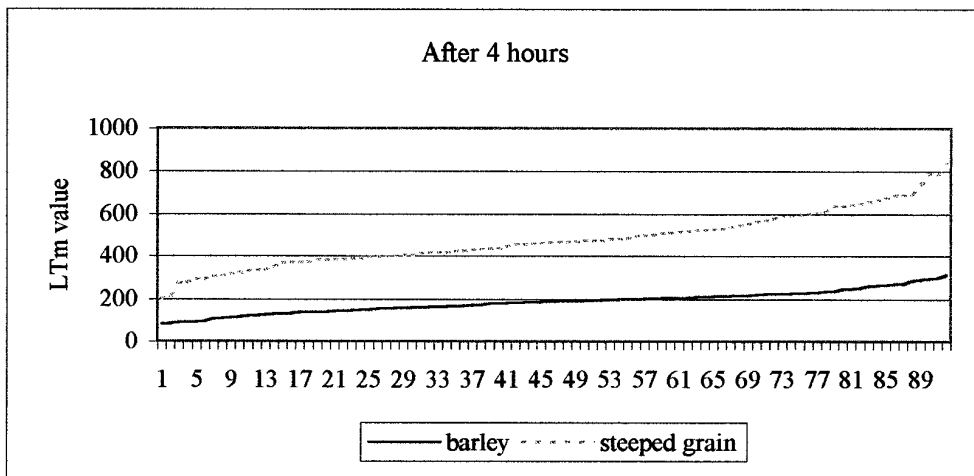
Figures 96-98 : LTm measurement during steeping (Fanfare)



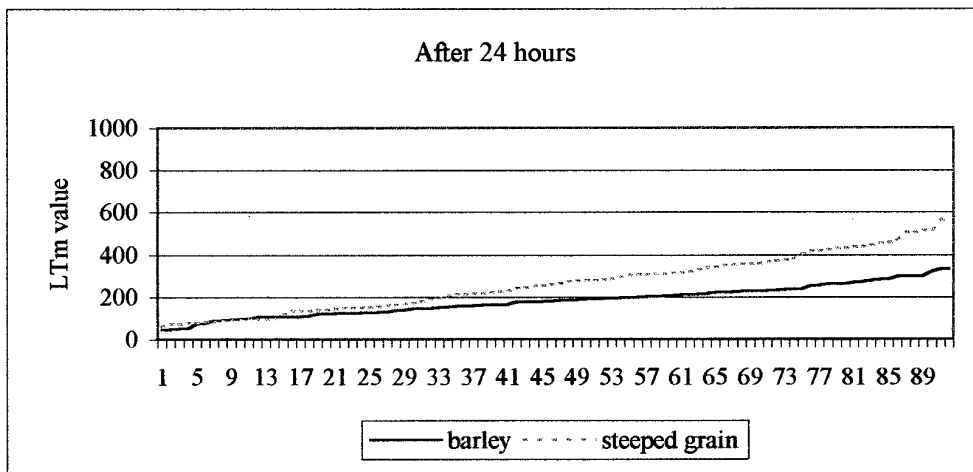
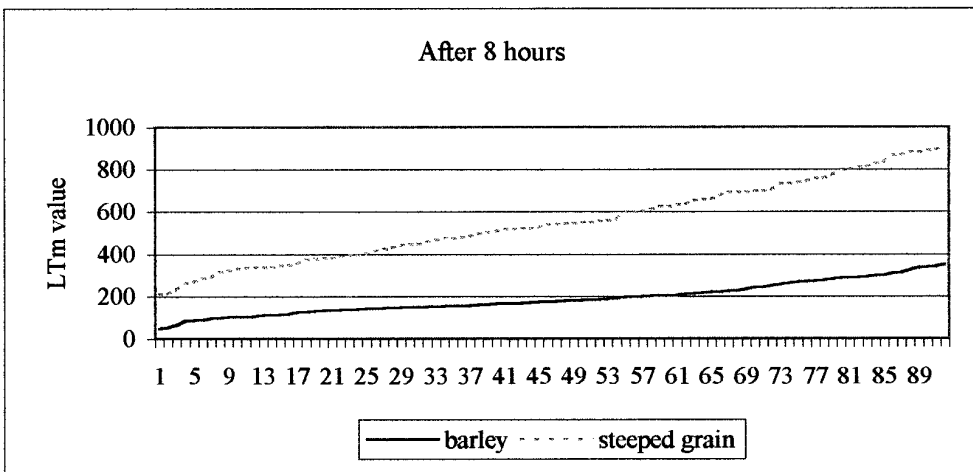
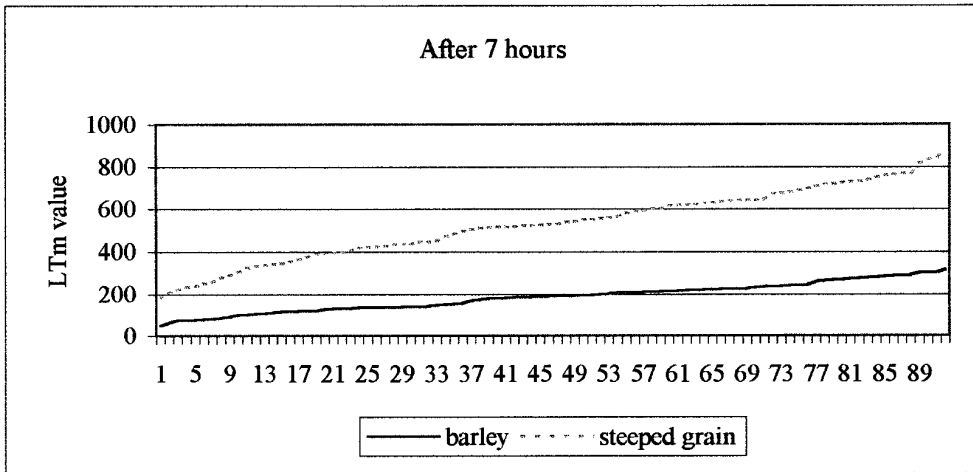
Figures 99-101 : LTm measurement during steeping (Fighter)



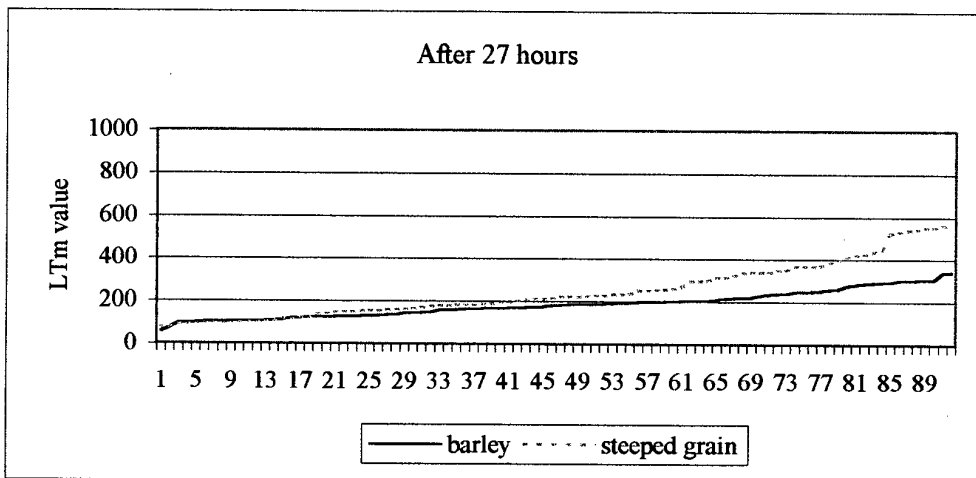
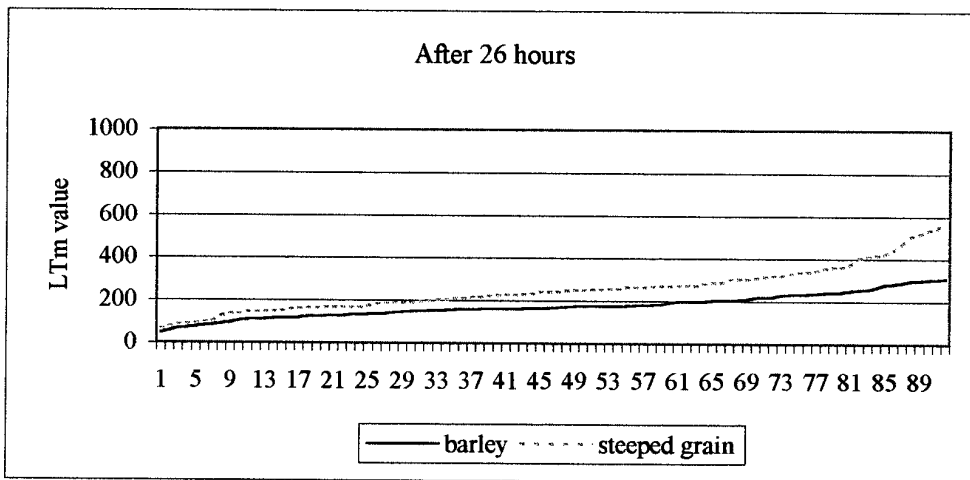
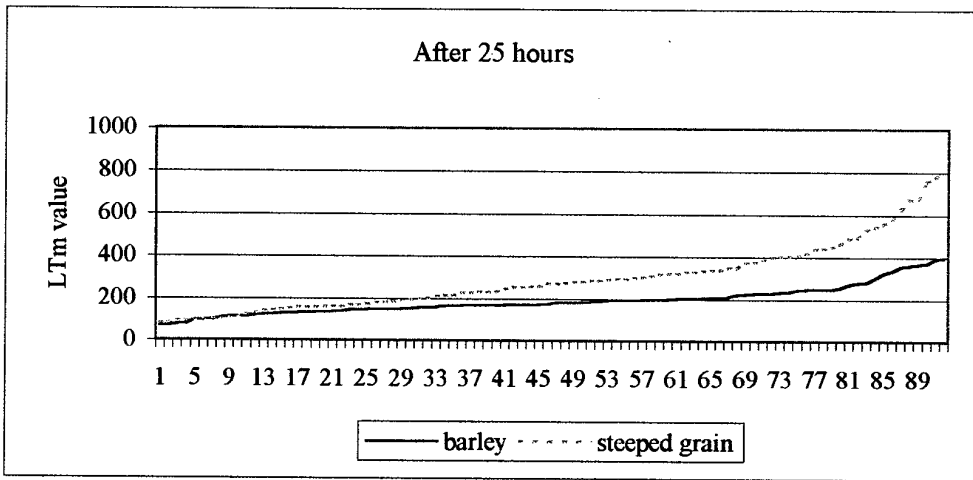
Figures 102-104 : LTm measurement during steeping (Fighter)



Figures 105-107 : LTm measurement during steeping (Fighter)

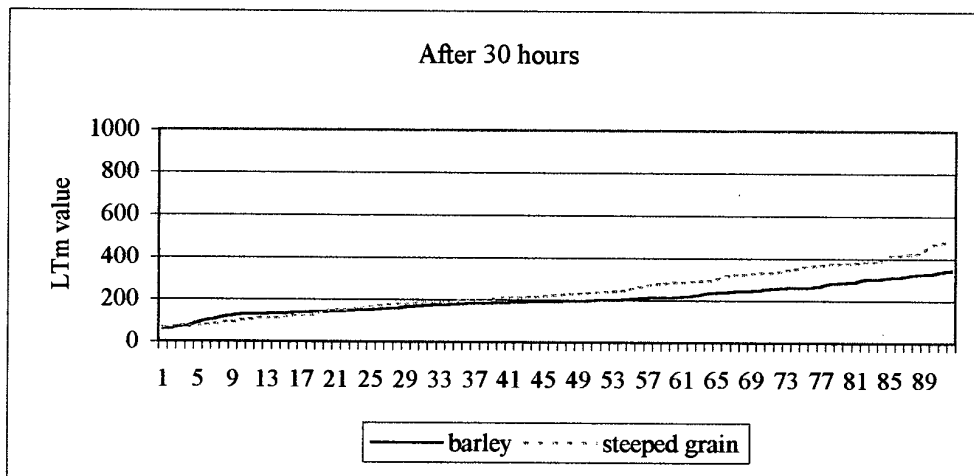
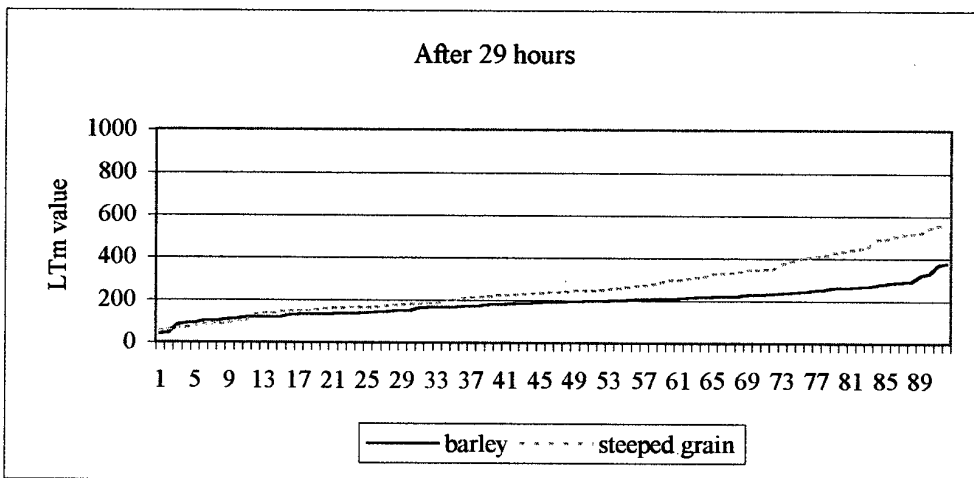
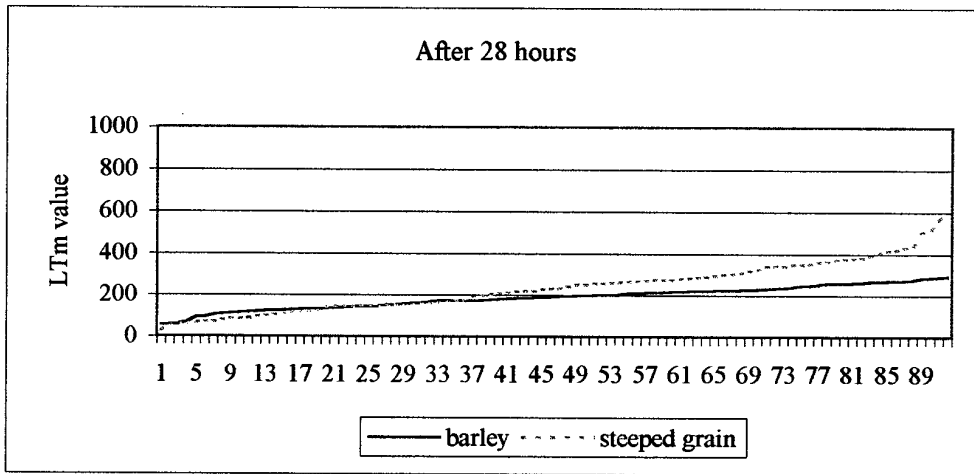


Figures 108-110 : LTm measurement during steeping (Fighter)

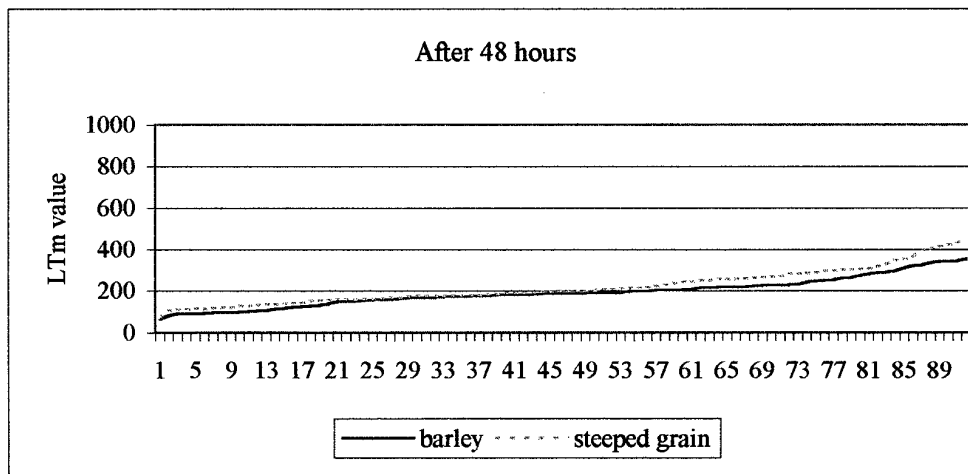
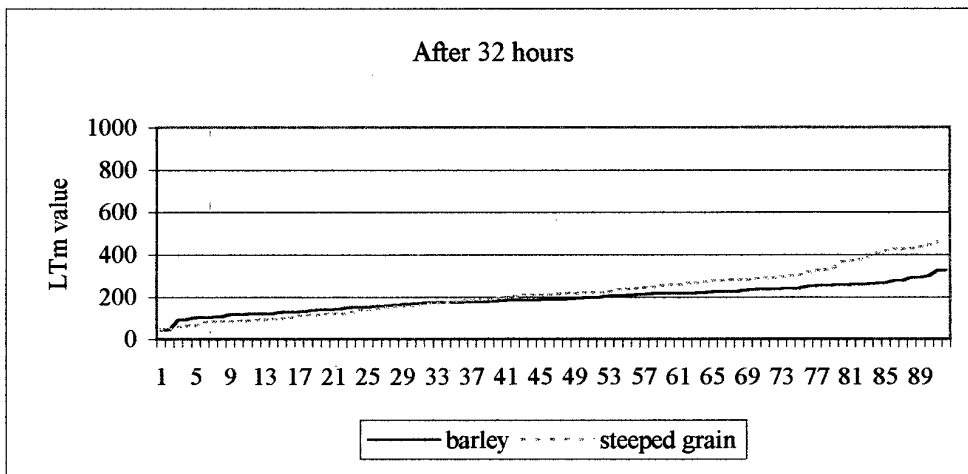
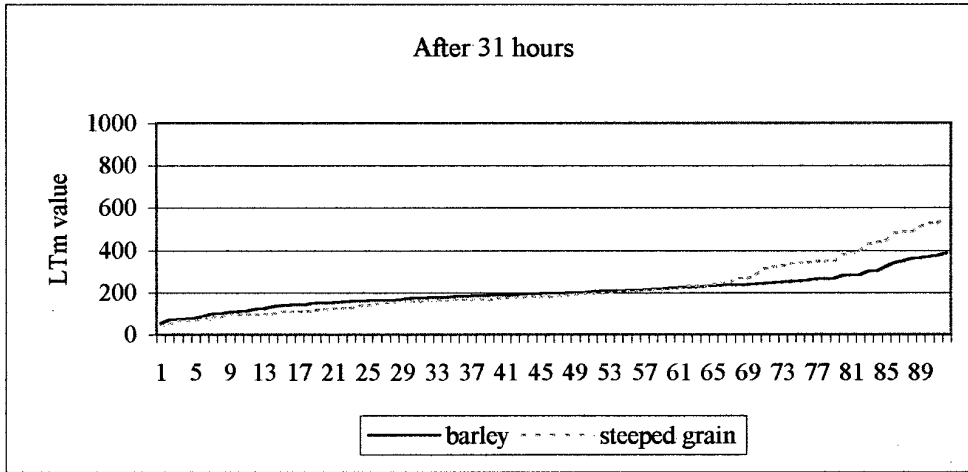




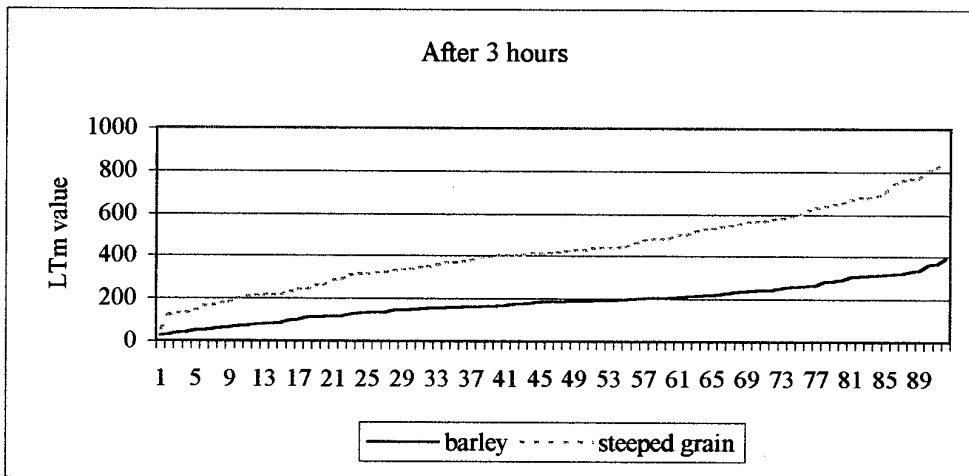
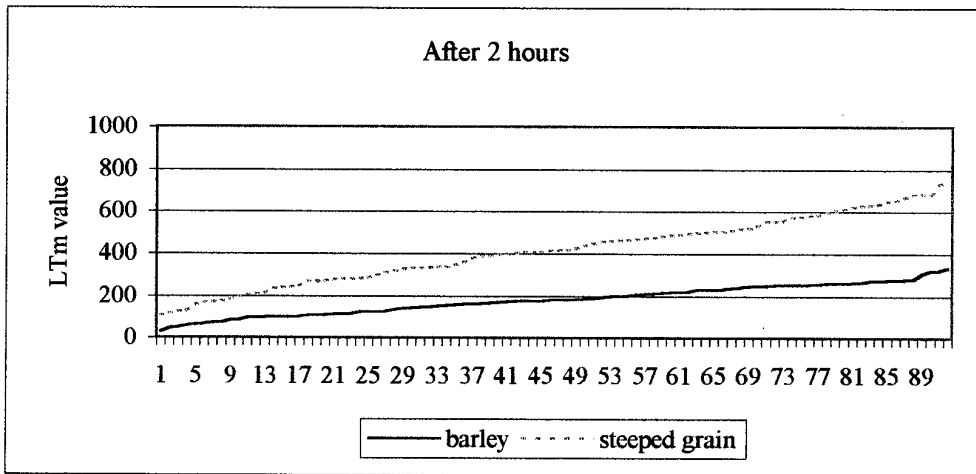
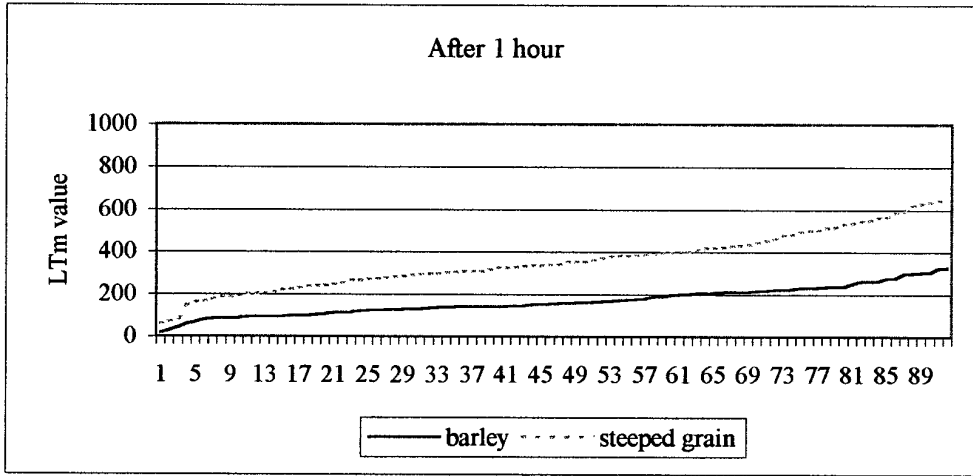
Figures 111-113 : LTm measurement during steeping (Fighter)



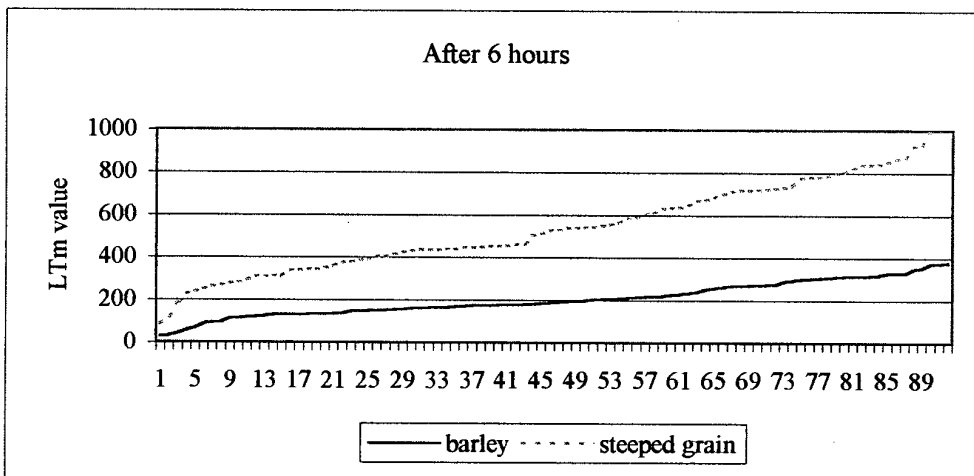
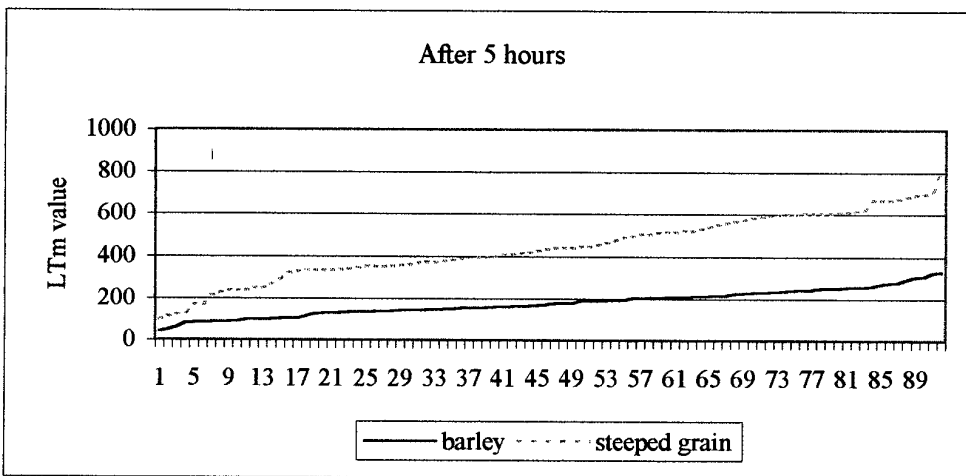
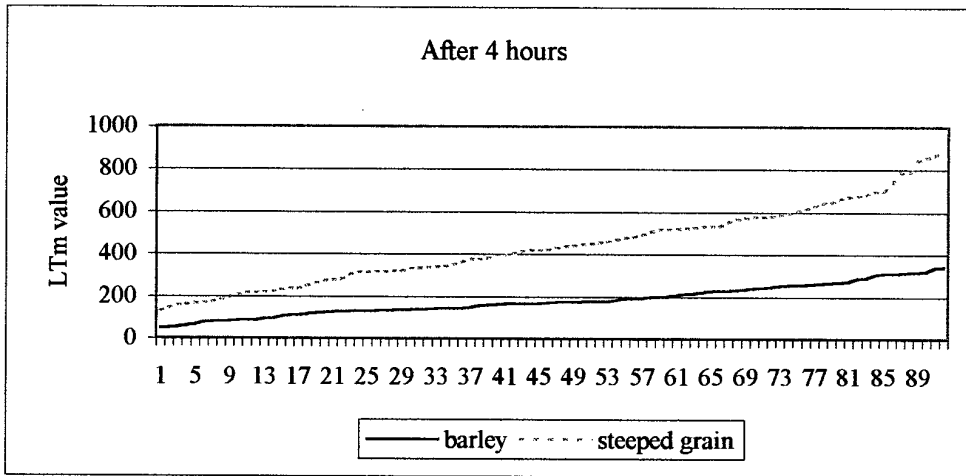
Figures 114-116 : LTm measurement during steeping (Fighter)



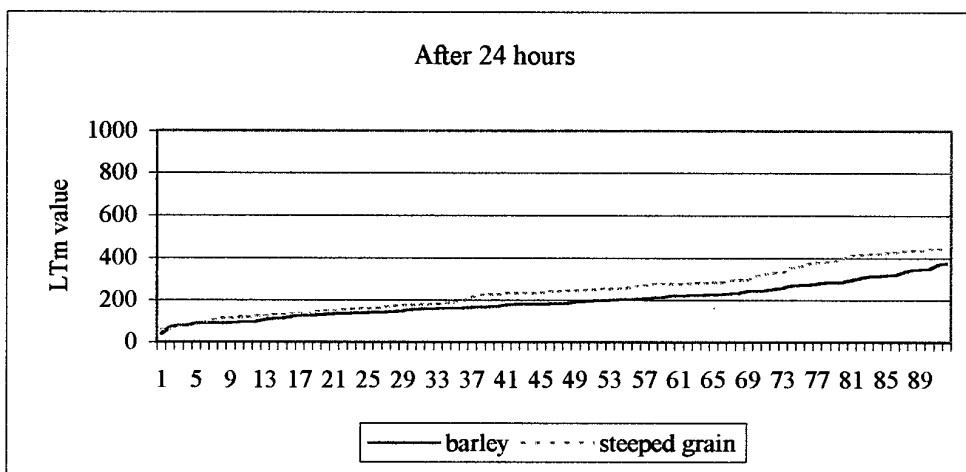
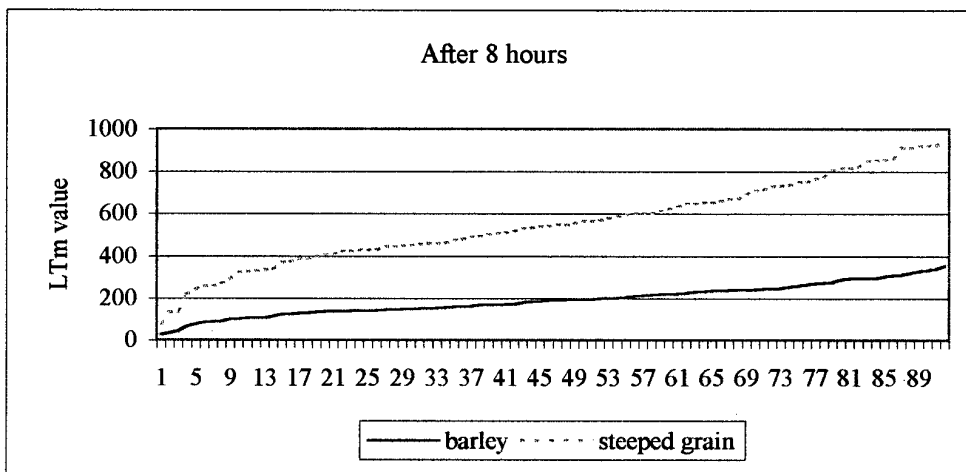
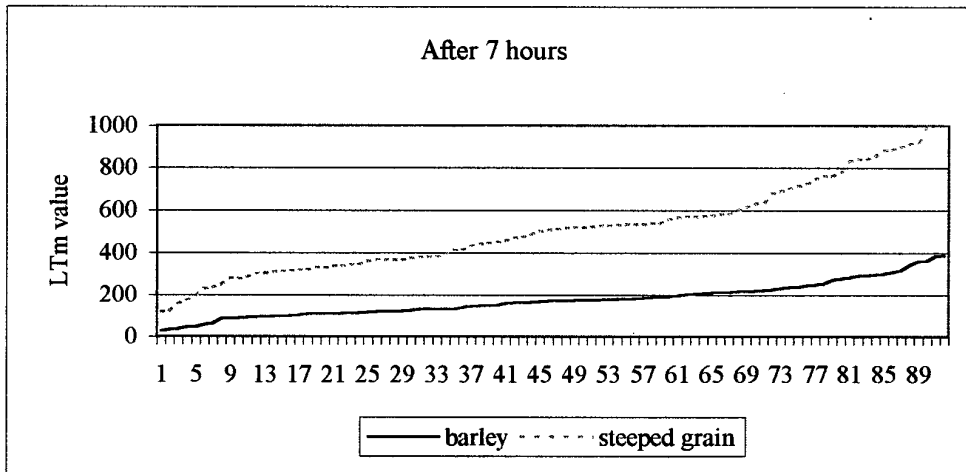
Figures 117-119 : LTm measurement during steeping (Halcyon)



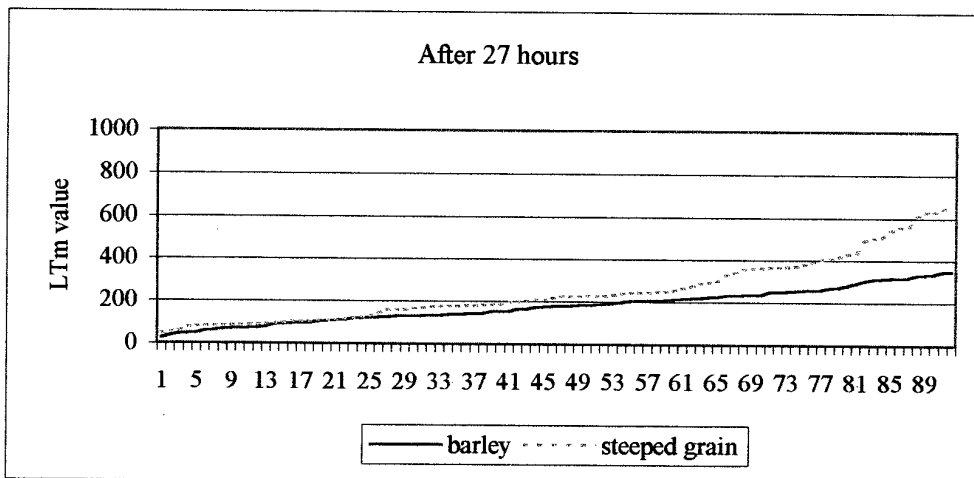
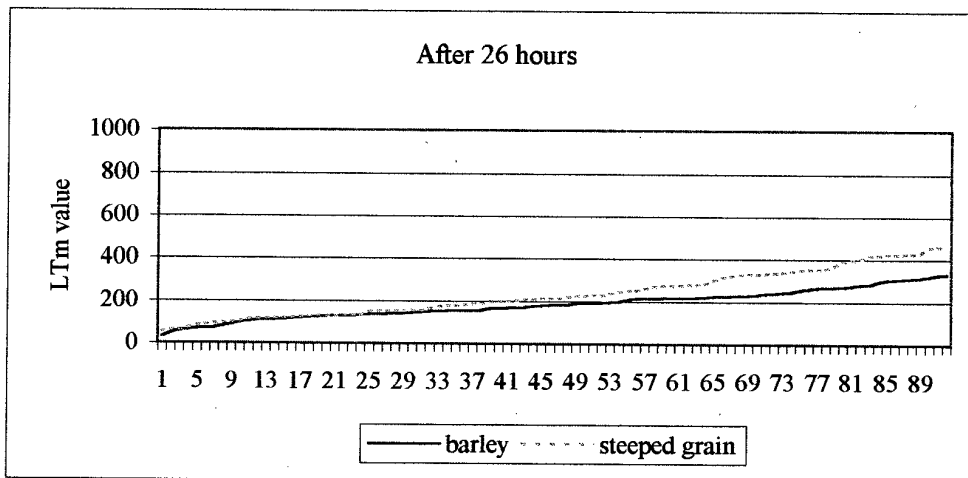
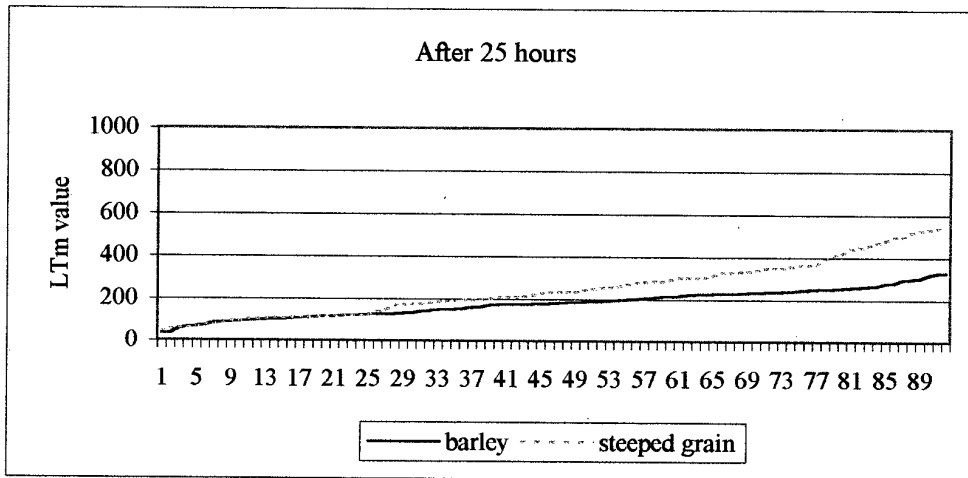
Figures 120-122 : LTm measurement during steeping (Halcyon)



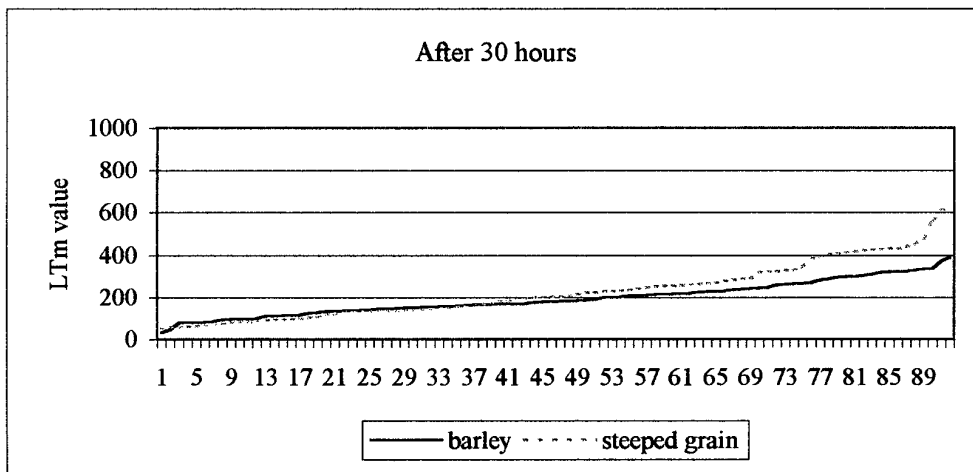
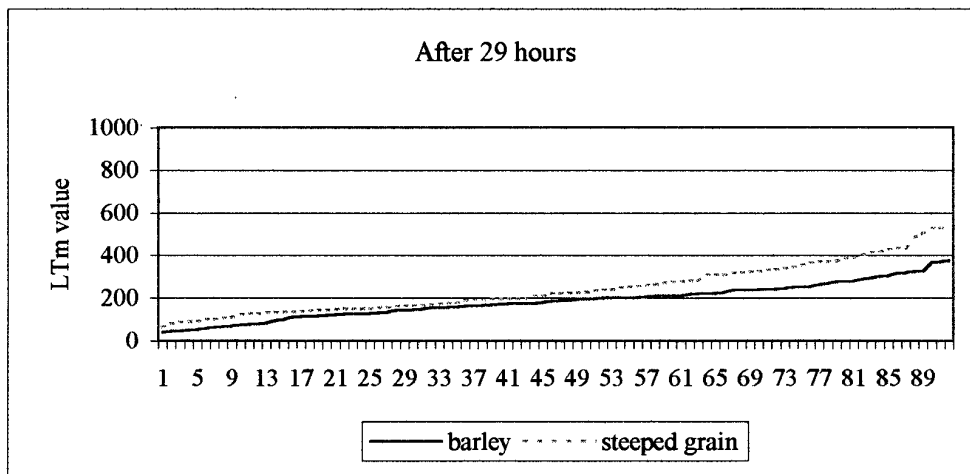
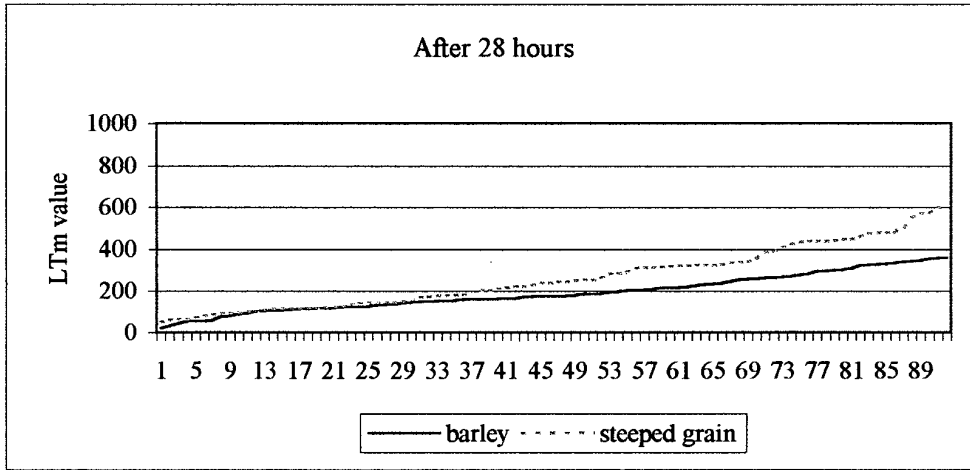
Figures 123-125 : LTm measurement during steeping (Halcyon)



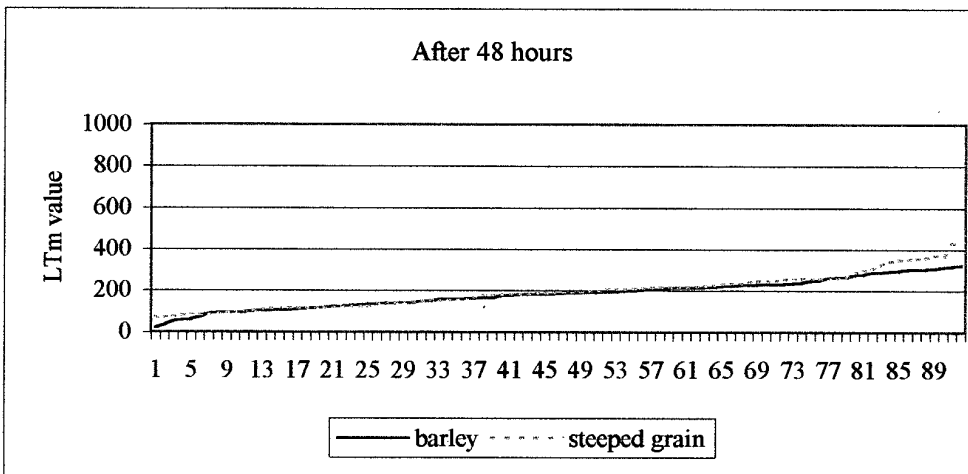
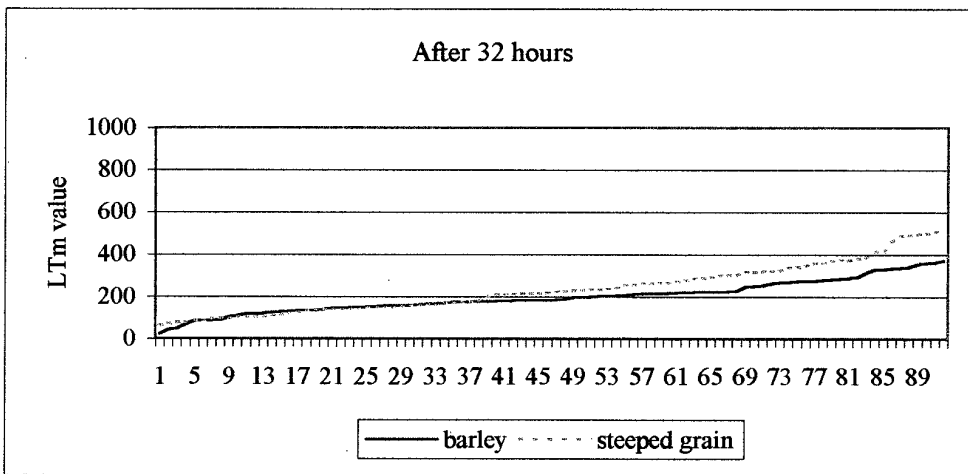
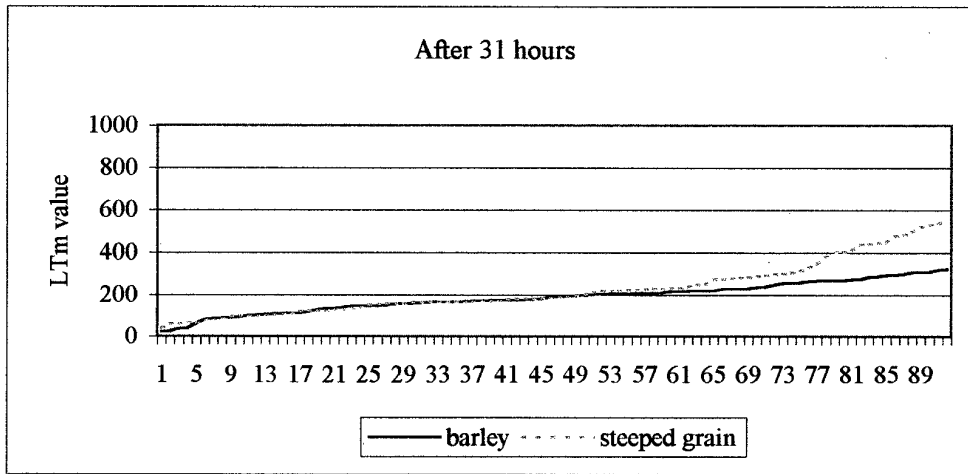
Figures 126-128 : LTm measurement during steeping (Halcyon)



Figures 129-131 : LTm measurement during steeping (Halcyon)

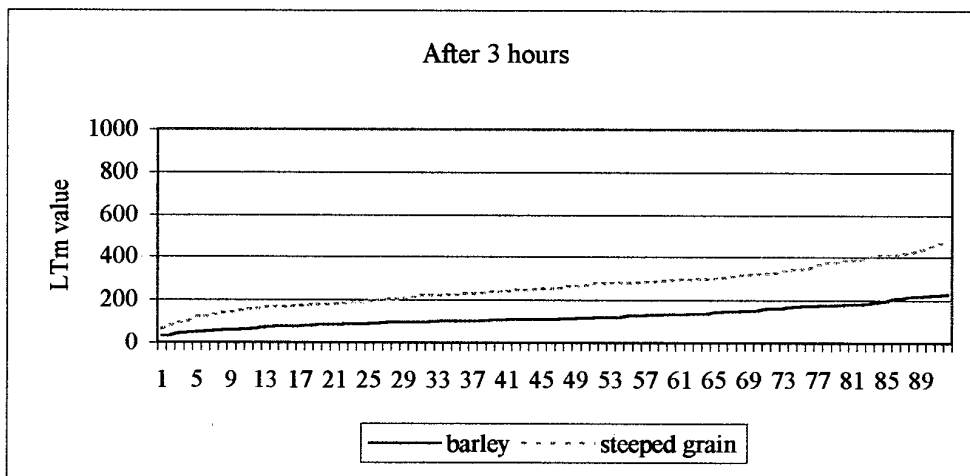
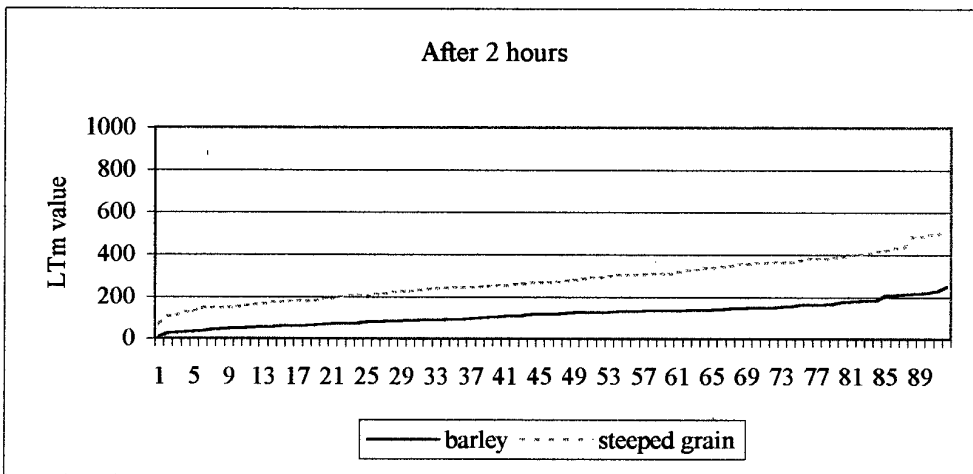
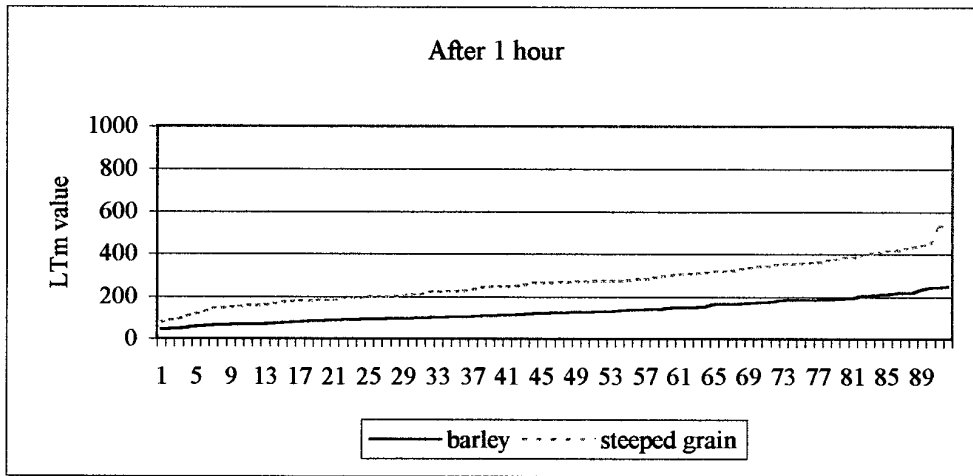


Figures 132-134 : LTm measurement during steeping (Halcyon)

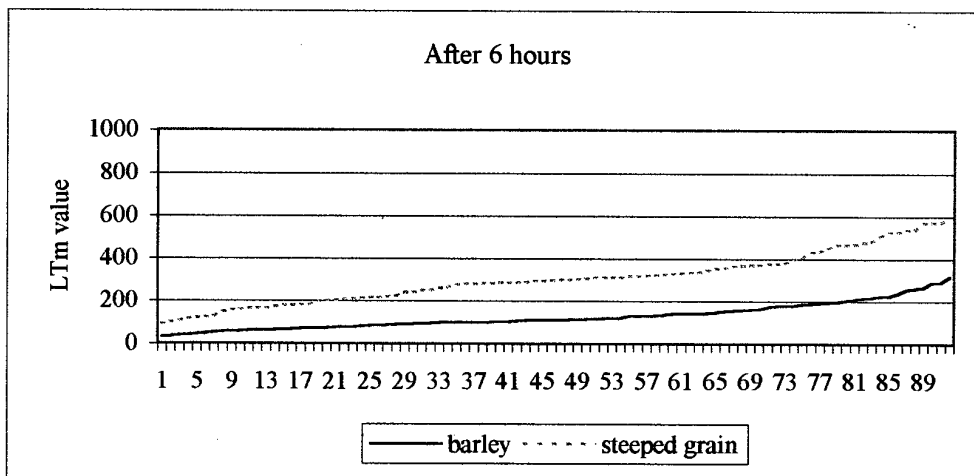
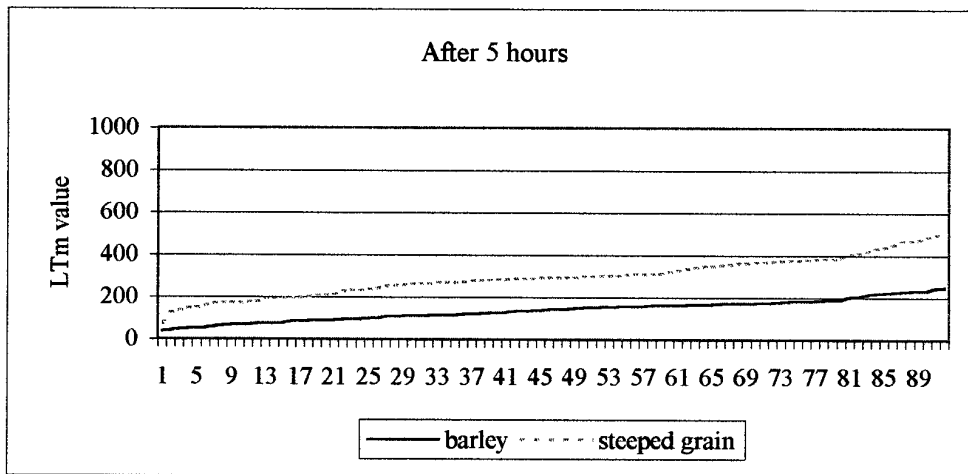
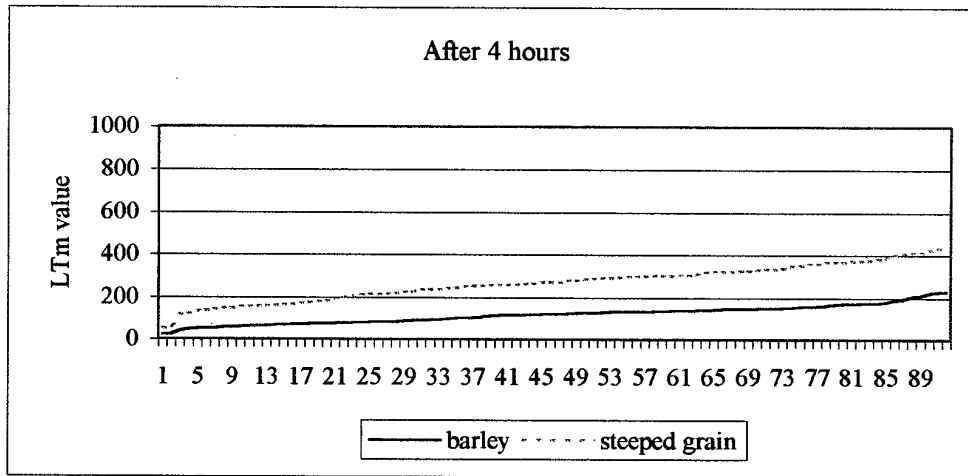




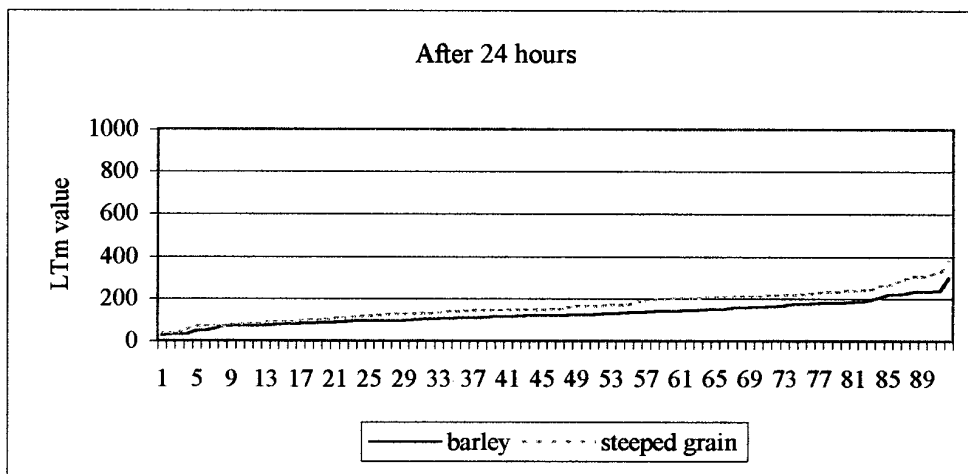
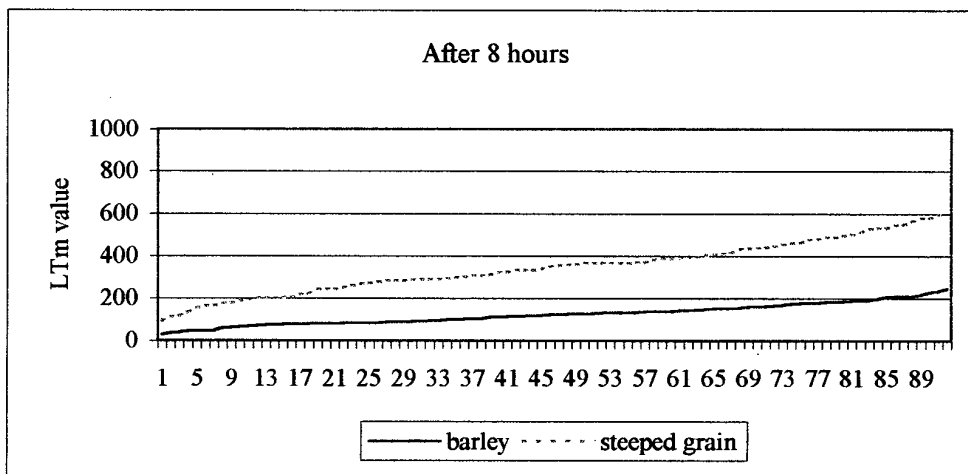
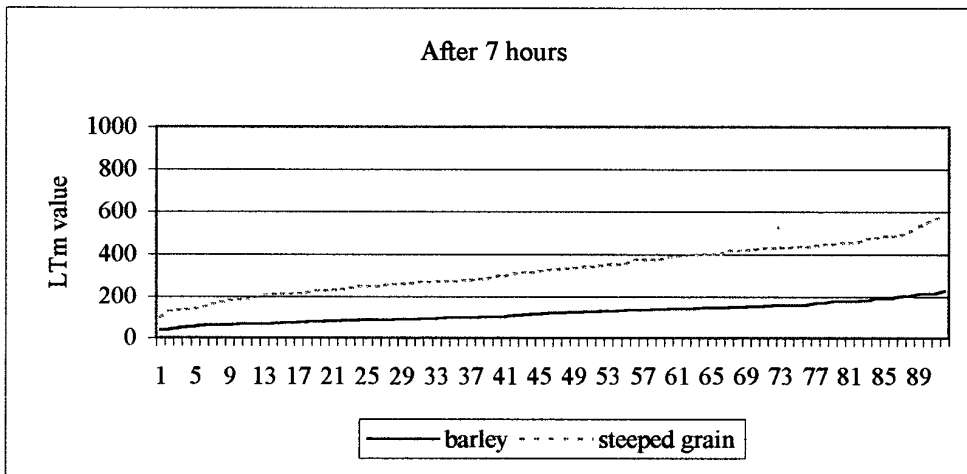
Figures 135-137 : LTm measurement during steeping (Regina)



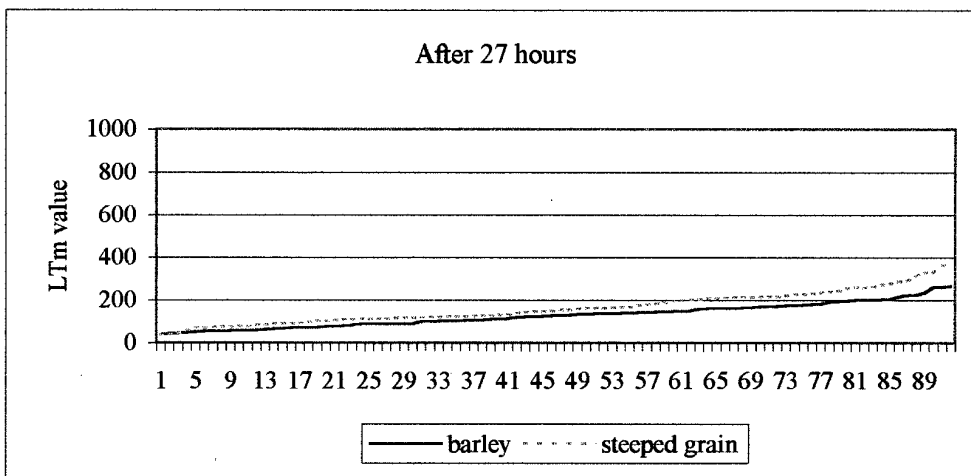
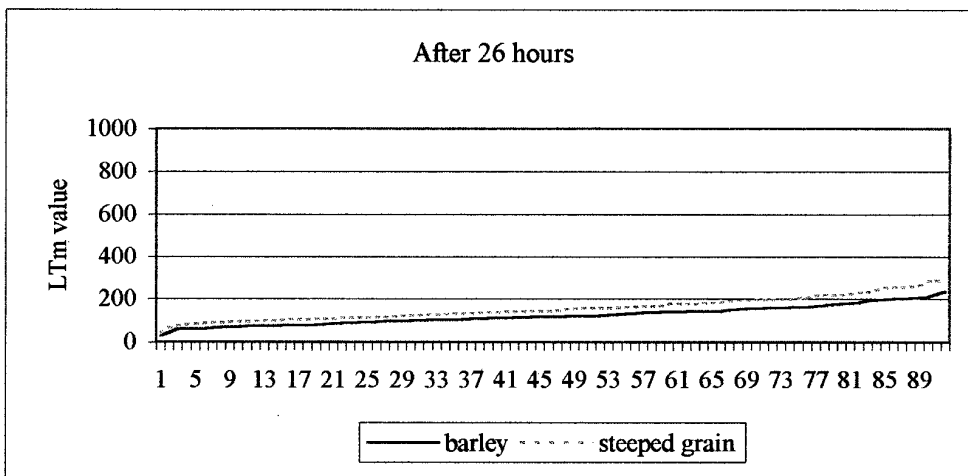
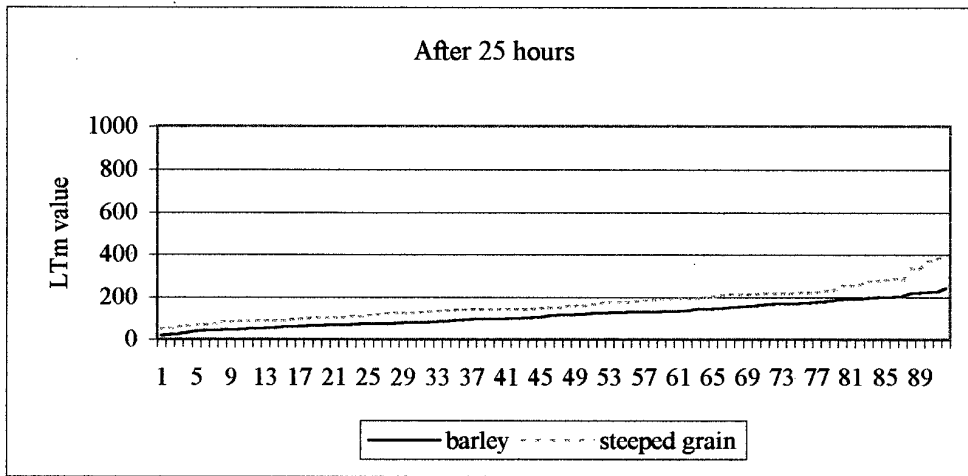
Figures 138-140 : LTm measurement during steeping (Regina)



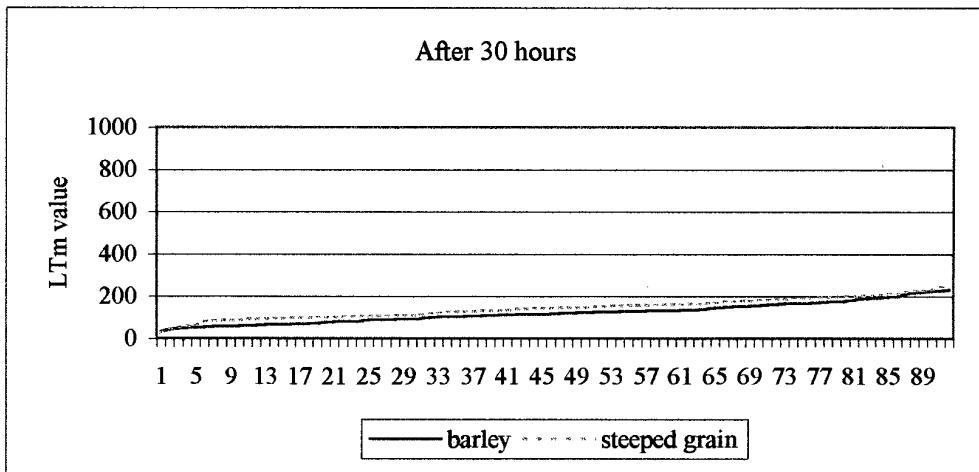
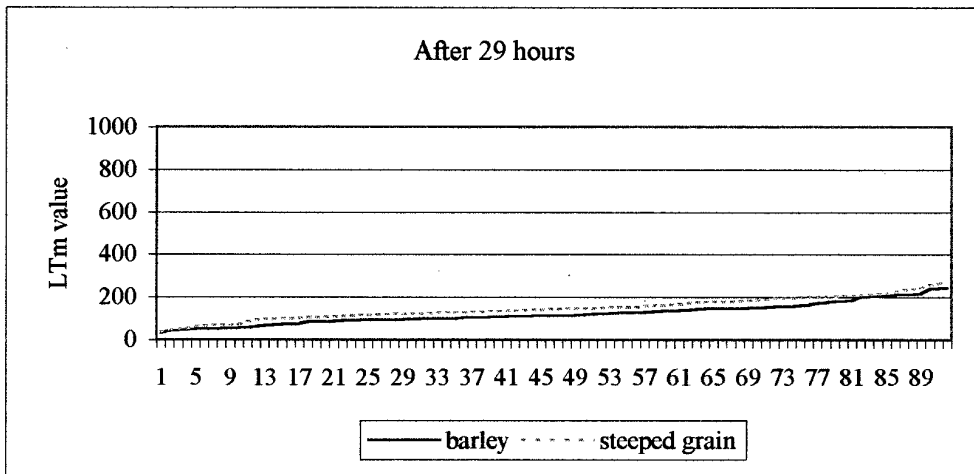
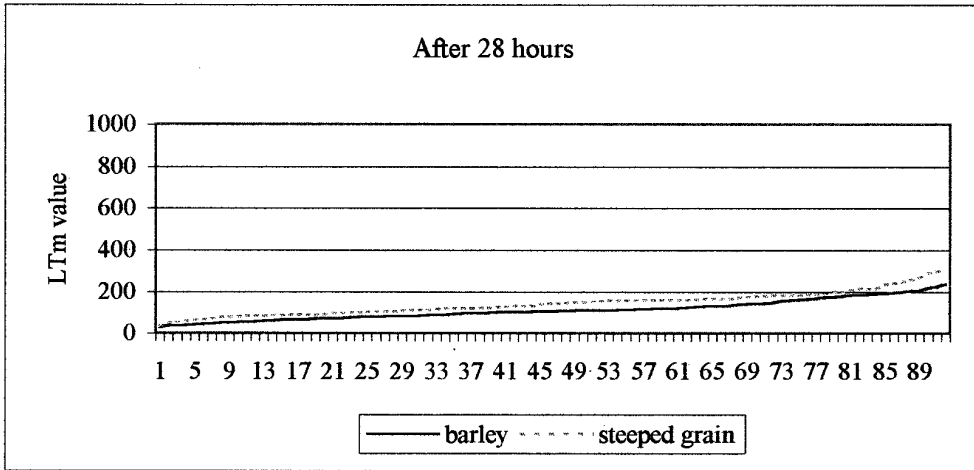
Figures 141-143 : LTm measurement during steeping (Regina)



Figures 144-146 : LTm measurement during steeping (Regina)



Figures 147-149 : LTm measurement during steeping (Regina)



Figures 150-152 : LTm measurement during steeping (Regina)

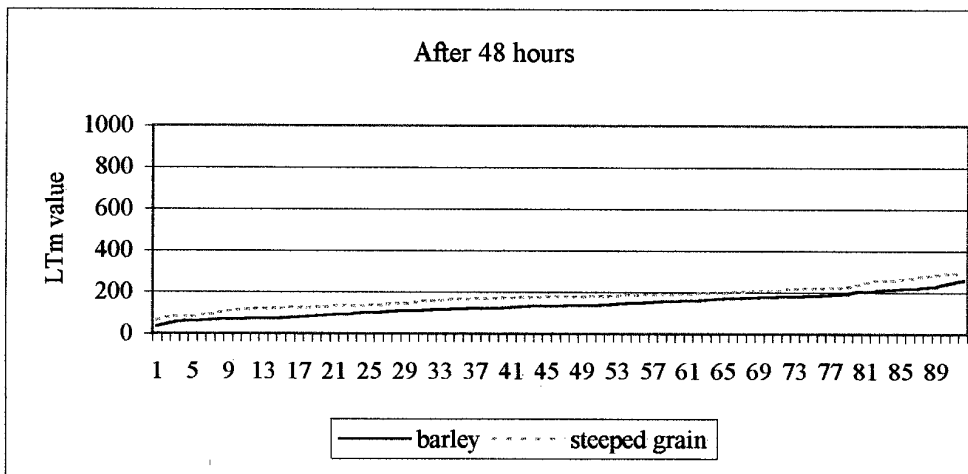
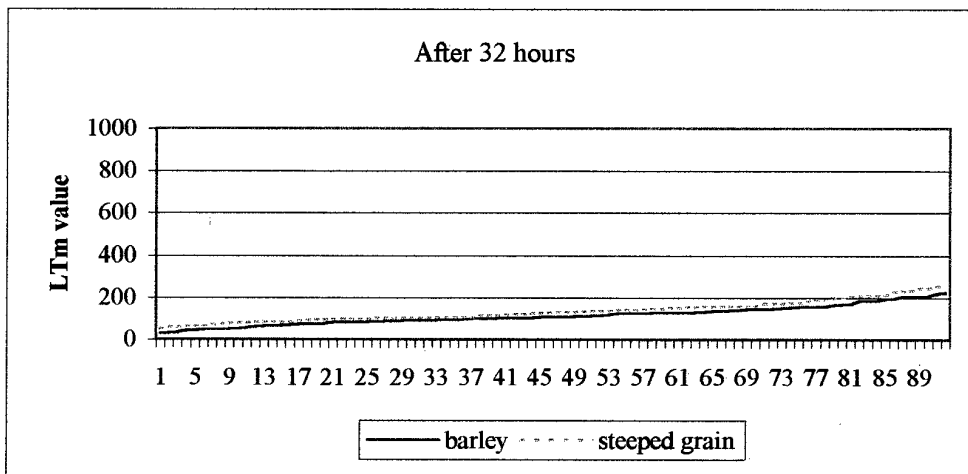
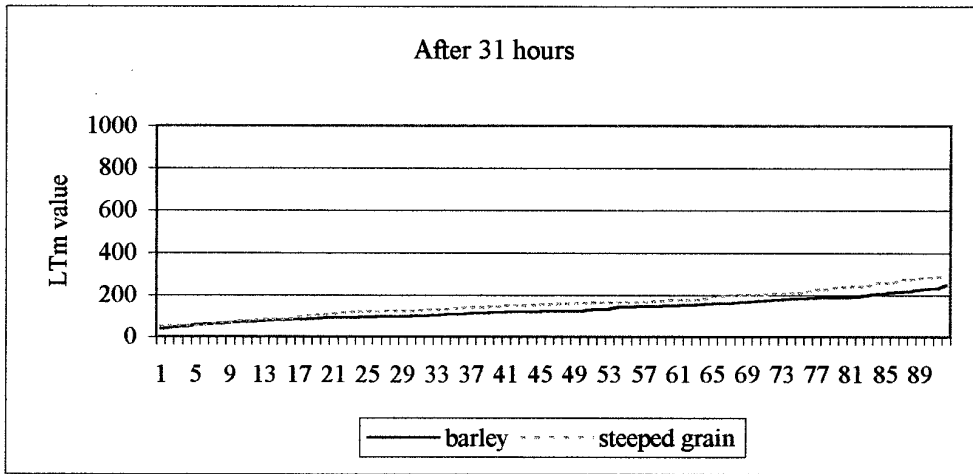


Figure 153 : L<sub>Tm</sub> measurement during steeping

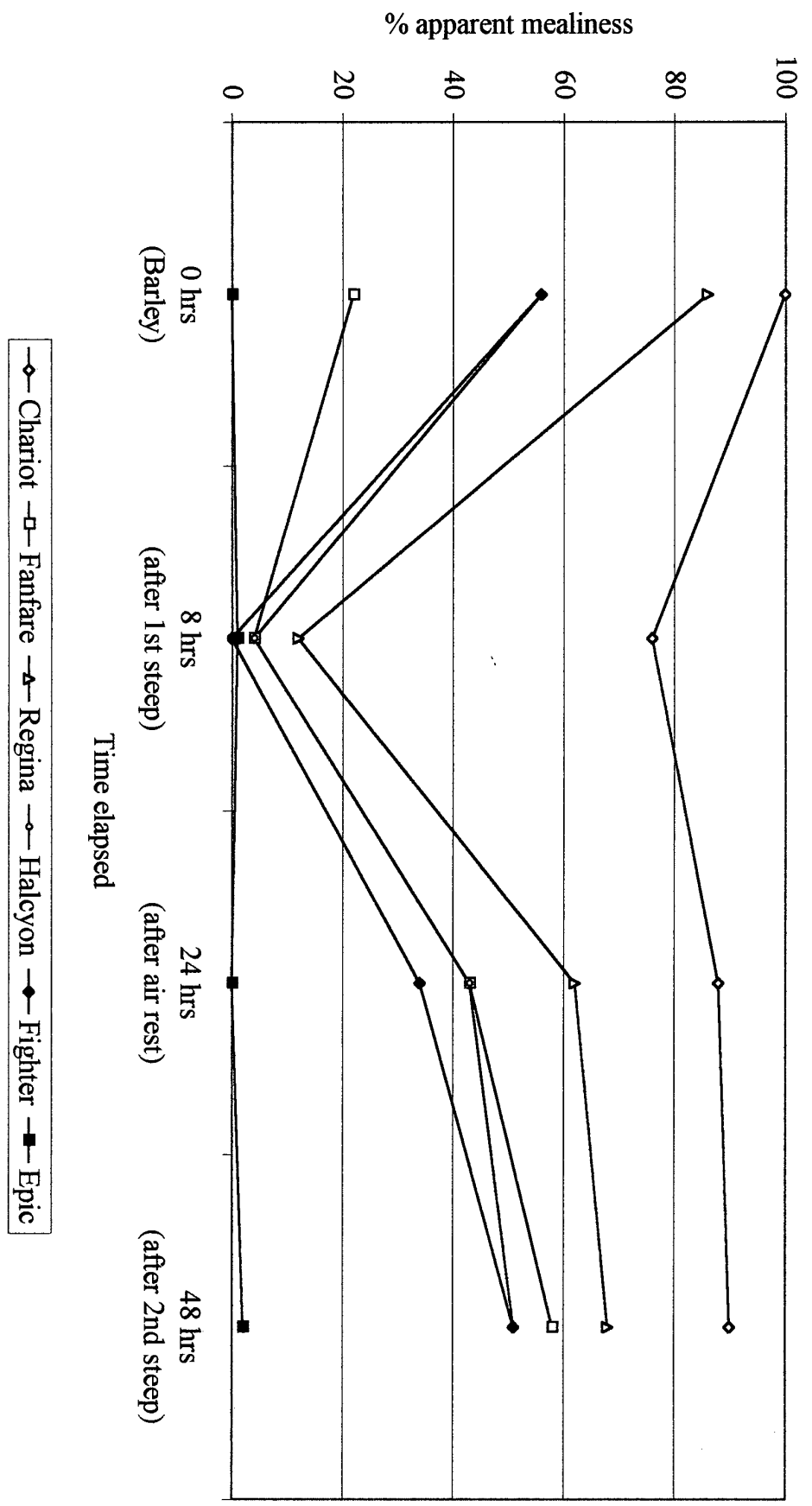


Figure 154 : Relationship between % apparent mealiness and Iodine Vapour Score

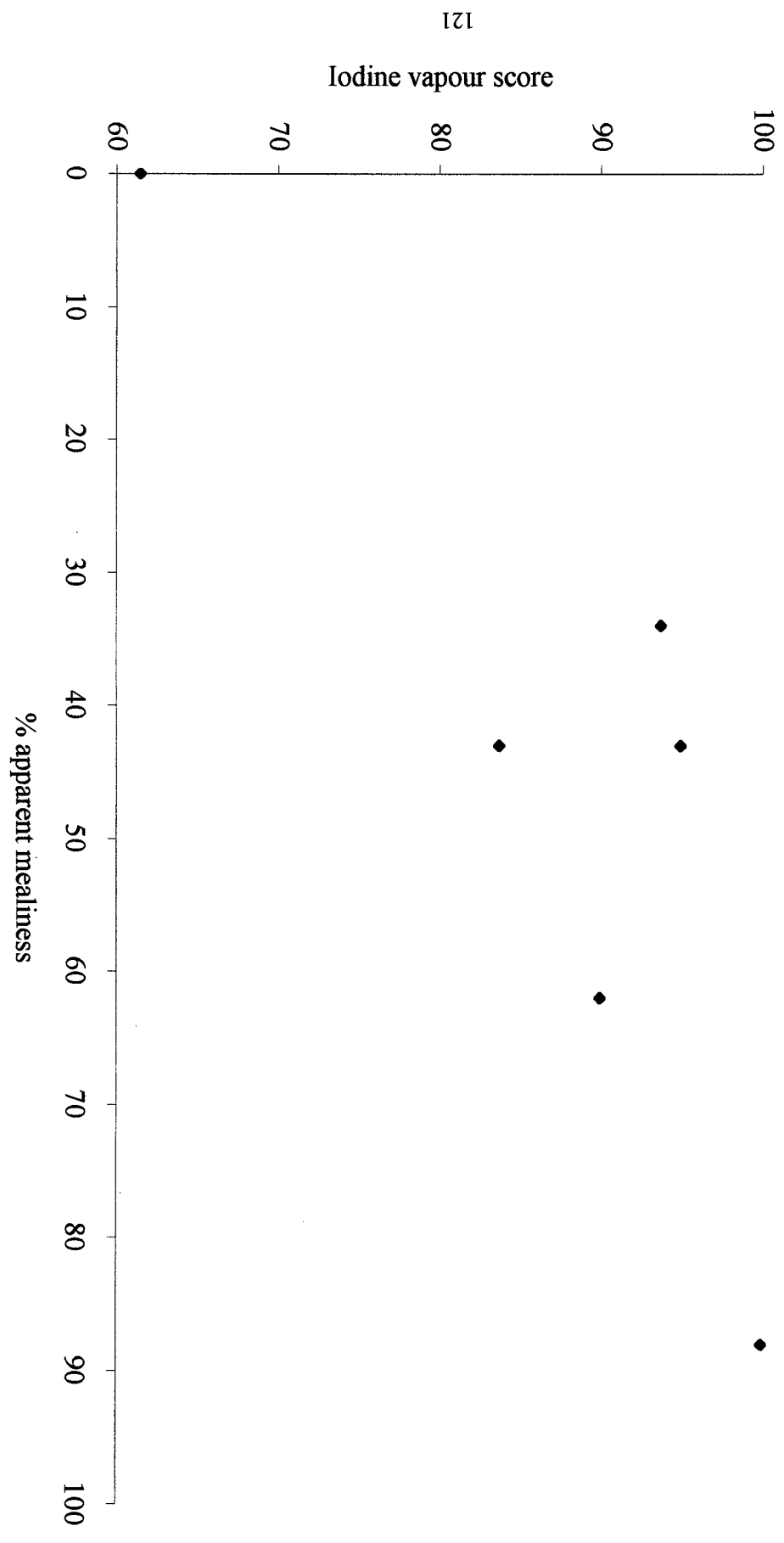




Figure 155 : Relationship between malt L<sub>Tm</sub> and Calcofluor modification

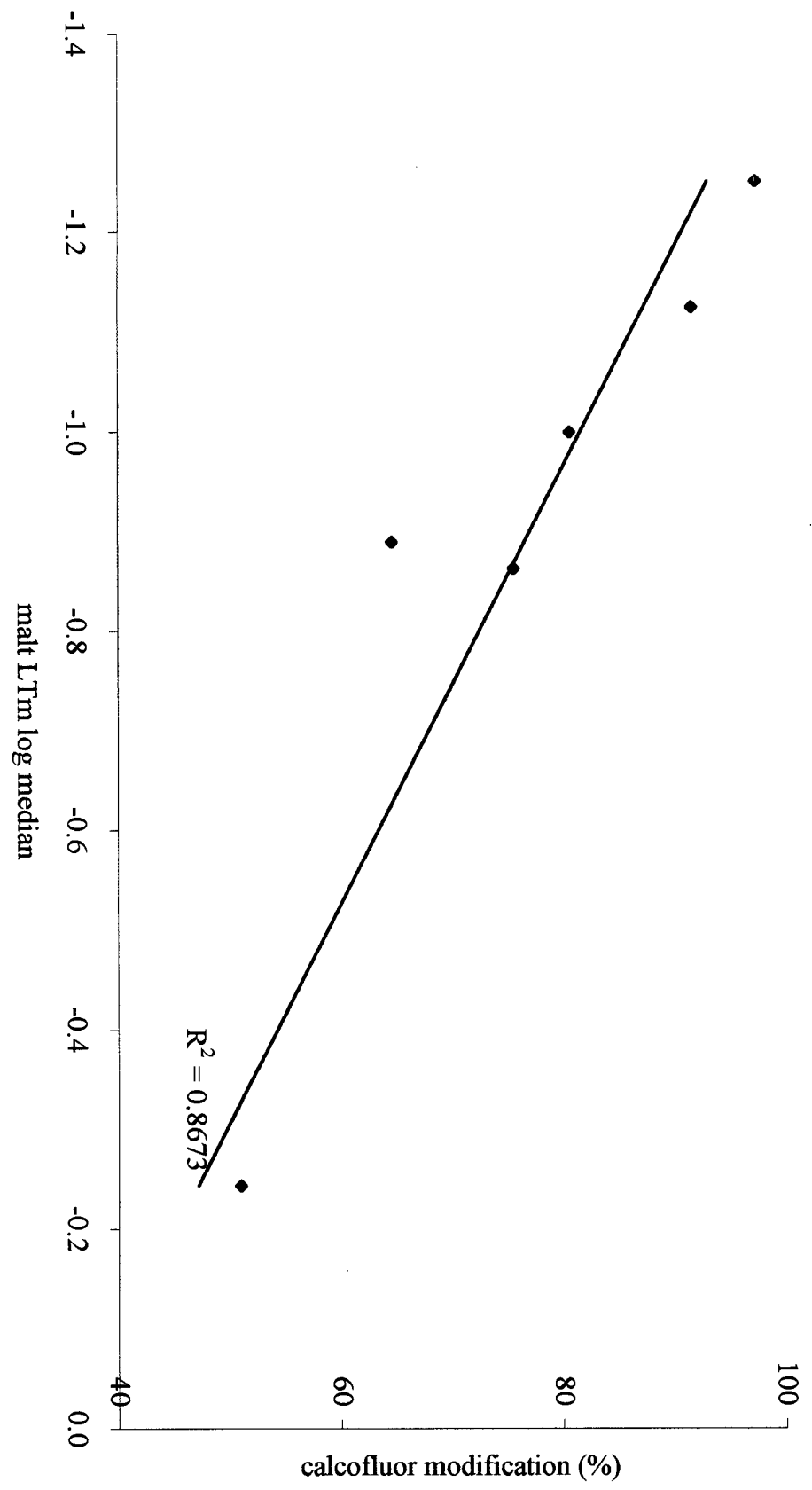


Figure 156 : Relationship between Extract and the proportion of chit malt

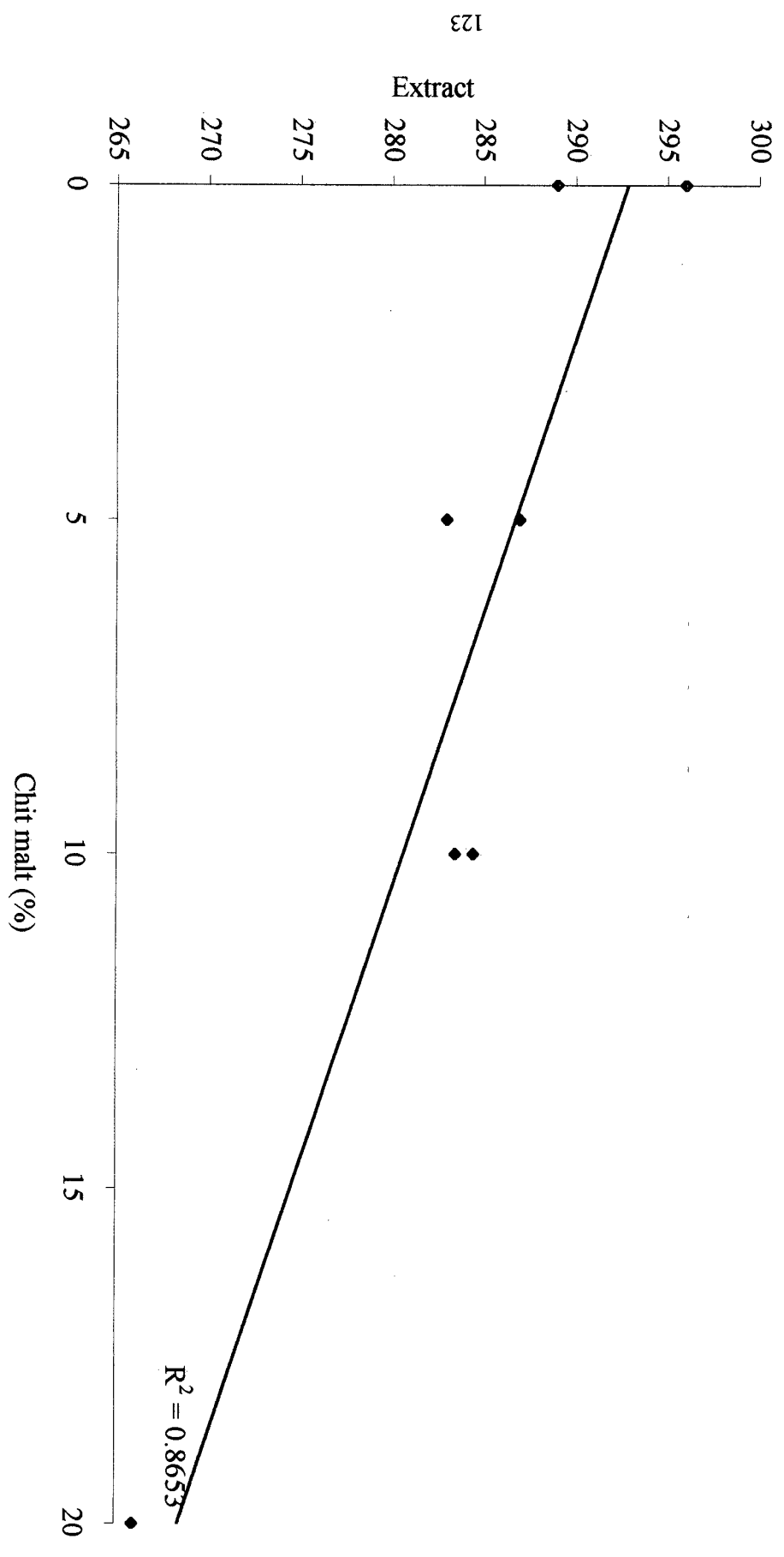


Figure 157 : Relationship between malt LTm and the proportion of chit malt

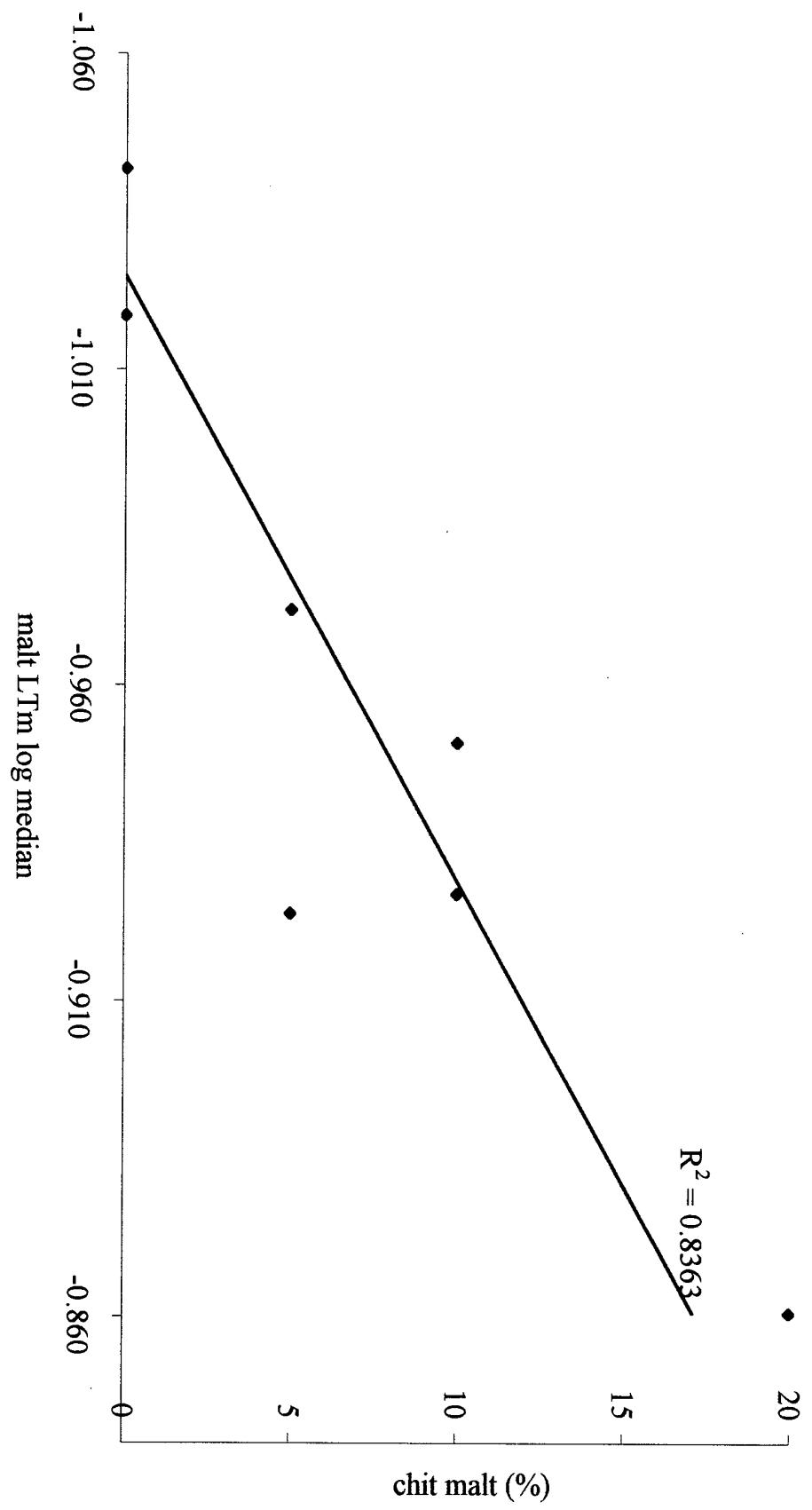
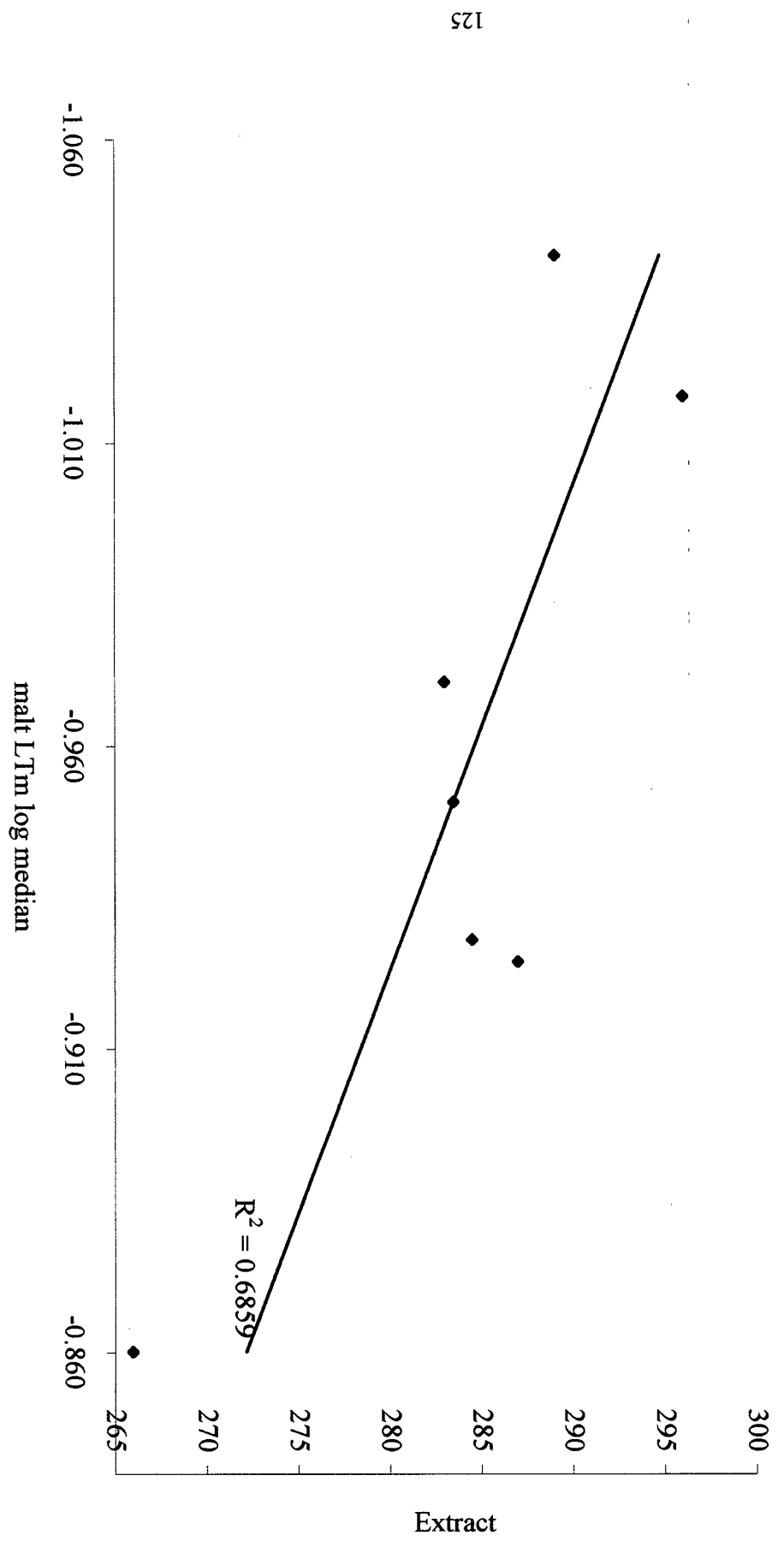


Figure 158 : Relationship between Extract and malt L<sub>Tm</sub> log median



## **APPENDIX A**

### **Preliminary investigation into characterising mealy and steely quality of barley by NIR**

**Fiona Bury  
10/11/98**

**Change Control: I. A. Cowe**

#### **SUMMARY**

It would appear that optical density may relate to malting quality parameters such as mealiness/steeliness of barley. From these preliminary experiments it may be possible to distinguish between mealy and steely barleys in the 850-1050nm region, although more samples with known mealiness scores would be necessary to take this work further.

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## **1. Objectives**

The objectives of this work was to investigate the possible classification of mealiness and steeliness of barley by NIR analysis.

## **2. Introduction**

Following a visit by Colin Eddison to the Brewing Research International facility (BRI), barley samples supplied by BRI and Foss were scanned on Foss Tecator instruments (NIRSystems 6500, and Infratec 1255 with single seed attachment) to determine the optimum wavelength for characterising mealy and steely barley samples.

### 3. Materials and Methods

Three samples (approx. 200g) were provided by BRI: -

Chariot - 80% mealy  
 Epic - >90% steely  
 Fanfare - process sample (intermediate)

A further fifteen UK barley samples were selected by variety from the Foss grainstore, giving a range of feed (steely), malt (mealy), and intermediate samples, in both spring and winter varieties. Five grains from each sample were cut and examined to quickly assess their quality (mealy or steely). The sample information is described in Table 1 below.

No.	Sample ID	Variety	Season	Quality	Quick Visual assessment of 5 grains.
1	BY0058	Pipkin	W	M	4 mealy, 1 steely
2	BY0049	Halcyon	W	M	Mealy, some with slight steely coat.
3	BY0131	Magie	W	I/M	Mealy
4	BY0167	Torrent	W	I	Mealy with steely coat.
5	BY0136	Fighter	W	I	Steely or steely coat.
6	BY0288	Intro	W	I/F	Mealy
7	BY0057	Bambi	W	I/F	Mealy
8	BY0050	Posuane	W	F	Mealy
9	BY0053	Fakir	W	F	Steely coat
10	BY0196	Camargue	S	M	Mealy
11	BY0192	Alexis	S	M	Mealy
12	BY0699	Vintage	S	I	Mealy/steely "variegated"
13	BY0207	Goldie	S	I	Steely
14	BY0187	Hart	S	F	Mealy/steely "variegated"
15	BY0102	Hart	S	F	4 mealy, 1 steely

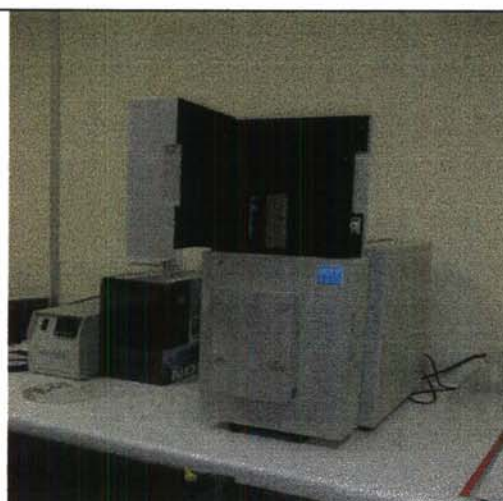
**Table 1. Foss barley sample information.**

These fifteen samples, along with the three BRI samples, were scanned on a NIRSystems 6500 in transmission, using the standard long rectangular cell as shown in Figures 1 and 2. Each sample was scanned twice using the same packing, then repacked and scanned twice more.



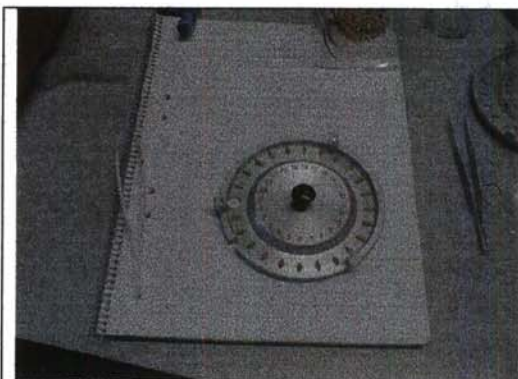


**Figure 1. 6500 rectangular cell.**



**Figure 2. 6500 instrument with cell.**

The three BRI samples, along with three Foss samples BY0058 (highest OD malt), BY0053 (lowest OD feed), and BY0699 (intermediate) were analysed on an Infratec 1255 with single seed attachment. The 1255 carousel (Figures 3 and 4) was filled twice giving 2 x 23 measurements for each sample.



**Figure 3. 1255 single seed carousel.**



**Figure 4. 1255 carousel in instrument.**

The spectral data was examined in Excel, and further data analysis was carried out in Unscrambler V6.1b.

## **4. Results and Discussion**

### **4.1 Visual Assessment**

The visual assessment carried out on the fifteen Foss barley samples was not a true test, as typically 100 grains should be examined by a trained eye. However, looking at five grains did illustrate that you cannot predict the quality of barley by the variety alone (as some samples that were classified as feed varieties appeared mealy, and vice-versa).

### **4.2 6500 data**

The data collected from the NIRSystems 6500 is shown below, in Figures 5 and 6. The colour coding used to identify the spectra is yellow for malting barleys (mealy), green for intermediate barleys, and brown for feed barleys (steely).

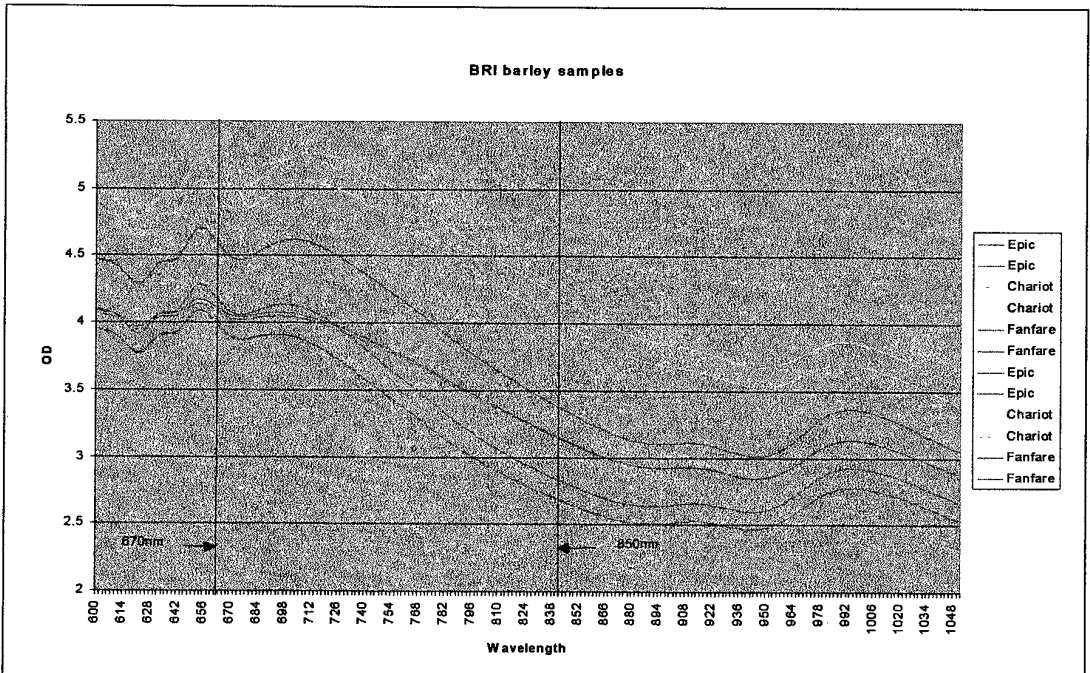


Figure 5. 6500 spectra of 3 BRI samples.

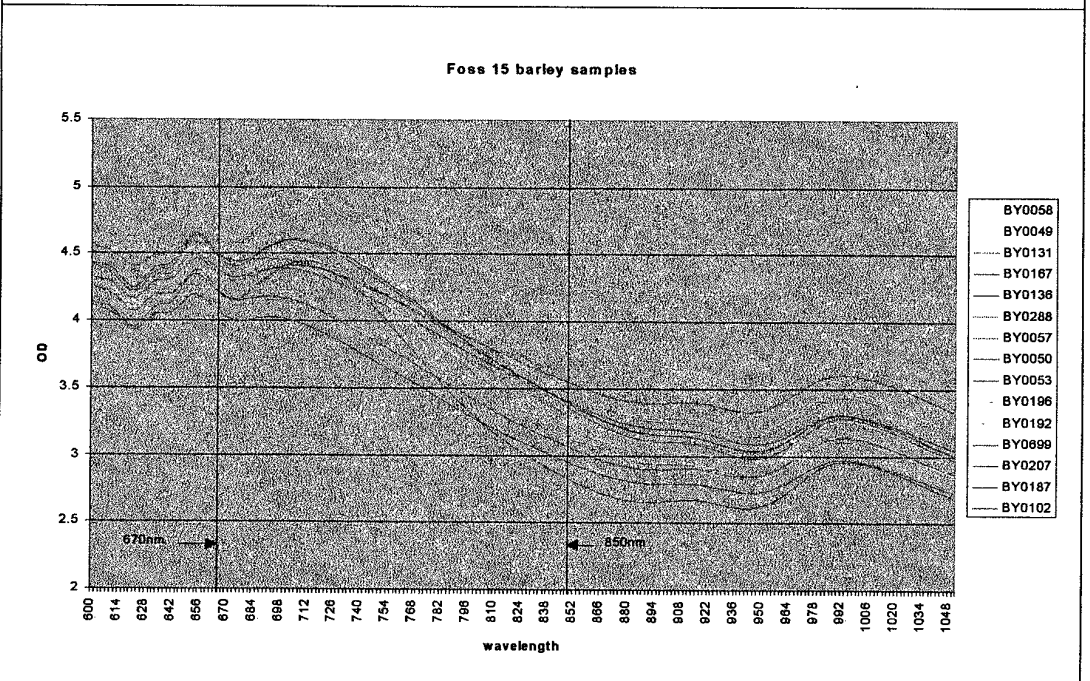


Figure 6. 6500 spectra of 15 Foss samples.

These plots illustrate that there is no unique information relating to malting quality at 670nm that cannot be measured elsewhere in the spectrum, and this does not distinguish between the different types of barley any more than the wavelengths currently measured by Foss instruments (850-1050nm). The three BRI samples (in Figure 5) clearly show a different optical density for mealy (highest) and steely

(lowest) samples, with the intermediate sample having an optical density between the two. It should be noted that the same samples can be measured very repeatably, however, repacking the sample can shift the optical density by up to 0.5 OD.

The fifteen Foss samples shown in Figure 6 have optical densities similar to those of the BRI samples, but the differentiation of malt and feed barleys is not so clear. Generally, the malting barleys have a higher OD, and the feed barleys are lower, but there is some crossover. These samples would need to be assessed properly (e.g. by examining 100 cut grains). Cutting the five grains shows that they are not always true to the varietal classification. This factor may also be confusing the picture.

### 4.3 1255 data

The spectra from the twenty-three single grains are shown in Figures 7-18 below.

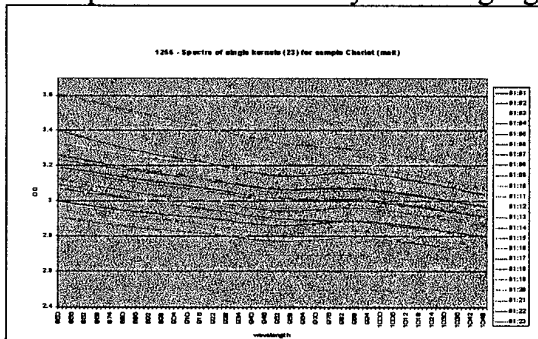


Figure 7. First 23 grains of Chariot.

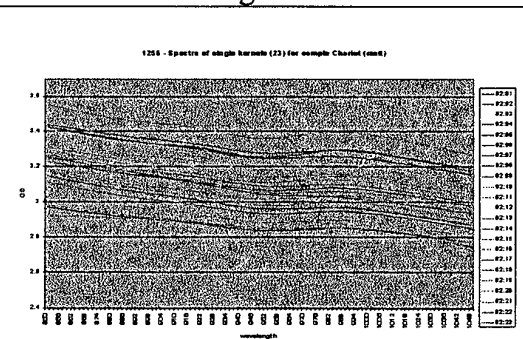


Figure 8. Next 23 grains of Chariot.

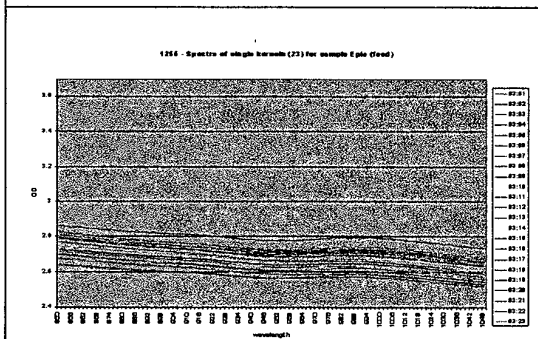


Figure 9. First 23 grains of Epic.

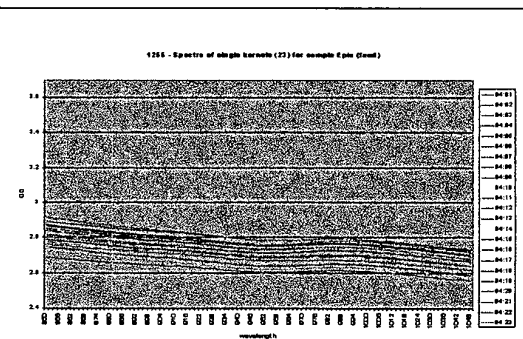


Figure 10. Next 23 grains of Epic.

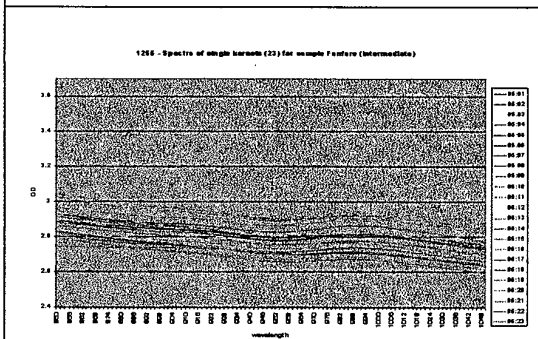


Figure 11. First 23 grains of Fanfare.

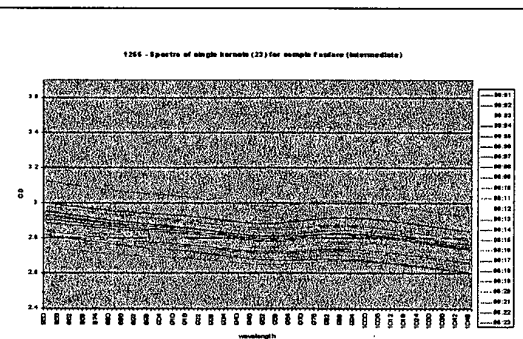


Figure 12. Next 23 grains of fanfare.

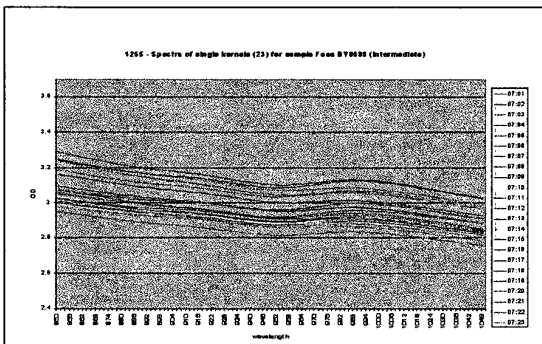


Figure 13. First 23 grains of BY0699.

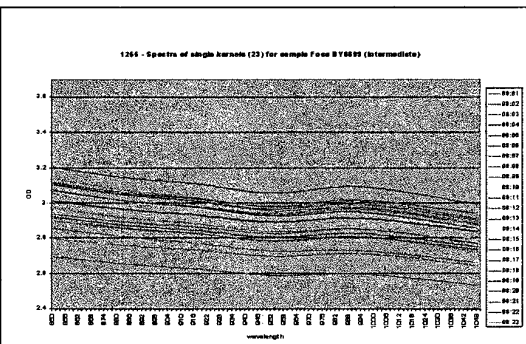


Figure 14. Next 23 grains of BY0699.

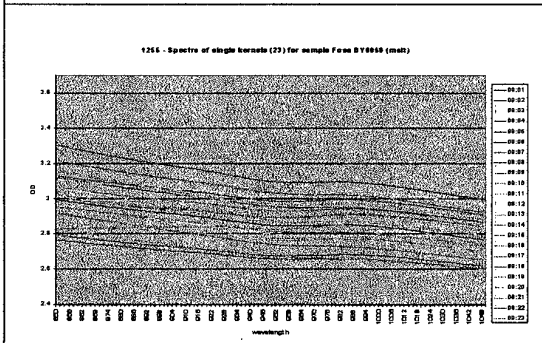


Figure 15. First 23 grains of BY0058.

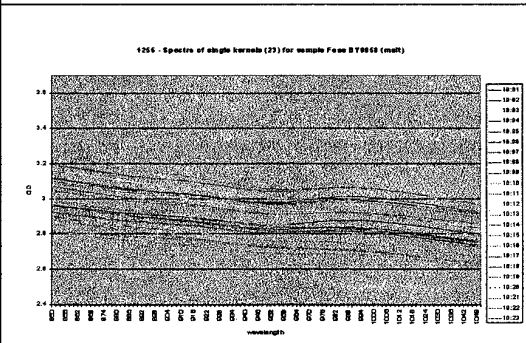


Figure 16. Next 23 grains of BY0058.

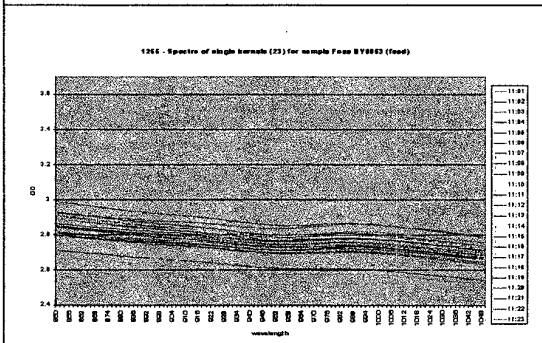


Figure 17. First 23 grains of BY0053.

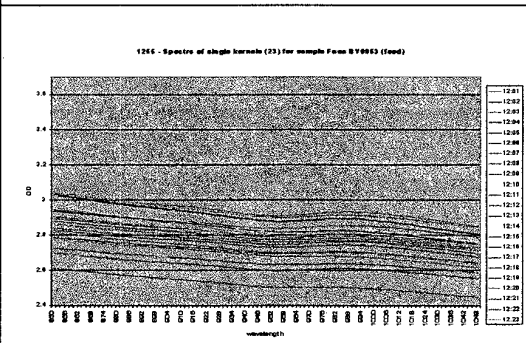


Figure 18. Next 23 grains of BY0053.

We see that the optical density again is higher for the malting barleys (Chariot and BY0058), and lower for the feed barleys (Epic and BY0053). The spread of optical densities over the twenty-three individual grains is up to 0.8 OD.

#### 4.4 Principal Component Analysis

The 6500 raw data for the repeats (2 repeats of 2 repacks) of the three BRI samples, and the fifteen Foss samples was combined in Unscrambler, and Principal Component Analysis (PCA) run on all 27 samples across the range 850-1050nm. Scores plots for early PC's are shown in Figure 19.

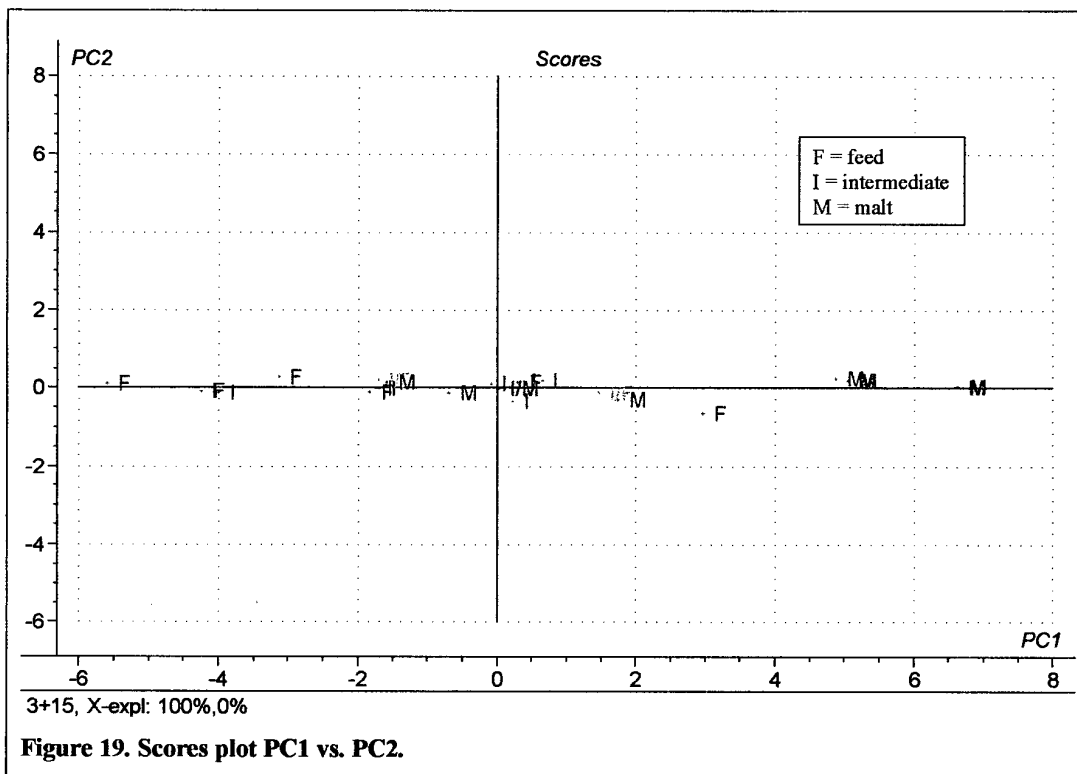
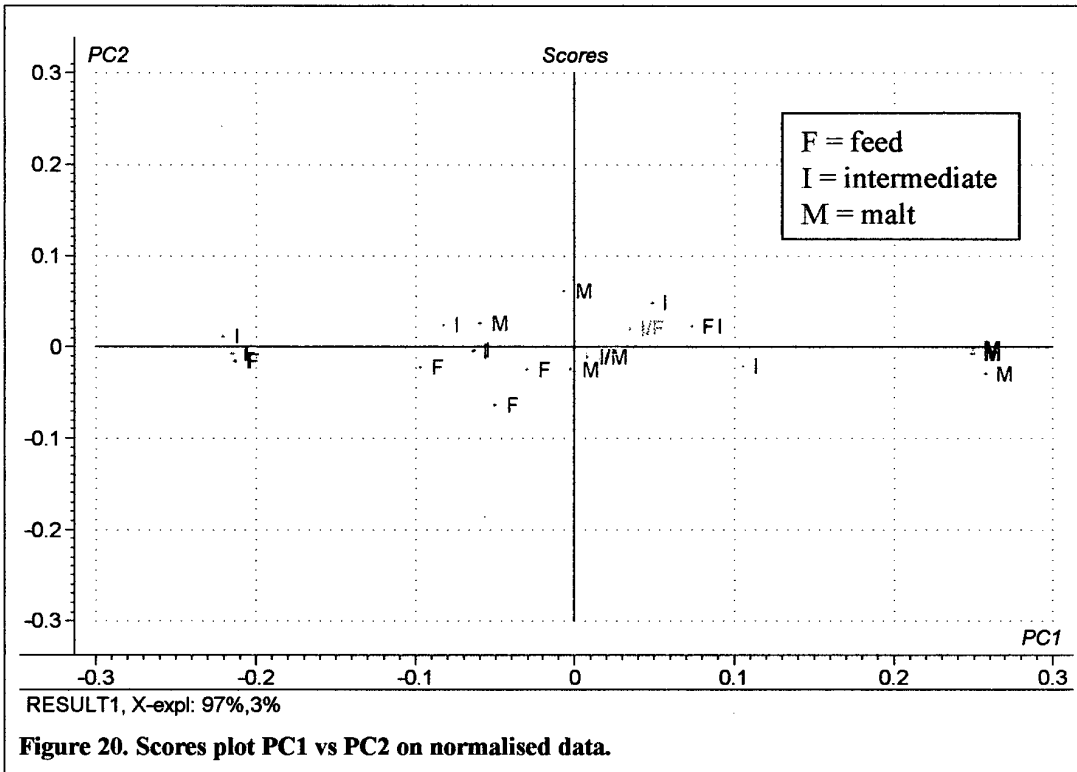


Figure 19 clearly shows the separation of malt and feed barleys. PC1 shows the greatest separation, which suggests that it is relating the optical density to the malting quality (as we identified from the raw spectra). We see that all the malting barleys are on the right hand side, apart from one sample BY0050 which is a feed barley (F), but which on visual assessment appears mealy (malt). The feed barleys are on the left, interrupted by one sample BY0136 classified as intermediate (I), but which visually appears steely. Later PC's showed no such clustering.

The Unscrambler data was then normalised between 850 and 1050nm (by dividing each point by the average for that spectrum), to scale the samples so they are centred around 1.0. PCA was then ran on the normalised spectra to see if it was still possible to differentiate between malt and feed barleys. The scores plot for PC1 vs. PC2 is shown in Figure 20.



The extreme right samples (Chariot and BY0058) are still differentiated as the more mealy samples, which agrees with looking at the raw data (they had the highest OD). The BRI feed sample Epic is the furthest left, and the nearest sample BY0136, although classified as intermediate, was visually assessed as steely. This suggests that malting quality can be estimated in the 850-1050nm region, not only on optical density.

## 5. Conclusions and Actions

From these preliminary experiments it may be possible to distinguish between mealy and steely barleys in the 850-1050nm region, although more samples with known mealiness scores would be necessary to take this work further.