PROJECT REPORT No. 100

THE EVALUATION OF METHODS FOR ASSESSING BARLEY VIABILITY

DECEMBER 1994

PRICE £5.00

PROJECT REPORT No. 100

THE EVALUATION OF METHODS FOR ASSESSING BARLEY VIABILITY

by

D. K. PARKER AND M. O. PROUDLOVE

Malt and Wort Production Team, BRF International,

Nutfield, Redhill, Surrey RH1 4HY

This is the final report of a four year project which commenced in April 1990. The work was funded by a grant of £186,764 from the Home-Grown Cereals Authority (Project No. 0001/1/90).

The Home-Grown Cereals Authority (HGCA) has provided funding for this project but has not conducted the research or written this report. While the authors have worked on the best information available to them, neither HGCA nor the authors shall in any event be liable for any loss, damage or injury howsoever suffered directly or indirectly in relation to the report or the research on which it is based.

Reference herein to trade names and proprietary products without stating that they are protected does not imply that they may be regarded as unprotected and thus free for general use. No endorsement of named products is intended nor is any criticism implied of other alternative, but unnamed products.

Contents

Page		
1	**Objectives**	
2	**Summary**	
3	**Abbreviations**	
4	1	**Introduction**
6	2	**Materials and Methods**
8	3	**Results and Discussion**
	3.1	ATP-bioluminescence as a measure of barley viability
	3.2	Malate dehydrogenase activity (MDH) as a measure of barley viability
	3.3	Single seed measurement
	3.3.1	MDH activity of single corns
	3.3.2	ATP content of individual corns
16	4	**Conclusions**
17	5	**Addendum**
	5.1	ATP content of single corns using a microplate luminometer
20	5.2	Membrane properties
21	5.2.1	Barley sterol measurement
22	5.3	Membrane permeability
23	5.3.1	Leakage of UV-absorbing material
24	5.3.2	Leakage of hydrogen ions
26	5.3.3	Leakage of potassium ions
28	5.3.4	Leakage of other ions
29	5.3.5	Conductivity measurements of seed exudates
31	5.4	Leakage of phosphatase enzymes
33	5.5	Conclusions
		References
		Figure Legends

Objectives

The aim of this project is to assess the suitability of novel techniques as rapid methods for measuring barley viability.

The technique must be simple, robust and rapid enough to be used at intake to the maltings.

Techniques under investigation involve detection of ATP - bioluminescence and measurement of specific dehydrogenase activity.

The accuracy of the measurement of viability is compared to viability determined using current IOB Recommended Methods.

Summary

A bioluminescence technique has been used to measure ATP extracted from barley. The results indicate a linear relationship between ATP and grain viability in barley samples of similar moisture content.

More ATP is extracted from barleys of higher moisture content and this increase is not due to enhanced microbial activity. The increase in ATP levels obtained after immersion of live grain was not seen with laboratory-damaged grain. Warm water immersion enhanced the extraction of ATP from corns but final levels extracted from undried barleys appeared to reflect structural differences between varieties rather than viability.

The differences in ATP levels (multicorn samples) between viable and damaged grain were too small, however, to be of use at intake.

The relationship between malate dehydrogenase (MDH) activity and viability was also investigated. Large differences in activity were seen between homogenates of live and damaged corn but, as in the case of ATP measurement, the problem of detecting a 1-2% difference in viability still applied.

It is clear that predicting viability will not be successful using a homogenate from a large number of corns. Any measurement of barley viability must be conducted on a corn by corn basis.

MDH activity is insufficiently selective for use as an index of barley viability. However, the ATP content of individual corns can be used to detect damaged grain amongst viable grain.

Abbreviations

ATP	-	Adenosine 5' - triphosphate
NADH	-	Nicotinamide adenine dinucleotide (reduced form)
NIAB	-	National Institute of Agricultural Botany
IOB	-	Institute of Brewing
GC	-	Germinative Capacity
GE	-	Germinative Energy
MDH	-	Malate dehydrogenase (E.C.1.1.1.37)
OAA	-	Oxaloacetic acid
TCA	-	Trichloroacetic acid

1. Introduction

Maltsters have expressed a need for a rapid, reliable test for measuring barley viability which can be carried out during intake to the maltings.

When barley arrives on site the maltster has a limited period in which to evaluate the quality of the corn. Moisture content, nitrogen content, screening, sieving and viability are just some of the vital tests performed on the grain.

Typically, a lorry load can be speared, the grain analysed and the results recorded within 30 minutes. The corn can be accepted or rejected on the outcome of the analyses.

Current rapid staining techniques [1] carried out at intake may overestimate viability, possibly due to heat-damage of grain during storage or drying [2]. This may result in the inadvertent purchase of reduced viability barley with subsequent financial loss.

The aim of this project is to identify reliable methods for use as a measure of barley viability.

During the first year of the project, the feasibility of using ATP-bioluminescence to measure viability was investigated.

Living cells contain ATP [3] and this can be measured using a luciferin/luciferase reaction to produce light. The amount of light generated by the reaction is directly proportional to the amount of ATP in the cells. The intention was to see whether this bioluminescence technique could be adapted to measure ATP as an index of viability. (It is well established that the ATP levels in microorganisms relates to their viability).

The IOB Recommended Method for rapid measurment of barley viability involves the staining of half-corns due to the reduction of a colourless tetrazolium salt to a red/pink formazan dye. The reducing power for this reaction is mainly present in the embryo and primarily comprises reduced coenzymes, such as NADH, which are products of dehydrogenase reactions.

The misleading results sometimes obtained with the tetrazolium test may be due to the relatively high stability of these coenzymes which may remain in the embryo long after the enzymes have been denatured, for example by heating.

A direct assay of the dehydrogenase enzymes, therefore, may reflect more closely the viability of the barley.

Malate dehydrogenase was selected for study as its reported levels were higher in barley than were other dehydrogenase enzymes [4].

2 Materials and Methods

Barley cultivars were NIAB and IOB Recommended List trial samples (winter and spring crops from 1989 and 1990). Undried barley samples from the 1990, 1991 and 1992 harvests were donated by Pauls Malt, Bass Maltings, United Distillers, Moray Firth Maltings and Albrew Maltings (now Carlsberg/Tetley Maltings).

Barley viability was assessed using both GC tests recommended by the IOB [1] : the peroxide-peeling test and the rapid staining method. Tests for GE were carried out on undried samples to assess levels of dormancy. Non-viable corns were prepared by heating pre-dried seed at 100°C for 20 hours. Non-viable corns used in individual determinations of ATP content and MDH activity were prepared by warming undried barleys in sealed foil bags at 45°C for several days.

ATP assay standards, reagents and methods of extraction and measurement from barley grains have been described previously [5]. All chemicals used were of the highest grade available.

Individual corns were crushed in 1ml TCA solution (0.05% (w/v)) in an Eppendorf tube. The contents were mixed and centrifuged for 3 minutes at 13500g. Extract and bioluminecence reagent (0.1ml) were added to a cuvette and placed in a luminometer. The light output was recorded and standardised with

the addition of ATP (1pmol).

The extraction and assay procedure for the measurement of malate dehydrogenase activity was as follows:

A sample of barley (25g) was ground in a coffee mill with cardice and further homogenised in 10ml phosphate buffer (0.1M, pH 7.5) using an Ultraturrax (Janke and Kunkel, Germany) at 2400rpm for 10s. The homogenate was centrifuged at 1000g for 10 minutes and the supernatant re-centrifuged at 600g for 3 minutes. The extract was kept on ice and diluted with ice-cold buffer before the assay.

The assay procedure for the measurement of malate dehydrogenase activity was that of Bergmeyer[6]. Diluted extract and NADH (0.235mM) were placed in cuvette (3ml) in a Philips PU8700 spectrophotometer. Baseline absorbance was measured at 340nm, the reaction was started by the addition of OAA (0.5mM) and the fall in absorbance monitored.

Individual corns were crushed and placed in 5ml phosphate buffer (0.1M, pH 7.5) and mixed using a Rotamixer. Extract (200μl) was placed in a microtitre plate well together with 15μM NADH (final concentration). The absorbance was read at 340nm using a Dynatech MR5000 microtitre plate reader. The reaction was initiated by the addition of OAA (0.5mM final concentration) and the reaction monitored by the drop in absorbance after 8 minutes. The absorbance values obtained with non-viable corns were compared to those for viable seed.

3 Results and discussion

3.1. ATP-Bioluminescence as a Measure of Barley Viability

A bioluminescence technique has been used to measure ATP extracted from a number of barley corns. No significant intervarietal differences were found between fourteen varieties of both spring and winter barleys of similar moisture content (10-12%) (Table 1).

There was, however, a significant decrease in ATP content when the grains were damaged by heating. Heat-damaged grain arriving at intake is rarely recognised as damaged until some months have passed. Samples of low viability grain were required, however, for use in these viability studies. Pre-dried grain appeared to be extremely resistant to damage using moderate wetting and heating regimes. A fairly severe treatment was necessary, therefore, to 'kill' the grain (100°C for 20 hours). Germination tests performed on the grain demonstrated that the treatment was successful in killing all of the grain (Table 2). There was, however, variable staining with tetrazolium, confirming that false positives can be obtained with this test.

The ATP content of the dead grain was less than half that found in live grain for all varieties.

Attempts to produce samples of differing viabilities proved problematic using small bulks. Live and dead barley samples were mixed, therefore, to obtain grain of calculated viability.

Extractable ATP from these samples correlated with viability (Figure 1) but the actual difference in ATP levels between barley of 95% and 100% viability were very small. Barley purchased for malting in the UK needs to be >95% viable (although some maltsters may stipulate a higher viability). Clearly, the measurement of ATP from an extract of a number of corns was not discerning enough for use at intake.

Undried barley, fresh from harvest, displayed higher extractable levels of ATP than were observed with dried grain (Figure 2). These higher ATP levels were found not to be the result of increased microbiological activity of microflora on the barley husk. Barley samples were washed with a microbial ATP extractant and the ATP levels compared to levels subsequently extracted from the barley. The amount of ATP extracted from barley microflora was <1% of levels extracted from barley.

The increase in ATP levels extracted from higher moisture grain was observed when dry grain was immersed in water (Figure 3). The ATP levels in damaged grain, however, were seen to decrease even though hydration followed a normal pattern (Figure 4).

Short immersion of grain appeared sufficient to enhance the differences between ATP levels extracted from live and dead grain. Figure 5 shows the results for one variety of barley (Nomad) immersed for 10 minutes at 40°C. The amount of extractable ATP from immersed, dead grain was not much greater

than that from unsoaked grain. The levels of ATP extractable from live, immersed grain, however, was enhanced six-fold.

More ATP was extracted from grain steeped at 55°C compared to 40°C. Samples of undried barley from the 1991 harvest were steeped for 10 minutes at 55°C and the levels of ATP extracted compared to viability as measured by the GC test (Table 3). There appeared to be little correlation between ATP levels and viability. Indeed, ATP levels seemed to relate more to variety than to any other parameter after immersion. This possibly reflects the differing physical characteristics of varieties, such as endosperm structure and tightness of husk, which may influence moisture uptake rate.

3.2 Malate Dehydrogenase (MDH) Activity as a Measure of Barley Viability

The initial rates of MDH activity for live and heat-treated grain (2.5g samples) are shown in Figure 6. Very little activity was displayed by heat-treated grain.

Samples of undried barley from the 1991 harvest were assayed for MDH activity and compared to viability, as measured using GC peroxide tests (Figure 7). A very weak correlation was observed between GC and MDH activity which is clearly inadequate for the screening of barley viability at intake.

3.3 Single Seed Measurement

Both the ATP and MDH assays appear unable to detect a 1-2% difference in viability using the existing protocols.

It is possible to calculate whether the precision required from the tests could actually be achieved using an extract from a number of corns (2.5g of ground barley represents around 50 seeds).

The likelihood of predicting the viability of a barley sample this way was studied using a spreadsheet. Table 4 displays a range of theoretical ATP values obtainable from the extraction of 100 seeds, homogenised together, with a ten-fold difference in the ATP content measured between live and dead corns. A variability factor of 5% is also included.

To distinguish between viabilities of, for example, 94% and 95%, the maximum ATP value given for 94% (993 units) must be smaller than the minimum value given for 95% (907 units). The results show this is not so and, therefore, the distinction could not be made.

Even if the ATP levels measured in live corns were 1000 times greater than those measured in non-viable corns, the distinction between consecutive viabilities could not be made (Table 5). Only if the errors involving the extraction and assay procedure were lowered to <0.2% , could the differentiation between viabilities be possible (Table 6). Variability

measurements as low as 0.2% would be impracticable. Indeed, the maximum number of seeds that could be extracted together to enable the resulting value to be related, with any certainty, to the number of viable seeds is, in this case, only 2 or 3 (Table 7).

This study confirms that any parameter measured as a potential indicator of viability must be conducted on individual corns.

3.3.1 MDH Activity of Single Corns

NADH absorbs at 340nm, whereas its oxidised form, NAD, does not. During the MDH assay, NADH is oxidised and MDH activity is measured by the subsequent drop in absorbance.
The activity of MDH was compared between viable and damaged corns. Samples of heat-damaged grain used in the previous studies were obtained by heating barley at very high temperatures. This severe treatment produced grain with a very low moisture content (2%) which is not representative of barley moisture intended for commercial use. Samples of damaged grain with higher moistures (16%) and a limited range of viabilities were obtained by warming undried grain in sealed foil bags (Table 8).

The sample held at 45°C for 72 hours had a moisture content of 16.3% and was used in all subsequent assays as a representative of non-viable grain.

High absorbance values (0.8-1.58) were obtained with damaged grain indicating low MDH activity (Table 9). Viable seed displayed lower absorbance values (<0.8) showing higher MDH activity. Two untreated corns gave high absorbance values and are apparently non-viable. One corn amongst the heat-treated seed appeared to have high MDH activity and is possibly a contaminant. It is unreliable, however, to directly compare MDH activities using final absorbance values as it does not allow for differences in the initial absorbance of the sample. A direct comparison of MDH activities can be made by measuring the drop in absorbance over 8 minutes. A high value will then indicate a high MDH activity.

For direct use in the microtitre plate system, smaller volumes are necessary. The volume of extracting buffer was reduced to 0.5ml and the absorbance drop at 340nm recorded for extracts from damaged and untreated corns. The results (Table 10) show the difference in MDH activity between viable and non-viable seed to be less marked than in the previous experiment. Activity of MDH measured for viable corns appeared to be consistent (0.5-0.6 absorbance drop) whereas the change in absorbance of non-viable corn extracts ranged from 0.25 to 0.72, giving variable MDH activiy.

Activity of MDH was measured on individual corns taken from a sample of barley (received from a maltings) which had only 75% GC.

The change in absorbance measured at 340nm ranged between 0.371 and 0.655 (Table 11). It was not possible, however, to select 12 or 13 potentially non-viable seeds from the 50 corns used. The differences in absorbance values obtained for low and high MDH activity corns were not sufficiently marked for absolute distinction.

Whether MDH activity is an appropriate enzyme to be studied as an indicator of viability is clearly questionable.

A sample of low viability barley (75% GC) was germinated over three days and the MDH activity measured in individual chitting and non-germinating corn (Table 12). Similar levels of MDH were measured in both germinating and non-germinating corn, suggesting that MDH activity was not sufficiently discriminative to be used as a viability measurement.

3.3.2 ATP Content of Individual Corns

There is a large difference in ATP levels present in viable and heat-damaged seeds measured individually (Table 13).

The method was repeated using a 75% GC barley sample (Table 14). Of twenty seeds, four corns could be selected with low ATP contents (denoted by *) which equated with 20% damaged seeds in the sample. Two other corns (*) displayed slightly lower ATP levels than viable seed suggesting an impaired germinative ability.

There were smaller differences in ATP levels between viable and naturally-damaged grain than were observed between laboratory-damaged grain and untreated grain but the differences were still sufficient to allow positive identification of damaged corn.

Large numbers of corns could be rapidly processed using a microtitre plate system. To fully complete the evaluation of this method, the ATP content of individual corns from the 1993 harvest should be measured using this system. In this way, the method could be judged to see whether it has the capability of identifying all damaged corns in a sample, thus providing a true viability measurement for use at intake.

4 Conclusions

ATP-bioluminescence can be used to measure ATP extracted from barley grain.

There is a linear relationship between ATP content and viability using homogenates from a number of corns. The differences in ATP levels between corns differing by 1% in viability is too small, however, for positive distinction. Differences in ATP levels between viable and laboratory damaged grain were sufficiently marked to allow non-viable grains to be identified on a corn by corn basis.

More assays need to be performed on grain of differing viabilities to evaluate the precision of this technique involving individual grain measurement (see Addendum).

The activity of malate dehydrogenase has been shown to be insufficiently selective for use as an index of viability, even on a corn by corn basis. It appears that the mechanism of damage to the grain during warming does not inactivate MDH completely.

The actual systems damaged by heating are unknown, but these studies show that grain can still possess active respiratory enzymes even though the mechanism for triggering germination within the grain has been lost.

5. ADDENDUM

5.1 ATP Content of single corns measured using a microplate luminometer

Grains were crushed and the ATP extracted as described in Materials and Methods (section 2.).

Extract (100μl) from 96 individual grains was pipetted into wells of a microtitre plate and placed into a microplate luminometer (EG+G Berthold, Model LB96P). Lumit-pm reagent (100μl) and ATP standard (1pmol) were injected automatically and a printout provided of light output per well before and after the addition of ATP. The amount of ATP present for each corn was calculated as before.

The accuracy of the procedure used to measure viability was assessed using barley samples which had been rejected from commercial maltings and were of differing viabilities.

5.1.1 Results and Discussion

Values calculated for ATP levels extracted per corn are presented for one of the barley samples (Table 15).

The indication is that non-viable corns could be selected using low ATP values (denoted by *).

A large degree of subjectivity still remained, however, in the selection of a cut-off point below which corns were considered non-viable. Nevertheless, delineators were chosen for each sample and the percentage of corns selected as damaged was calculated. The viability of the sample determined using this procedure was compared to the viability measured at intake to the maltings (using the rapid staining method[1]) and the current viability determined using the GC test[1] (Table 16).

Although the viabilities of the barley samples measured using the microtitre plate method were similar to those determined using the peroxide test, it was obvious that the differences in the ATP levels between viable and non-viable grain required enhancement.

The procedure was repeated with the grain steeped in warm water (40°C) for 30 minutes before ATP extraction. The ATP values obtained after steeping were greatly increased (Table 17) compared to the unsteeped values (Table 15). It was easier, therefore, to select non-viable corns (denoted by *).

The ATP values obtained for one particular reject sample were low before and after steeping (Table 18), suggesting that the grain was dead. This was confirmed by the results of the peroxide test, during which none of the corns germinated. This sample was chosen, therefore, to determine a threshold level of ATP, below which individual corns would fail to germinate.

The upper confidence limit (95%) was calculated (using $x + 1.96\sigma$) and this value (20.6) was used as a delineator. All corns with calculated ATP values ≤ 20.6 were considered to be non-viable. The percentage viability for each sample was redetermined and compared to the viability measured using the peroxide test (Table 19). A better correlation was observed between the measurements of viability using the GC test and the microtitre plate method (r=0.93) than using the tetrazolium staining method (r=0.89)(Figure 8).

5.1.2 Conclusions

The measurement of ATP extracted from individual grains can be used to select non-viable corns and, consequently, to determine the viability of a barley sample.

Greater accuracy is achieved if the sample is steeped before the ATP is extracted.

The period of steeping (30 minutes) and the measurement of ATP using the microtitre plate method ensures that this is a rapid test to perform. Currently, the most time consuming aspect of the test is the crushing of the corns, the extraction of ATP from each grain and the transfer of an equal amount of extract into the microtitre plate wells whilst ensuring minimum cross-contamination.

The extraction and assay procedures described for single corns are not performed to their optimum but are a compromise for

use in the microtitre plate system.

The small volumes used in the assay necessitate the use of a lower concentration of TCA for the extraction of ATP. This lower TCA concentration, in turn, extracts less ATP per corn. Higher TCA concentrations, however, will prevent the bioluminescence reaction from occurring and would, therefore, increase preparation and handling time because of the need to dilute each extract before measurement.

Nevertheless, the technique has demonstrated that ATP-bioluminescence can predict barley viability, although, currently, it is more expensive to perform than the existing intake test. The results presented (Figure 8) also demonstrate that the measurement of viability using ATP-bioluminescence is more accurate than measurements using tetrazolium staining.

5.2 Membrane Properties

Heat damage in grain may denature key enzymes and affect essential membrane constituents. It has been demonstrated, however, that some respiratory enzymes (such as malate dehydrogenase) survive in non-viable barley (see section 3.3.1). Aspects of membrane composition have therefore been studied using untreated and heat-treated corn.

5.2.1 Barley Sterol Measurement

Sterol measurement can be used as a vitality indicator for yeast[7]. Sterols are important membrane components involved in nutrient transport. A new, rapid method of measuring sterol content in membranes has been developed using a filipin complex which is measured spectrophotometrically[7]. Sterols react stoichiometrically with the antibiotic filipin, causing a characteristic change in the absorption spectrum of the filipin molecule (Figure 9). The magnitude of the change depends on the amount of sterol present.

Sterols comprise 2-3% of barley lipids which, in turn, allow for 3-4% of the barley dry weight. The rapid method for measuring yeast sterol levels was applied to determine whether the technique could be used as an index of barley viability.

Materials and Methods

Barley (2.5g) was ground in a coffee mill and homogenised in distilled water (10ml) containing Triton-X-100 (0.03g/l) using an ultraturrax.

The homogenate was ultrasonicated (20KHz, 0.3KW) for 15 minutes on ice and the sterols were extracted using n-heptane (10ml). The solvent was evaporated under pressure and the sterols resuspended in n-butanol (0.5ml). Buffer (5ml, 1M Tris; 0.03g/l Triton-X-100; pH7) and filipin complex (50µl, 2.2mM in dimethyl formamide) were mixed in a glass tube and the absorbance determined (340nm, 1cm quartz cell). n-Butanol (25µl) was added

to control tubes and barley sterol extract (25μl) was added to sample tubes. The tubes were incubated for 1 hour at 25°C and the absorbances (340nm) read again.

Results

There appeared to be very little difference in absorbance values after 60 minutes incubation for any of the sterol extracts from live grain or dead grain (Table 20). There also was no change in the absorption spectrum of filipin (Figure 10). It was concluded, therefore, that barley sterols were not extracted using this method.

Further preparation of the barley would be required to ensure sterol extraction and this would increase the time taken for the test to be completed. It was concluded that the measurement of barley sterols using this method would not be suitable as a rapid test for barley viability.

5.3 Membrane Permeability

It is well documented that a number of compounds leach out of imbibing seeds[8-15]. These compounds include inorganics[8] and organics[9-15]. Seed viability or vigour falls as the rate of solute leakage increases and this is associated with increased membrane permeability or loss of membrane integrity. The analysis of seed exudate has been used to assess seed quality[10] and seed viabilty[11,14,15].

5.3.1 Leakage of UV-absorbing Material

Organic materials which are known to leach out of steeped seeds include sugars[9] and amino acids[10]. Other compounds, such as phenols and nucleic acids (which can be measured spectrophotometrically at 260nm) may also leach out of seeds.

The absorbance value at 260nm, of seed exudates steeped for 6-10 hours, has been reported to be sufficient for the prediction of membrane integrity and seed viability of crops such as chickpeas and cotton[11].

The absorbance of exudate from live and dead corns was evaluated to see whether it could be used as an index of barley viability.

Method

Barley corns were steeped individually (1.5ml) for 20 minutes and the absorbance of each exudate read at 260nm (0.5cm quartz cell).

Results

The absorbance values for live and dead corn exudates are presented (Table 21). Statistical analysis confirmed that there was no significant difference between the mean values obtained from the samples ($p=0.05$) after 30 minutes or 3 hours.

Unlike the case with other crops[11], the absorbance value of barley seed exudate cannot be used as a rapid prediction of viability.

5.3.2 Leakage of Hydrogen Ions

Measurement of the pH of seed exudate has been used to predict the germination of soybeans[12].

Indicator dyes can be used to determine pH, with spectrophotometric measurement. The assay was adapted to assess rapidly the pH of a large number of seed exudates using microtitre plate technology.

The pH value of exudates from steeped corn was evaluated in live and dead grain.

Method

Barley corns were steeped individually (0.5ml) for 30 minutes. Cresol purple (10μl, 0.1%, (w/v) in 10% ethanol (v/v)) was added to wells in a microtitre plate followed by the addition of steep water (200μl). The absorbance values were read at 450nm.

Results

The absorbance values of the exudates from live, dead and 75% viable barley samples were determined using individual corns (Table 22).

Statistical analysis of the results (Student's T test, p=0.05) indicated that there were no significant differences in the mean values obtained for exudates from 75% viable corn compared to the live or dead samples.

The partition value to distinguish viable and non-viable

soybeans is reported to be pH 5.8[12]. Barley corns were steeped in different pH solutions (phosphate buffer, 0.1M, pH 5.0-6.8) and the absorbances were measured after the addition of cresol purple (100μg). Again, no significant differences were observed between exudates from live and dead corns (Figure 11).

Barley appears to have excellent buffering capacity and it was postulated that different ions may be actively pumped out of the grain in response to different external pH, particularly those outside the pH range encountered naturally by barley in the field.

Corns from live and dead barley samples were steeped in acidic conditions (0.1M citrate buffer pH 3.0) and in alkaline conditions (0.1M phosphate buffer pH 10.0). Bromophenol blue (100μg) was used as the indicator dye for exudates steeped at pH 3.0 and thymol violet (100μg) for the measurement of exudates at pH 10.0. A spectral scan of bromophenol blue gave absorbance maxima at 436nm and 592nm. Mean absorbance values obtained at 405nm and 570nm (filters available for microplate reader) for exudates of live and dead corn were not significantly different (Student's T test, $p=0.05$) whereas those measured at 630nm were significantly different (Table 23).

At pH 10.0, the scan of thymol violet gave absorbance maxima at 293nm, 420nm and 594nm. There were no statistical differences in absorbance measurements of exudates from live and dead corn at either 570nm or 630nm (Table 24).

Overall, the results indicate that there is little difference in the pH of exudates from live and dead barley grains. Some potential for the prediction of seed viability, however, may lie in the determination of exudate absorbance from grain steeped at pH 3.0 and measured spectrophotometrically (630nm) using bromophenol blue as the indicator dye.

The absorbance values obtained for the exudates were low, however, and the differences between exudate pH from live and dead corns were very small (Figure 12). It was considered that the distinction between viable and non-viable corns using the pH of seed exudate was insufficient for a direct measurement of barley viability.

5.3.3 Leakage of Potassium Ions

Large amounts of potassium are required for normal plant growth[13]. Potassium has a general function in the regulation of water in plant cells and serves as an osmotic adjustor[13]. Certain enzymes have also demonstrated a requirement for potassium[13].

Leakage of potassium ions has been reported in imbibing seeds[8] and it was postulated that potassium leakage may be greater in 'dead' corn, which may reflect damaged membranes or increased permeability.

Method

Viable and dead (heat-treated at 45°C for 3 days in sealed bags) barley samples (25g) were steeped (50ml) for 16 hours and the potassium ion concentration in the steep water was determined using an ion-specific electrode (Orion potassium electrode, Model 93-19). The electrode was calibrated using standard solutions of potassium chloride.

Results

The calibration curve for the electrode shows a linear response over the range 0.01-100mM KCl (-140 to +50mV)(Figure 13).

Higher concentrations of potassium ions leached out of non-viable corn compared to viable corn (Table 25) when barley samples were steeped *en masse*.

For the accurate determination of viability, measurements must be conducted on a single corn basis. The electrode, however, required a minimum steep volume of 30ml in a 50ml beaker to accommodate the potassium electrode, the reference electrode and a thermometer. The concentration of potassium ions leaching out of a single corn into 30ml water was too low to distinguish between individual live and dead corns, even after employing a special low level calibration (Figure 14). This type of electrode, therefore, is not suitable for the measurement of barley viability based on potassium ion leakage.

5.3.4 Leakage of other ions

The presence of other ions leaching out of grains during steeping was determined using ion-exchange chromatography (Dionex). The concentrations of monovalent cations (potassium (K^+), sodium (Na^+) and ammonium (NH_4^+)) and divalent cations (magnesium (Mg^{++}) and calcium (Ca^{++})) were measured in leachates from viable, non-viable (heat-treated) and 75% viable barley samples (25g in 50ml water).

Results

All of the cations investigated were found to be present in barley leachate (Table 26). Of those cations, 85-90% comprised potassium and higher levels leaked out of heat-treated corn. The concentrations of ammonium and magnesium ions were also higher in leachates from dead corns, but the differences were not significant when compared to potassium leakage.

The analysis of leachates from single corns by chromatography, however, is impractical. A method for determining ion leakage from single corns is required to assess the potential of the method for measuring barley viability.

5.3.5 Conductivity measurements of Seed Exudates

The previous observations concerning ion leakage demonstrate that varying concentrations of ions leach out of seeds depending on their physiological status. By applying a small electrical voltage to the leachate, the size of the resultant current will reflect the concentration of ions in solution and, therefore, the viability of the seed.

The electrical conductivity of seed exudate has been used to detect membrane leakage and seed viability in various crops[11,14,15].

Electrolyte leakage from single seeds can be determined using an automatic seed analyser[14,15]. Individual seeds are placed in a soaking tray containing 100 small cells. Water is added to the tray and the current determined in each cell using a multi-electrode head. High electrical currents indicate serious leakage and possible damage.

To predict the germination of a seed lot, a theoretical breakpoint in current level is selected that will divide the population into 'live' and 'dead' seeds. Current levels above this partition value indicate 'dead' or 'non-viable' seeds.

Method

A selection of barley samples rejected from commercial maltings was steeped and the conductivity of each corn exudate determined using an Automatic Seed Sorter (Model ASA 610, Agro Sciences Inc., Michigan). Barley samples were sieved over 2.5mm to overcome differences in grain size and the same volume of water (2ml) was added to each cell.

Results

There was little correlation between viability measured by conductivity after 30 minutes steeping and the actual viability of the barley samples even when a number of partition values were used (Table 27).

Generally, higher conductivities were present in cells containing low viability corns (corns were germinated on moist filter paper after the assay), but one sample had very low conductivity values and was competely non-viable (Table 28). It was considered, therefore, that the measurement of viability using the conductivity of the leachate was not suitable for barley.

These results corroborated findings of other authors who highlighted problems associated with the routine application of conductivity measurements of individual seeds[16] and the failure to predict *Brassica* seed germination using conductivity measurements[17].

5.4 Leakage of Phoshatase Enzymes

Phosphatases hydrolyse a variety of orthophosphate esters. These enzymes are important for the hydrolysis of phosphate-containing compounds to provide a free phosphate pool for metabolic pathways[18].

Phosphatases have been localised in the cytoplasm of aleurone cells and are associated with intracellular membranes. Leakage of phosphatases was investigated as a possible sign of membrane damage in non-viable seeds.

Method

Samples of live and dead (heat-treated at 45°C for 3 days in sealed bags) barley were surface sterilised for 20 minutes in sodium hypochlorite solution (1% (v/v)). Traces of chlorine were removed by washing the grain with acid (1mM HCl) and the acid was neutralised by rinsing with sterile water until grain suface pH was pH 5-6.

Corns were plated onto nutrient agar containing *p*-nitrophenol phosphate (0.1% (w/v) in 0.05M glycine buffer pH 9.0) under aseptic conditions.

Live and dead whole corns were selected, together with half corns, corns with embryos absent, huskless corns and artificially damaged corns. The proximal ends were embedded into the agar.

After 5 hours incubation (14°C), the plates were examined. Phosphatase leakage was visualised by the presence of a yellow ring (*p*-nitrophenol) around the grains.

Results

Figure 15 shows phosphatase leakage from live barley corns and Figure 16 from dead barley corns.

The results demonstrate clearly that phosphatase enzymes only leak from physically damaged corns. Whole intact corns, whether dead or alive, do not appear to leak phosphatases.

Phosphatase enzymes, however, do survive in heat-treated, non-viable grain (cf. MDH) and will leak out if the grain has been physically damaged.

Interestingly, the leakage of sugars from seed also correlates with mechanical damage rather than seed viability[9].

The leakage of phosphatases from whole grain, therefore, whilst not suitable as an indicator of viability, could have potential for determining the percentage of damaged corns in a sample. In this way, the extent of post-harvest damage or the efficiency of the abrasion process could be monitored.

5.5 Conclusions

The objectives of this project were to assess the suitability of various *rapid* methods for measuring barley viability.

The ATP content of individual corns appears to be a good indicator of viability. Currently, however, a method for the extraction of ATP from a large number of barley corns individually, yet simultaneously, is required for this method to be suitable for use as a rapid viability test at intake.

Measurement of grain constituents or potential damage for the prediction of barley viability proved to be less successful.

The rapid method used to determine yeast sterol levels was not suitable for the extraction of barley sterols. Further extraction preparations would increase the time taken to complete the assay. The measurement of single corns would also be very time consuming. It was considered that this method was not a practical proposition for the determination of barley viabilty.

The leakage of various compounds from seed during imbibition has been used to predict germination in a number of plant species. Although the determination of leachate parameters, including the absorbance value at 260nm, the pH and conductivity measurements, have been used successfully to predict germination in various crops, none appeared to correlate with barley

viability. The reason for this failure to mimic the results obtained with other seeds could be the presence of the fused testa/pericarp and husk on barley. The husk constitutes between 9-14% dry weight barley and is lignified and abrasive because of silica in the outer epidermis. The husk and pericarp protect the inner, living tissues and resist swelling during imbibition[15]. The seed-coat (testa) and surrounding layers (1-3%) make up a selectively permeable system and divides the interior from the exterior of the grain. Salts, sugars and microbes do not pass through this layer in healthy, undamaged grain and nutrients are reported not to pass out[15], although this work appears to refute this statement. Generally, it can be considered that barley is unlike other seeds, particularly because of the presence of the husk. The husk appears to restrict water and ion movement and may act to buffer leachate. Determinations of viablity which involve measuring components of barley leachate, therefore, appear unlikely to provide an accurate prediction of viability.

References

1) The Institute of Brewing, Recommended Methods of Analysis, London, 1982.

2) Anon. Malting Barley News (1990), 12, 2.

3) Stanley, P.E. (1989). Society for Applied Bacteriology p1-10, Technical series 26, Blackwell Scientific Publications.

4) Mandl, Von B., Wullinger, F., Fucher, A. and Piendl, A. Brauwissenschaft, (1970) 23, 175.

5) Parker, D.K. and Proudlove, M.O. Journal of the Institute of Brewing, (1993) 99, 327.

6) Bergmeyer, H.U., Methods of Enzymic Analysis, (1963) Academic Press,

7) Rowe, S.M., Simpson, W.J. and Hammond, J.R.M. Letters in Applied Microbiology, (1991) 13, 182.

8) Lott, J.N.A., Cavdek, V. and Carson, J. Seed Science and Technology (1991) 1, 229.

9) Abdul-Baki, A.A. and Anderson, J. Crop Science, Jan./Feb. (1970) 10, 1, 31.

10) Hendricks, S.B. and Taylorson, R.B. Plant Physiology (1976) 58, 7.

11) Deswal, D.P. and Sheoran, I.S. Seed Science and Technology (1993) 21, 179.

12) Peske, S.T. and Amaral, A. Seed Science and Technology (1986) 14, 151.

13) Rains, D.W. Plant Biochemistry, 3rd Edition. Edited by Bonner, J. and Varner, J.E. Academic Press (1976) 569.

14) Steere, W.C., Levengood, W.C. and Bondie, J.M. Seed Science and Technology (1981) 9, 567.

15) Siddique, M.A. and Goodwin, P.B. Seed Science and Technology (1985) 13, 3, 643.

16) Hepburn, H.A., Powell, A.A. and Mathews, S. Seed Science and Technology (1984) 12, 2, 403.

17) Thornton, J.M., Powell, A.A. and Mathews, S. Annals of Applied Biology (1990) 117, 129.

18) Akiyama, T. and Suzuki, H. Z. Pflanzenphysiol. Bd. (1981) 101, 5, 131.

19) Briggs, D.E. Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology, Edited by Shewry, P.R., The Alden Press Ltd. (1992) 369.

Figure Legends

Figure 1 ATP extracted from barleys of differing viabilities.

Figure 2 The contribution of microbial ATP to extracted ATP levels from undried barleys.

- -△- Barley ATP
- -○- Microbial ATP

Figure 3 The effects of immersion on ATP levels extracted from dry barley.

- -□- ATP content of barley
- -○- Moisture content of barley

Figure 4 Water uptake and ATP release in dead grain.

- -□- ATP content of barley
- -○- Moisture content of barley

Figure 5 The effect of immersion for 10 minutes at 40°C on ATP levels extracted from live and dead barley.

Figure 6 Malate dehydrogenase activity from live and dead barley.

- -□- Live grain
- -○- Dead grain

Figure 7 MDH activity present in undried barley of differing viabilities.

Figure 8 Comparison of viability measured by tetrazolium and ATP content.

- ——— - Expected relationship
- ○ - Viability measured by tetrazolium
- ▲ - Viability measured by ATP content

Figure 9 Spectral changes due to the sterol-filipin reaction.

——— 0 minutes

- - - - 60 minutes

Figure 10 Sterol-filipin extracion from live and dead barley (no difference between lines for live and dead barley at 0 minutes and 60 minutes).

Figure 11 Calibration curve for cresol purple (100μg).

pH of exudates from live corn - ● -

pH of exudates from dead corn - ■ -

Figure 12 Calibration curve for bromophenol blue (100μg)

pH of exudates from live corn - ○ -

pH of exudates from dead corn - ■ -

Figure 13 Calibration curve for Potassium Selective Electrode

Figure 14 Low level calibration curve for potassium ion measurement.

- □ - Calibration curve
- ▲ - Potassium leakage from live corn
- ■ - Potassium leakage from dead corn

Figure 15 Phosphatase leakage from live corns

- ◍ - potassium leakage

(area of *p*-nitrophenol)

Figure 16 Phosphatase leakage from dead corns

- ◍ - potassium leakage

(area of *p*-nitrophenol)

Table 1 Germination, moisture content and ATP content of NIAB spring (s) and winter (w) barleys

Cultivar	GC (%)		Moisture	ATP
	Peroxide	Tetrazolium	(%)	(pmol/seed)
s Alexis	100	100	11.4	68
s Atem	100	100	11.6	60
s Blenheim	100	100	10.9	57
w Clarine	99	100	11.8	78
s Corniche	100	100	10.6	62
s Digger	100	100	11.1	57
s Doublet	99	99	11.0	73
w Finesse	100	100	11.6	74
w Frolic	99	100	11.8	68
w Gypsy	99	100	11.8	87
s Hart	100	100	10.6	79
s Klaxon	100	100	11.1	76
w Marinka	98	99	11.6	83
w Melusine	100	100	11.5	80
s Natasha	100	100	11.4	64
s Nomad	100	100	12.1	66
s Nugget	100	100	11.4	67
w Pastoral	100	100	11.8	77
w Plaisant	100	99	11.7	57
s Prisma	100	100	11.0	78
w Puffin	97	100	11.6	77
s Regatta	99	100	11.3	83
w Sarah	100	99	11.4	81
w Torrent	98	100	11.8	62
s Triumph	100	100	10.4	66
w Vixen	99	100	11.7	70
w Waveney	100	100	11.4	62

Table 2 ATP content, germination potential and Tetrazolium staining in a range of spring barleys heat-treated at 100°C

Cultivar	GC (%)	Tetrazolium stain (%)	ATP content (pmol/seed)
Alexis	0	4	27
Atem	0	12	28
Blenheim	0	26	34
Corniche	0	3	28
Digger	0	0	27
Doublet	0	7	26
Hart	0	20	22
Klaxon	0	12	16
Natasha	0	7	20
Nomad	0	2	15
Nugget	0	18	30
Prisma	0	0	31
Regatta	0	7	23
Triumph	0	4	25

Table 3

Levels of ATP extracted from undried barleys of differing viabilities steeped at 55°C for 10 minutes

Variety	GC (%)	ATP Content (nmol/g dry wt.)
Camargue	97	6.2
Blenheim	95	20.4
Tyne	94	8.2
Blenheim	93	18.2
Camargue	92	6.9
Triumph	90	4.3
Triumph	85	4.5

Table 4 Possibility of distinguishing between 94% and 95% viability

Units of ATP (live): 10
Units of ATP (dead): 1
Total number of seeds: 100

5% variability

Viability of sample	Range of ATP values Max	Min
100	1050	950
99	1041	942
98	1031	933
97	1022	924
96	1012	916
95	1003	907
94	993	899
93	984	890
92	974	882
91	965	873
90	956	865

Table 5 Distinguishing between viabilities using 100 seeds

100 seeds extracted and assayed
5% variability

Difference between live and dead corn	Range of values 94% viability Max	95% viability Min
5	500	456
10	993	907
50	4941	4517
100	9876	9030
1000	98706	90255

Table 6 Distinguishing between viabilities using 100 seeds

100 seeds extracted and assayed
Variable errors

Difference between live and dead corn	Error(%)	Range of values 94% viability Max	95% viability Min
5	0.2	477	479
50	0.1	4711	4750
100	0.5	9453	9458
1000	0.5	94477	94530

Table 7 Prediction of viability
- any number of seeds

10 units difference between live and dead seeds
5% variation

		Range
2 seeds:	2 live	19 - 21
	1 live	11 - 12
	2 dead	1.9 - 2.1
3 seeds:	3 live	29 - 32
	2 live	20 - 22
	1 live	11.4 - 12.6
	3 dead	2.9 - 3.2
5 seeds:	5 live	48 - 53
	4 live	39 - 43
	3 live	31 - 34
	2 live	22 - 24
	1 live	13 - 15
	5 dead	4.8 - 5.3

Table 8 Levels of ATP extracted from heat-treated barleys

Sample	GE (%)	Tetrazolium stain(%)
Control (18.4 % moisture)	97	97
45°C 64 hrs	77	97
45°C 72 hrs (16.3 % moisture)	0	0

Table 9 Malate dehydrogenase activity of untreated and heat-treated corn (45°C)

Untreated corn Abs_{340nm}	Heat-treated corn Abs_{340nm}
0.512	1.062
0.456	1.219
0.682	1.189
0.423	0.579
0.358	0.951
1.182	1.081
0.591	1.116
0.792	1.533
1.042	1.437
0.548	1.314
0.891	1.489
0.765	1.581
0.299	1.131
0.234	0.870
0.274	0.876
0.252	0.875
0.268	0.850
0.269	0.802
0.243	0.867
0.235	0.860
0.252	0.870
0.247	0.875
0.330	0.843

Table 10 MDH Activity of individual corns
(extracted into 0.5 ml buffer only)

Untreated corn	Heat-treated corn
0.587	0.337
0.509	0.553
0.633	0.246
0.620	0.254
0.572	0.703
0.630	0.722
0.618	0.716
0.576	0.612
0.543	0.376
0.602	0.384

Absorbance drop at 340nm

Table 11 MDH activity from individual corn

75% GC (25% damaged)

0.544	0.445	0.636	0.410	0.450
0.454	0.624	0.487	0.553	0.534
0.469	0.503	0.465	0.472	0.491
0.464	0.461	0.475	0.475	0.497
0.470	0.472	0.459	0.553	0.402
0.434	0.461	0.466	0.452	0.438
0.515	0.451	0.633	0.467	0.371
0.458	0.458	0.655	0.363	0.497
0.504	0.567	0.532	0.448	0.491
0.463	0.475	0.650	0.467	0.493

Absorbance drop (340 nm)

Table 12 MDH activity of steeped grain
(7:17:7:17:1 steep regime)

Chitted grain	Non- chitted grain
0.466	0.551
0.322	0.573
0.453	0.219
0.414	0.598
0.340	0.555
0.462	0.623
0.399	0.472
0.382	0.577
0.439	0.523
0.503	0.556

Absorbance drop at 340nm

Table 13 ATP content of individual corn
(extracted in lml 0.05% (w/v) TCA)

	Untreated corn ATP (pmol)	Heat-treated corn ATP (pmol)
1	13.4	0.13
	13.7	0.12
	13.0	0.13
	13.9	0.13
	13.8	0.14
2	5.6	0.66
	5.4	0.67
	5.9	0.74
	6.4	0.68
	5.5	0.70

Table 14 ATP content of 20 individual corns

75% GC

RLU =Relative Light Units

Sample + reagent (RLU)	+ ATP std (RLU)	ATP (pmol/seed)	
4918	26546	2.27	
4470	27473	1.94	(*)
7859	31498	3.32	
2776	27538	1.12	*
8673	29488	2.92	
5061	28255	2.18	
2519	23651	1.19	*
2404	25166	2.91	
14909	35972	7.08	
4092	28582	1.67	(*)
4431	20409	2.76	
6137	23745	3.39	
6925	25111	3.81	
5542	22767	3.22	
2277	29797	0.85	*
8887	23656	3.31	
1825	23336	0.85	*
5065	22890	2.84	
7321	23814	4.44	
4339	22124	2.44	

Table 15 ATP levels (pmol) extracted from single corn

1	8.9	25	11.1	49	13.5	73	9.5
2	11.1	26*	5.5	50	11.5	74	10.2
3*	6.1	27	9.2	51	7.9	75	10.1
4	8.8	28	10.1	52	13.2	76	8.3
5	7.1	29	7.6	53	9.0	77	11.8
6	8.6	30	8.6	54	28.4	78	12.6
7	10.2	31	8.0	55	10.4	79	10.1
8	8.7	32	10.7	56*	6.2	80	9.0
9	7.2	33*	6.6	57	9.2	81	11.8
10	8.0	34	11.3	58	14.4	82	11.1
11*	6.3	35	9.3	59	8.4	83*	6.0
12	7.6	36	9.1	60	8.4	84	14.1
13	7.1	37*	6.9	61	10.7	85*	6.9
14	10.5	38	10.4	62*	6.9	86	11.4
15*	6.9	39	7.0	63	11.6	87	13.2
16	12.0	40	7.6	64	7.9	88	9.3
17	8.6	41	11.5	65	10.5	89	9.9
18*	6.6	42	14.5	66	10.5	90*	7.0
19	10.1	43	11.4	67	9.5	91	8.7
20	10.8	44*	6.8	68	11.7	92	8.8
21	9.0	45	8.7	69	12.0	93	11.8
22*	4.1	46	12.8	70	13.1	94	9.2
23	10.9	47	9.7	71	7.3	95	10.0
24	8.6	48	7.2	72	7.1	96	9.1

Table 16 Prediction of germination of barley samples using the microtitre plate method for measuring ATP levels in single grain compared to germination determined at intake (tetrazolium staining) and current germination (peroxide tests and tetrazolium staining)

	Viability estimation using different methods			
Reject sample	Intake (%)	Peroxide (%)	Tetrazolium stain (%)	ATP Test[a] (%)
7	93	82	51	87
15	30	27	41	27
21	68	87	73	65

[a]Viability (%) from ATP measurement;
viable corn contains >20.6pmol ATP.

Table 17 ATP levels (pmol) extracted from single corn after steeping (30 mins)

1	132	25	70	49	107	73	62
2	225	26	150	50	179	74	133
3	472	27*	14	51*	9	75	94
4	251	28	145	52	51	76	72
5	276	29*	17	53	30	77*	8
6	37	30	135	54	69	78	72
7	160	31*	17	55	26	79	65
8	225	32	97	56	49	80	50
9	173	33	185	57	40	81	94
10	77	34	33	58	155	82	35
11	187	35	64	59	86	83	109
12	191	36	47	60	53	84	89
13	22	37*	16	61	116	85	155
14	65	38	29	62	86	86	133
15	40	39	82	63	119	87*	7
16	132	40	26	64	164	88	56
17	46	41	67	65	191	89	130
18	146	42*	5	66	34	90	56
19	139	43	168	67	72	91	104
20	116	44	153	68	135	92	200
21	42	45	217	69	87	93	51
22	146	46*	18	70	116	94	186
23	230	47	84	71	85	95	110
24	304	48	178	72*	12	96	141

Table 18 ATP levels (pmol) extracted from single corn

Before steeping				After steeping (30 minutes at 40°C)			
1	6.5	21	6.2	1	6.5	21	52.7
2	4.4	22	4.2	2	3.7	22	9.1
3	4.6	23	7.8	3	2.9	23	3.2
4	4.0	24	9.3	4	3.7	24	3.6
5	6.5	25	4.8	5	12.6	25	1.5
6	4.0	26	7.0	6	4.7	26	1.9
7	6.9	27	7.7	7	4.4	27	3.5
8	6.8	28	6.5	8	9.6	28	1.3
9	3.7	29	3.8	9	5.8	29	34.9
10	9.3	30	7.0	10	5.7	30	21.8
11	8.3	31	3.6	11	18.9	31	1.8
12	4.6	32	20.1	12	3.9	32	1.5
13	8.7	33	6.8	13	9.7	33	14.9
14	5.3	34	5.4	14	2.9	34	17.9
15	4.9	35	5.7	15	12.0	35	1.5
16	5.9	36	3.5	16	2.7	36	4.1
17	9.8	37	3.8	17	3.6	37	1.6
18	8.5	38	5.4	18	4.1	38	59.5
19	9.3	39	10.8	19	5.6	39	10.0
20	5.0	40	4.4	20	2.8	40	4.6

Table 19 Prediction of germination of barley samples using the microtitre plate method for measuring ATP levels in single grain compared to current germination determined by peroxide tests, 4ml tests and tetrazolium staining.

	Viability estimation using different methods			
Reject sample	GC H_2O_2 (%)	GE 4ml Test (%)	Tetrazolium stain (%)	ATP test[a] (%)
43	91	88	83	91
41	52	50	63	66
44	98	93	100	100
7	82	80	51	92
11	0	0	18	8
1	80	59	90	91
3	88	74	94	98
Brewster	95	97	96	96

[a] Viability (%) from ATP measurement;
viable corn contains >20.6pmol ATP

Table 20 Barley sterol measurement

Sample	A340nm 0min	A340nm 60 min	Change in A340nm
Control	1.411	1.446	0.035
Live corn	1.426	1.368	-0.058
Dead corn	1.336	1.321	-0.015
75% viable corn	1.322	1.322	0.056

Table 21 UV-absorbing materials leaked from single corns

	Germinated	Absorbance (260nm) 'Live' corn	Germinated	'Dead' corn
1	Y	0.191	N	0.203
2	N	0.282	N	0.135
3	N	0.206	N	0.254
4	Y	0.173	N	0.235
5	Y	0.220	N	0.237
6	N	0.212	N	0.162
7	N	0.438	N	0.238
8	Y	0.255	N	0.133
9	Y	0.374	N	0.223
10	Y	0.347	N	0.175
11	N	0.440	N	0.284
12	Y	0.375	N	0.201
13	Y	0.431	N	0.223
14	Y	0.292	N	0.214
15	Y	0.233	N	0.265
16	Y	0.252	N	0.229
17	Y	0.314	Y	0.273
18	Y	0.244	N	0.194
19	Y	0.301	N	0.307
20	Y	0.352	N	0.243

Corns germinated on moist filter paper - Yes (Y) or No (N)

Table 22 Leakage of hydrogen ions from whole grains

	Absorbance (450nm)		
Corn	Live	Dead	75% viable
1	0.490	0.931	0.596
	0.403	0.789	0.571
2	0.506	0.589	0.488
	0.406	0.557	0.54
3	0.542	0.567	0.665
	0.642	0.522	0.594
4	0.549	0.738	0.729
	0.550	0.721	0.591
5	0.526	0.728	0.652
	0.484	0.725	0.696

Table 23 Absorbance values of exudates from single barley corns steeped at pH 3

Corn number	Control	Live corn	Dead corn
1	0.079	0.088	0.128
2	0.081	0.079	0.114
3	0.079	0.084	0.116
4	0.079	0.082	0.104
5	0.072	0.088	0.106
6	0.073	0.079	0.109
7	0.095	0.084	0.100
8	0.085	0.089	0.097
9	0.076	0.082	0.104
10	0.055	0.087	0.100
11	0.079	0.084	0.096
12	0.075	0.081	0.106

Bromophenol blue (10mg/ml) read at 630nm

Table 24 Absorbance range for exudates from live and dead corns at pH 10 determined by thymol violet

Sample	Absorbance range		
	405nm	570nm	630nm
Control	0.260 - 0.327	0.192 - 0.307	0.151 - 0.234
Live corns	0.265 - 0.301	0.219 - 0.265	0.160 - 0.187
Dead corns	0.277 - 0.351	0.227 - 0.277	0.159 - 0.190

Table 25 Potassium leakage from steeped grain

25g grain in 50ml water

Viability	Electrode reading (mV)	K^+ concentration (mM)
Live seed	-69	7.4
75% viable seed	-63	9.2
Dead seed	-56	12

Table 26 Ions leached from whole grain measured by Dionex

Sample	Na^+ (mg/l)	NH_4^+ (mg/l)	K^+ (mg/l)	Ca^{++} (mg/l)	Mg^{++} (mg/l)
Live grain	15	5	323	25	12
	19	5	386	26	13
75% viable grain	23	7	470	25	11
	26	6	506	26	12
Dead grain	16	13	624	20	24
	14	12	588	20	23

Table 27 Viability of barley samples determined by conductivity measurements

GC - Peroxide (%)	pv100	pv150	pv200
0	100	100	100
52	59	76	90
60	96	97	100
74	45	73	81
80	98	100	100
88	94	99	100
91	61	90	96
98	70	92	96
98	94	99	100

pv = partition value

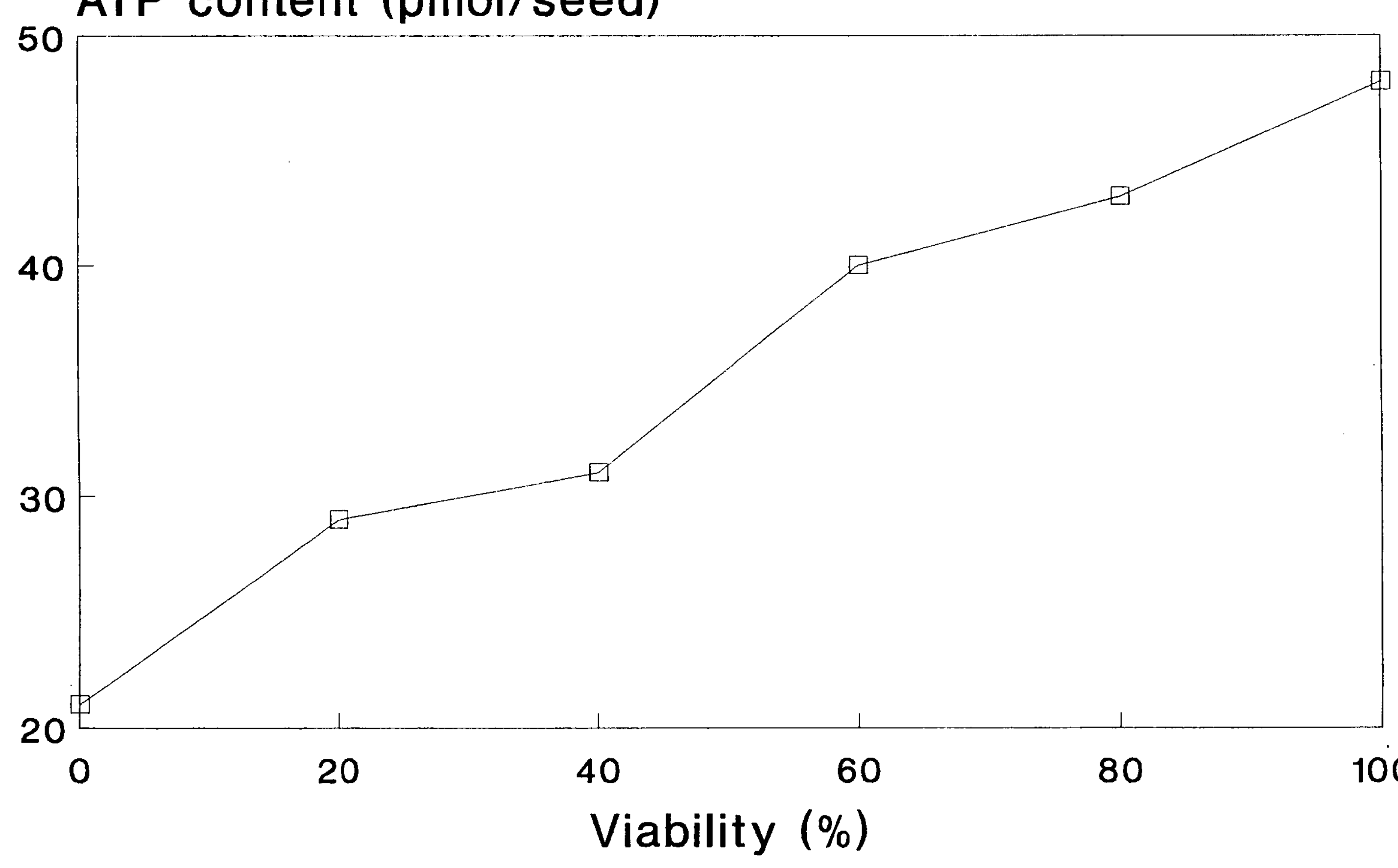
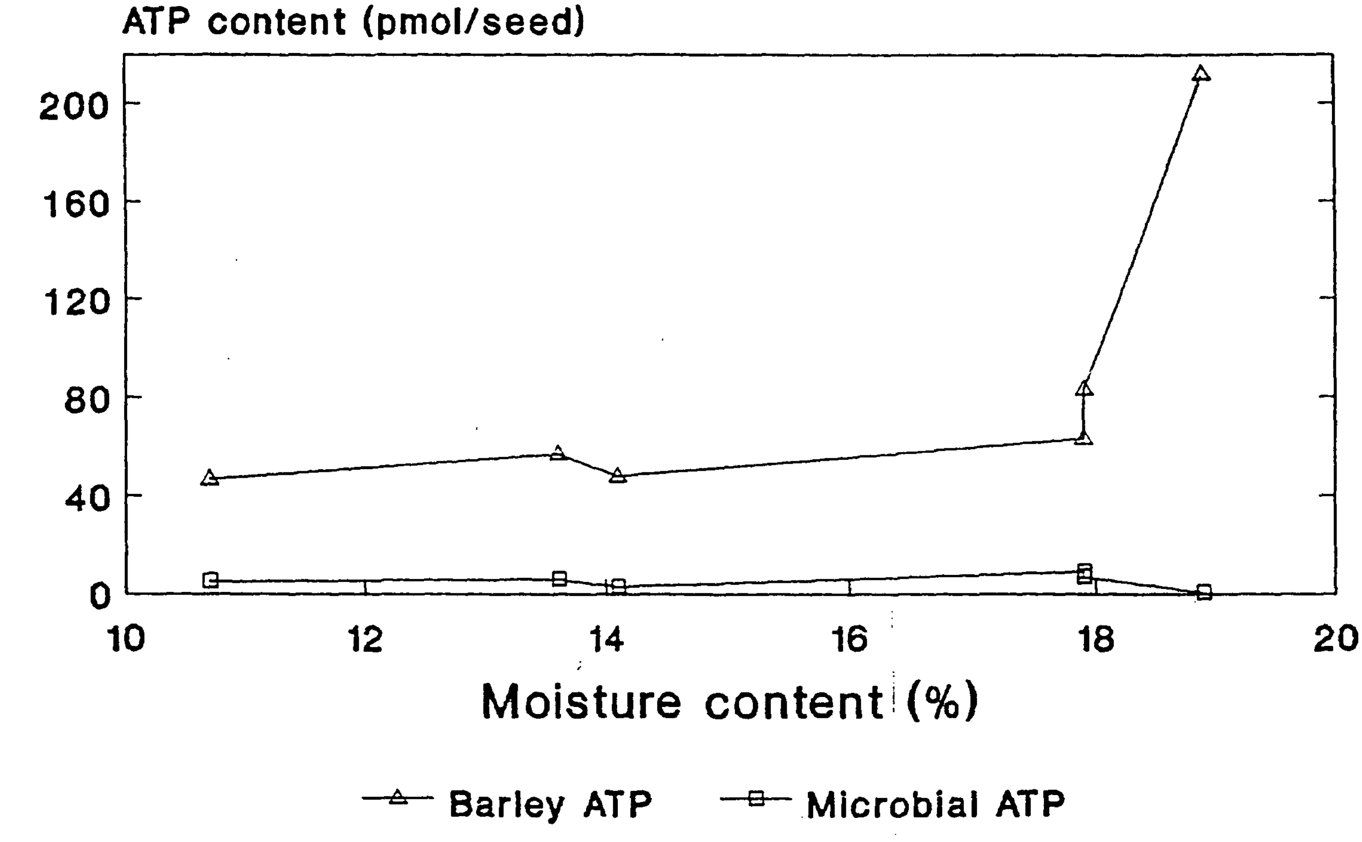


Figure 1 The ATP content of barleys of differing viabilities

Figure 2 The contribution of microbial ATP to extracted ATP levels from undried barley samples



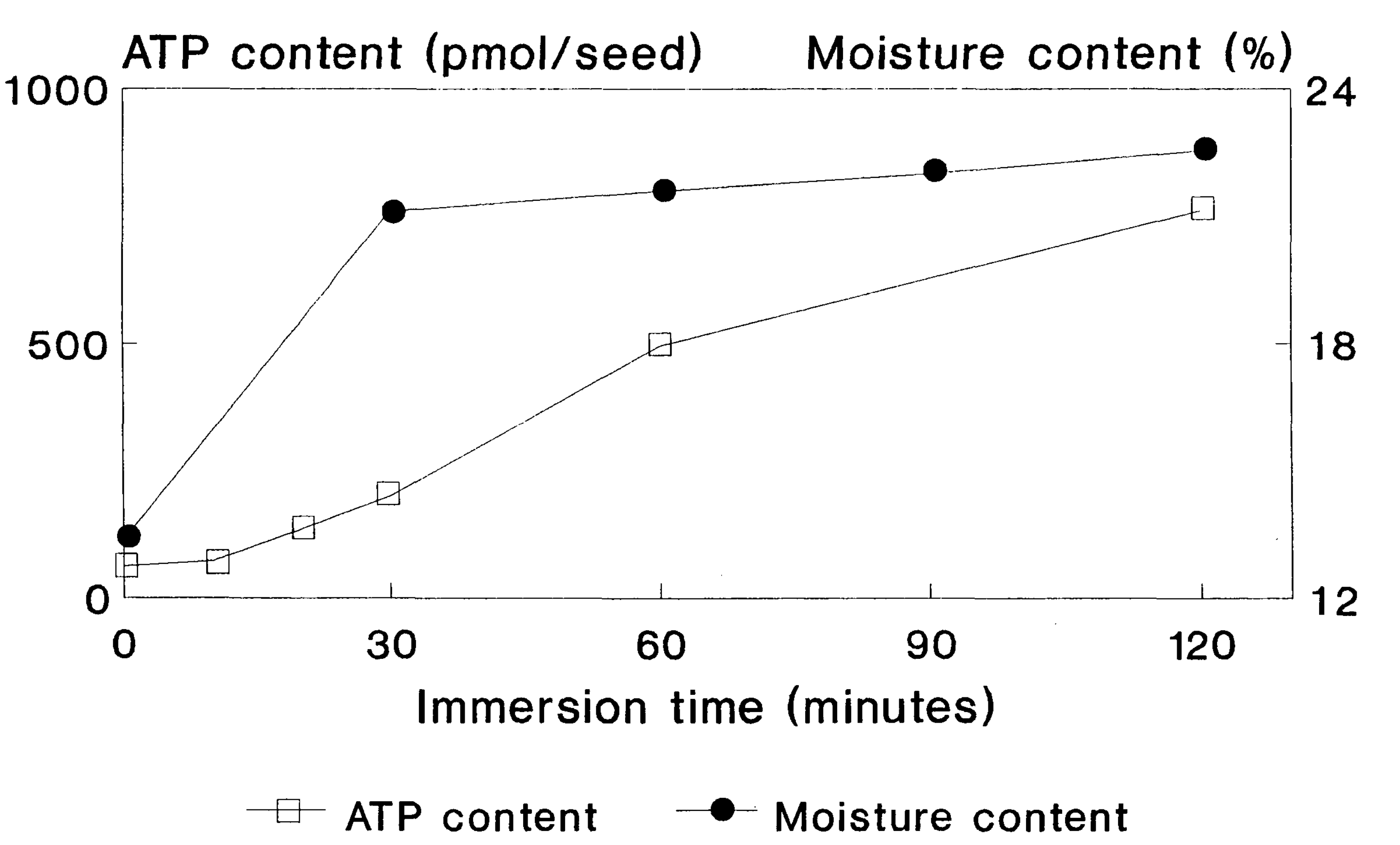


Figure 3 The effect of immersion on ATP levels extracted from dry barley

Figure 4 Water uptake and ATP release in dead barley

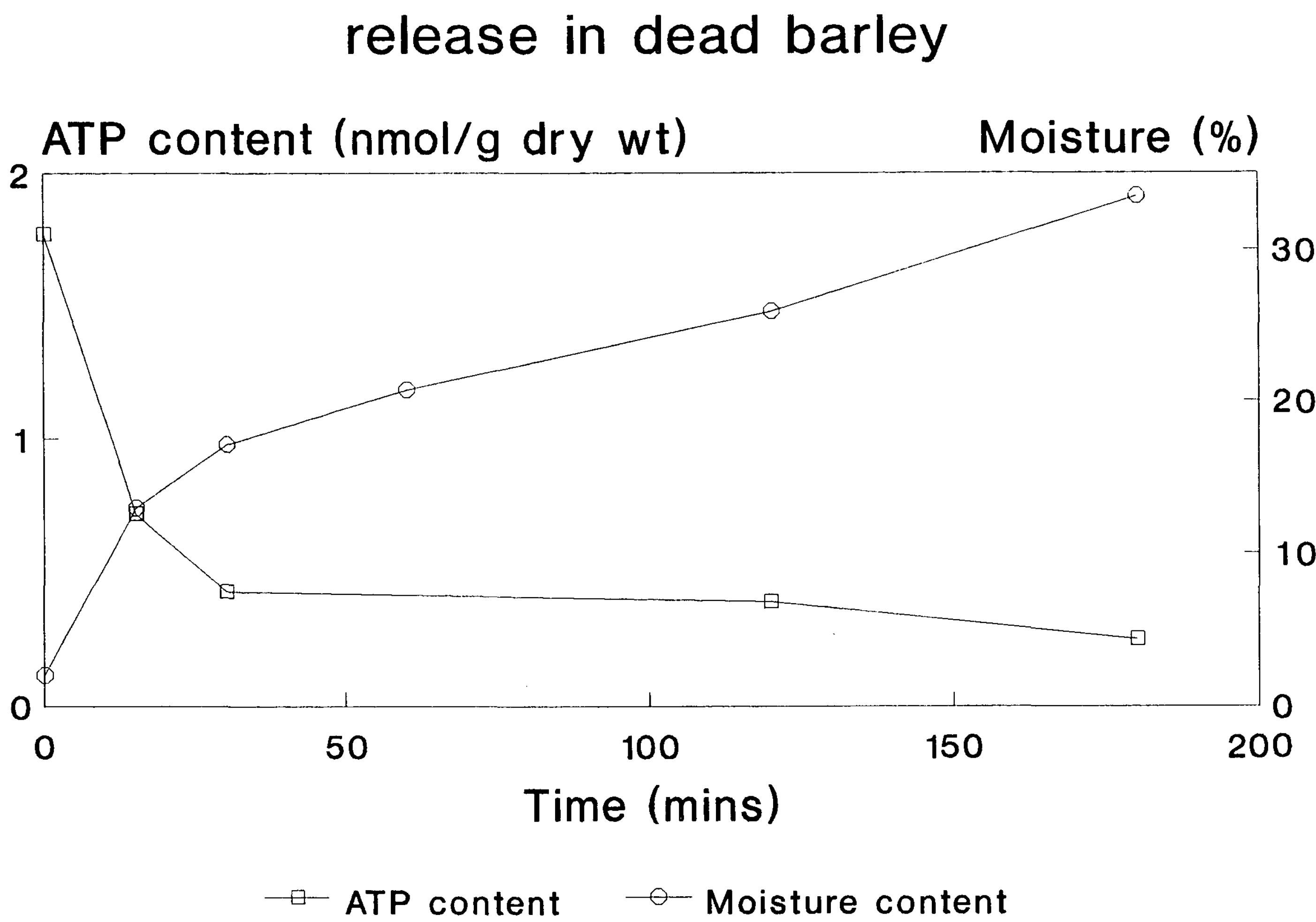
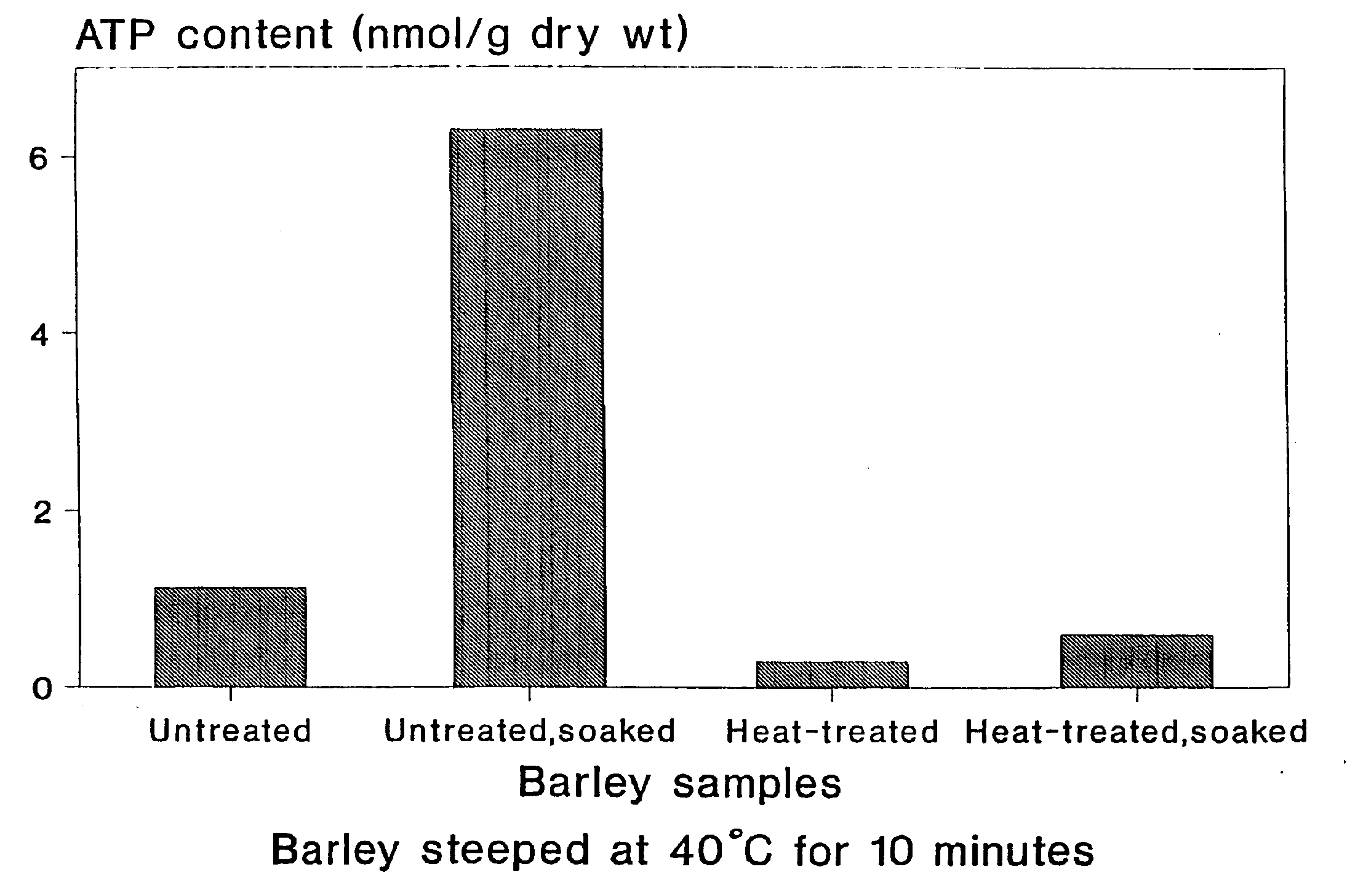


Figure 5 The effect of immersion on ATP levels extracted from live and dead corn



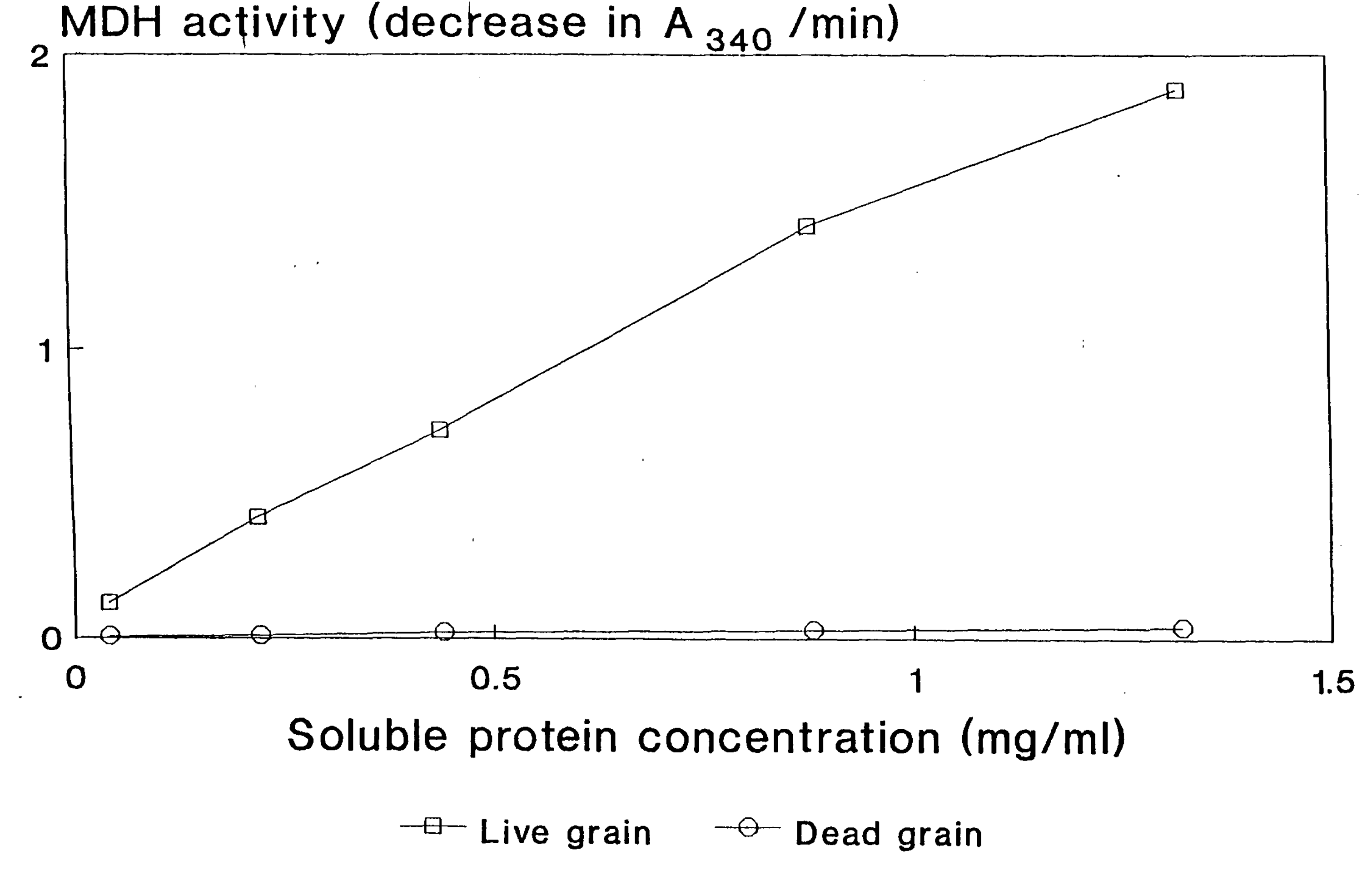


Figure 6 MDH from live and dead barley
MDH activity (decrease in A 340 /min)
2
1
0
0
0.5
1
1.5
Soluble protein concentration (mg/ml)
Live grain
Dead grain

Figure 7 MDH activity present in undried barleys of differing viabilities

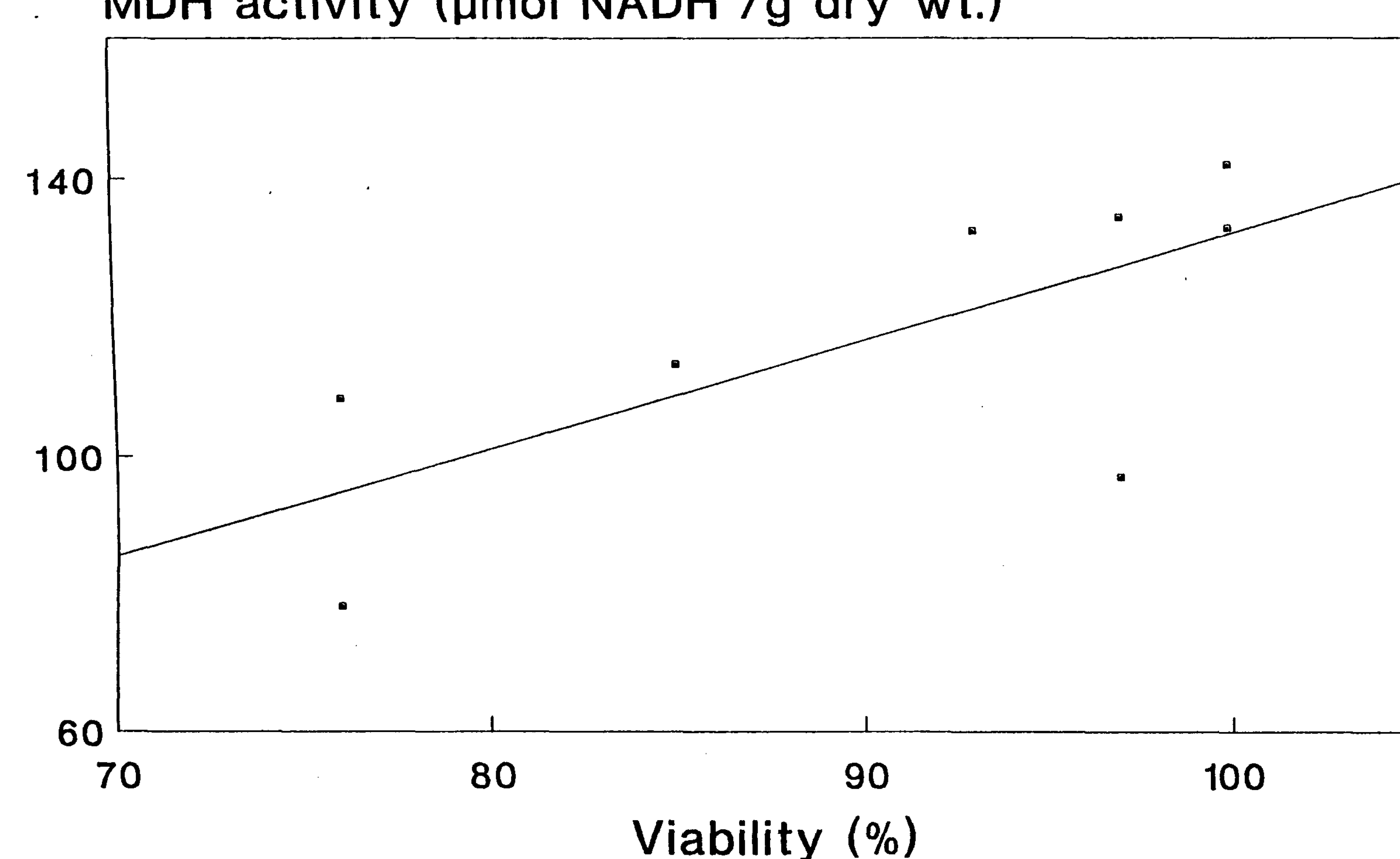


Figure 8 Comparison of viability measured by tetrazolium or ATP content

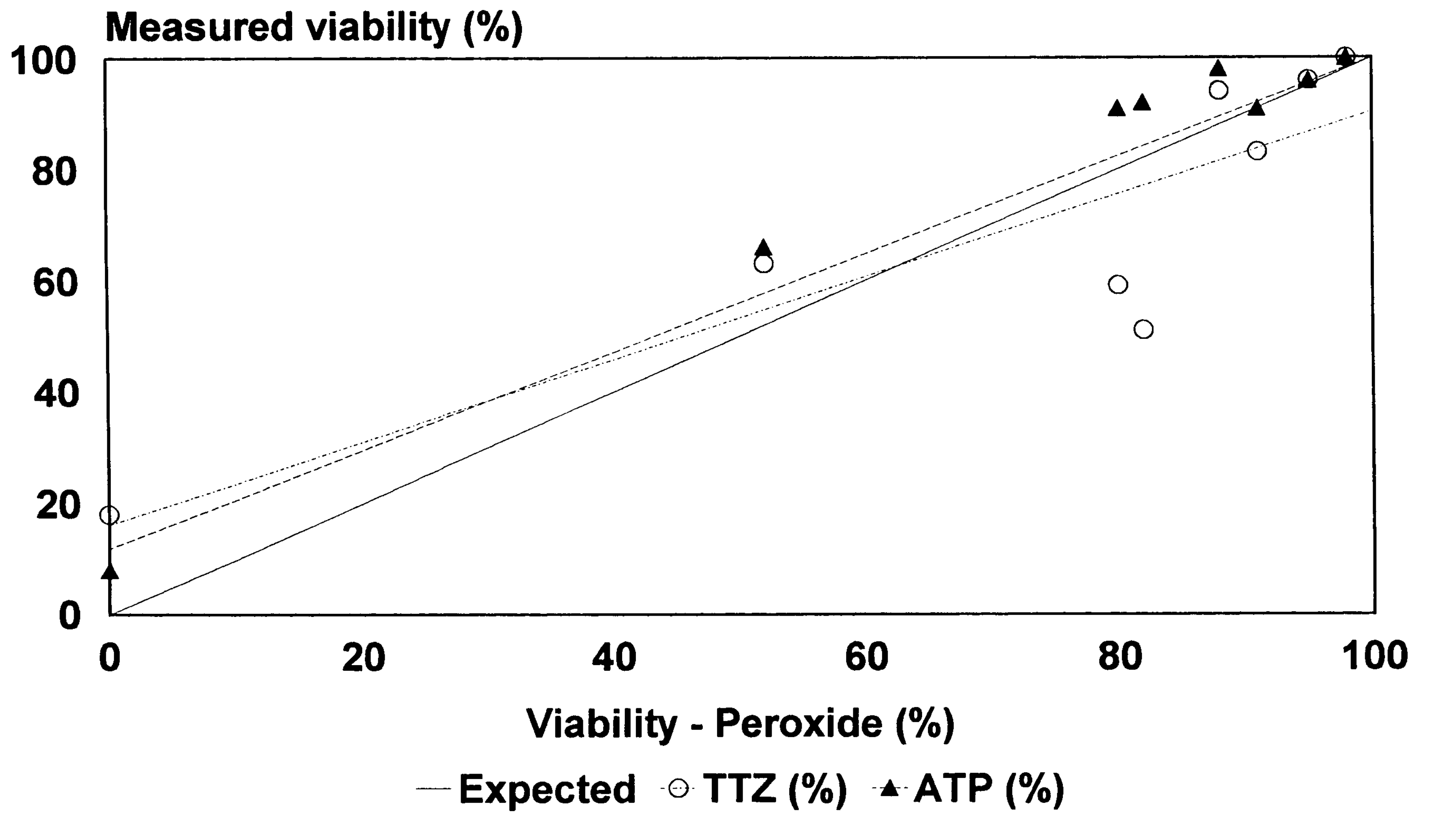


Figure 9 Spectral changes due to ergosterol filipin reaction

—— 0 min - - - - 60 min

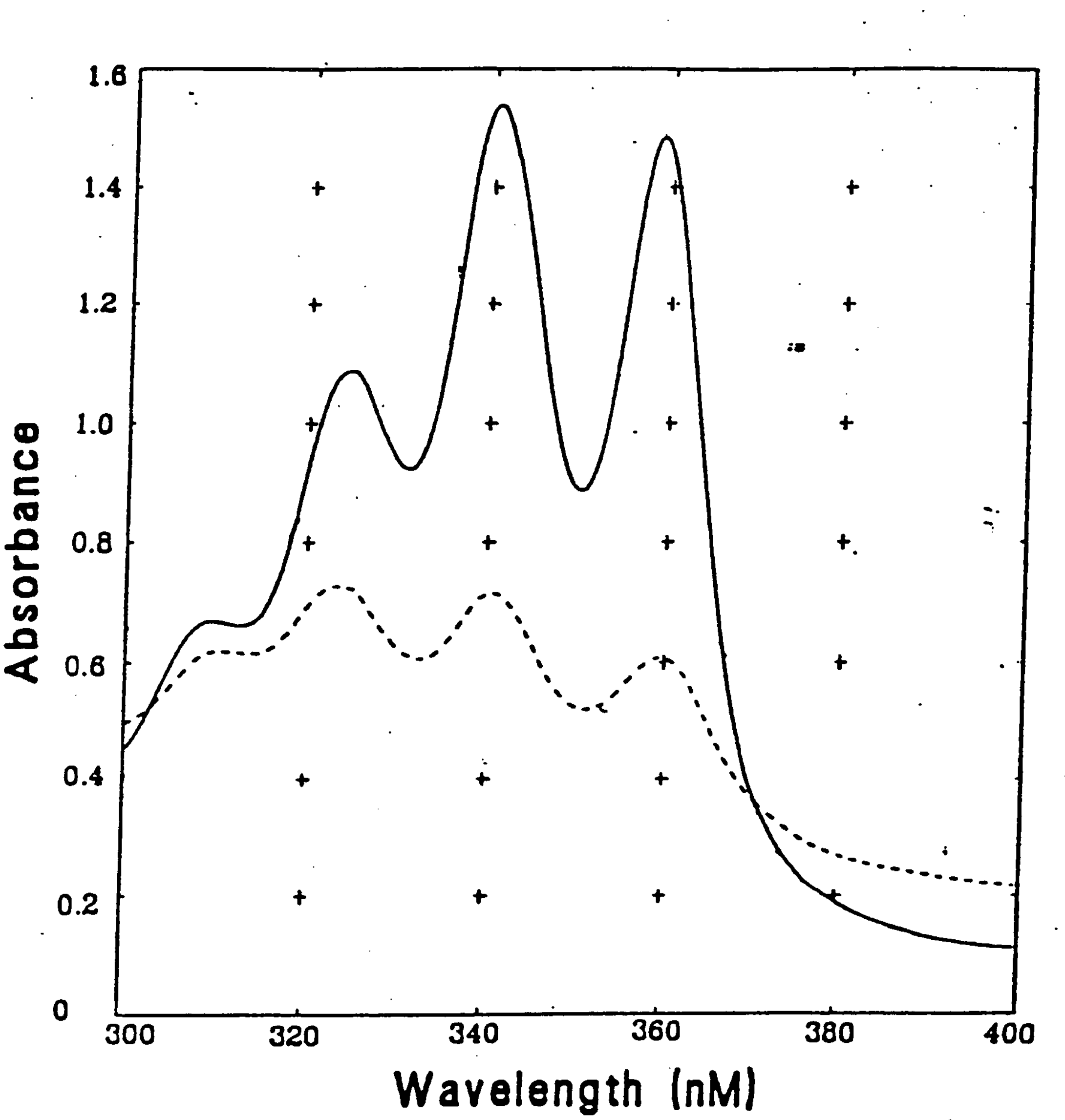


Figure 10 Sterol- filipin extraction from live and dead barley

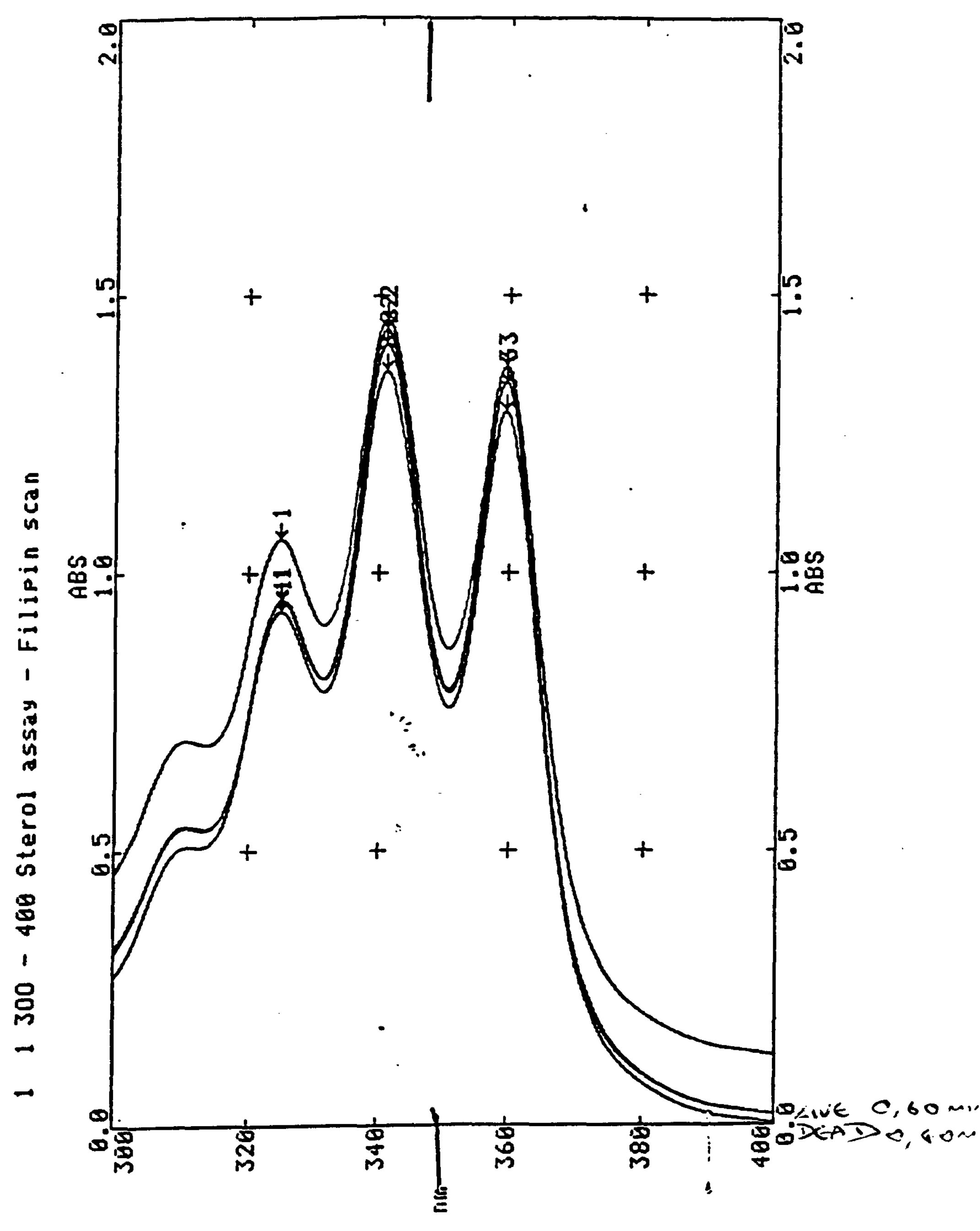


Figure 11 Calibration curve for cresol purple (100µg)

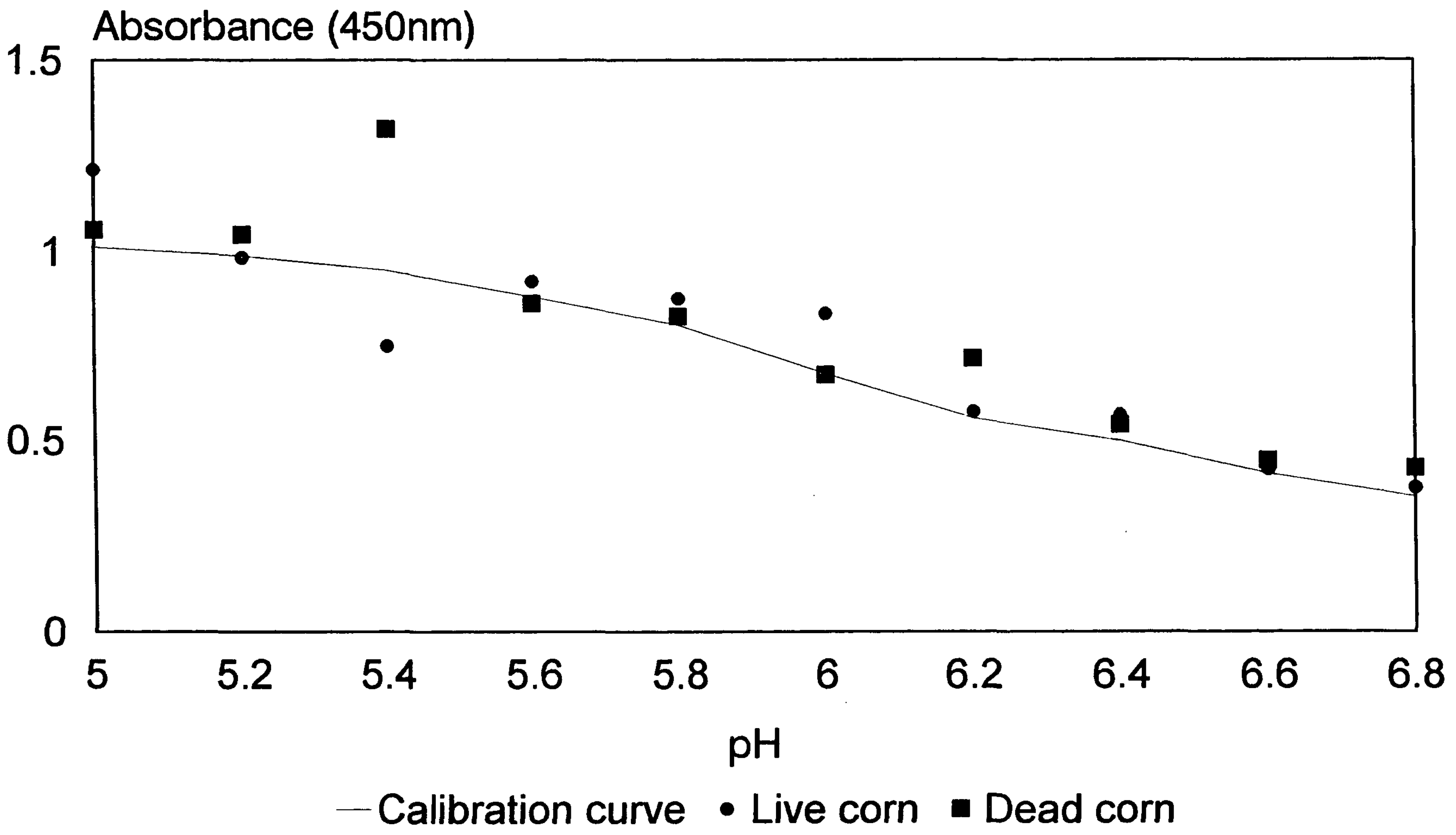


Figure 12 Calibration curve for bromophenol blue (10mg/ml, pH 3) read at 630nm

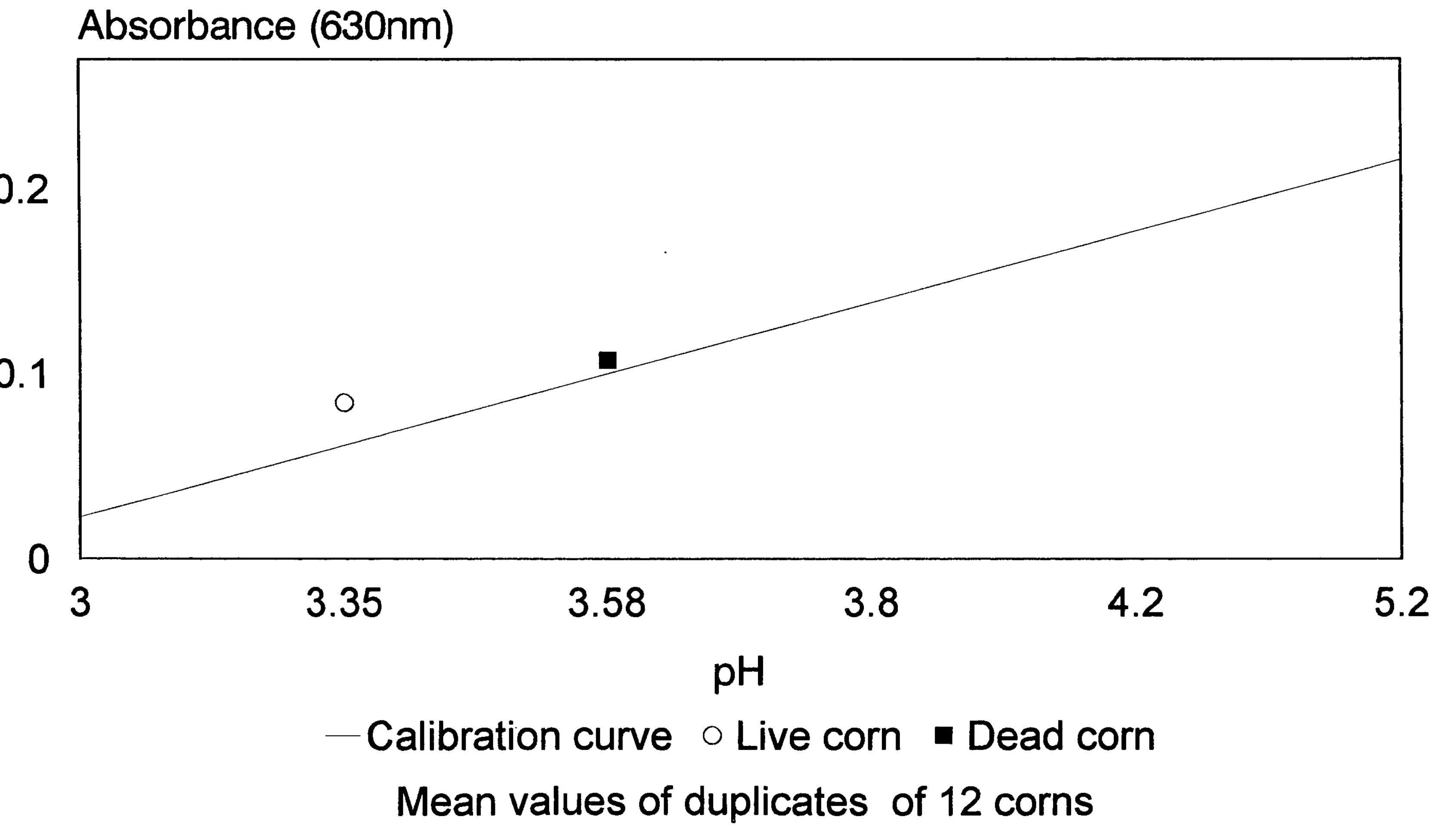


Figure 13 Calibration curve for Ion Selective Electrode

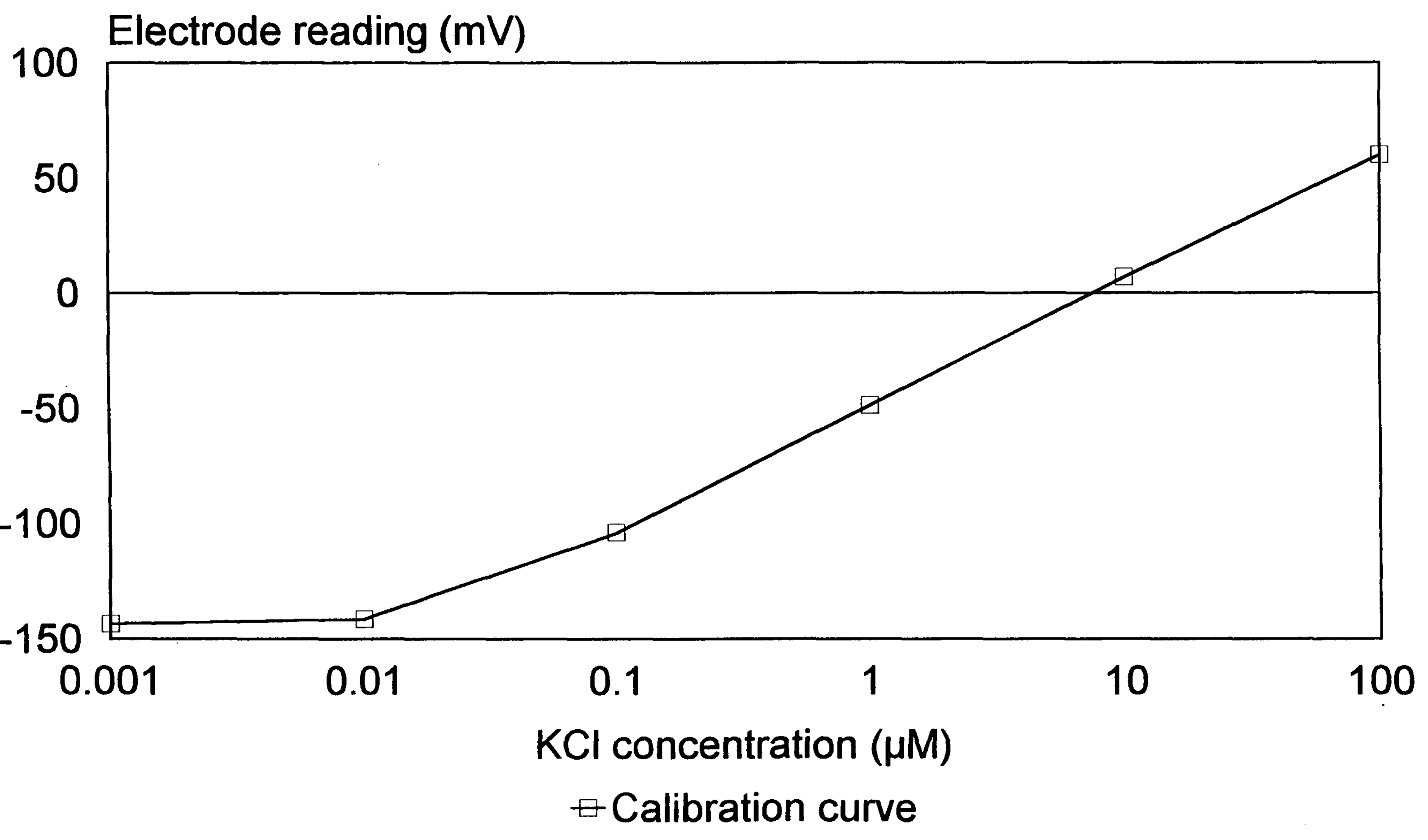


Figure 14 Low level calibration for potassium leakage from single corn

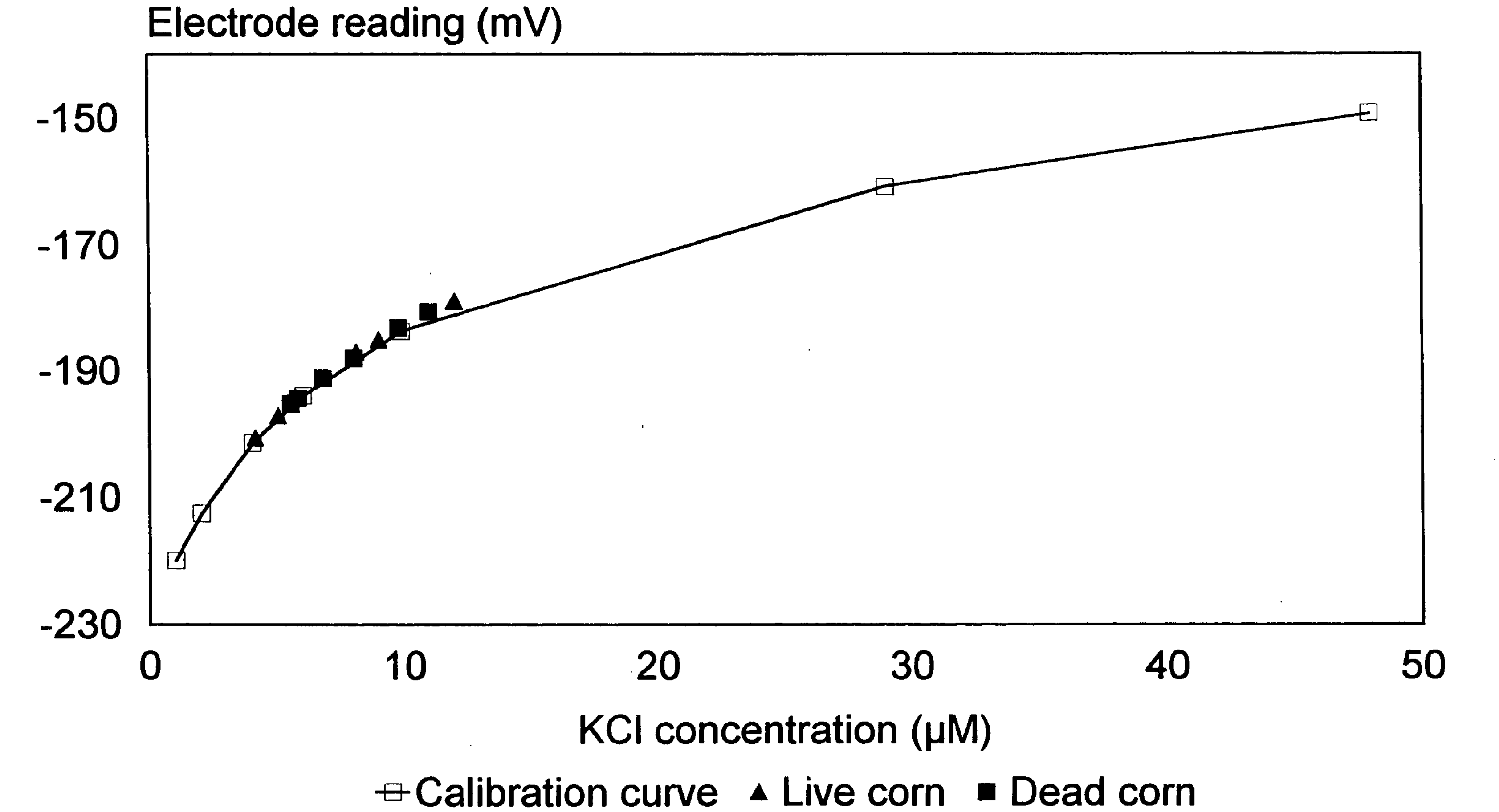
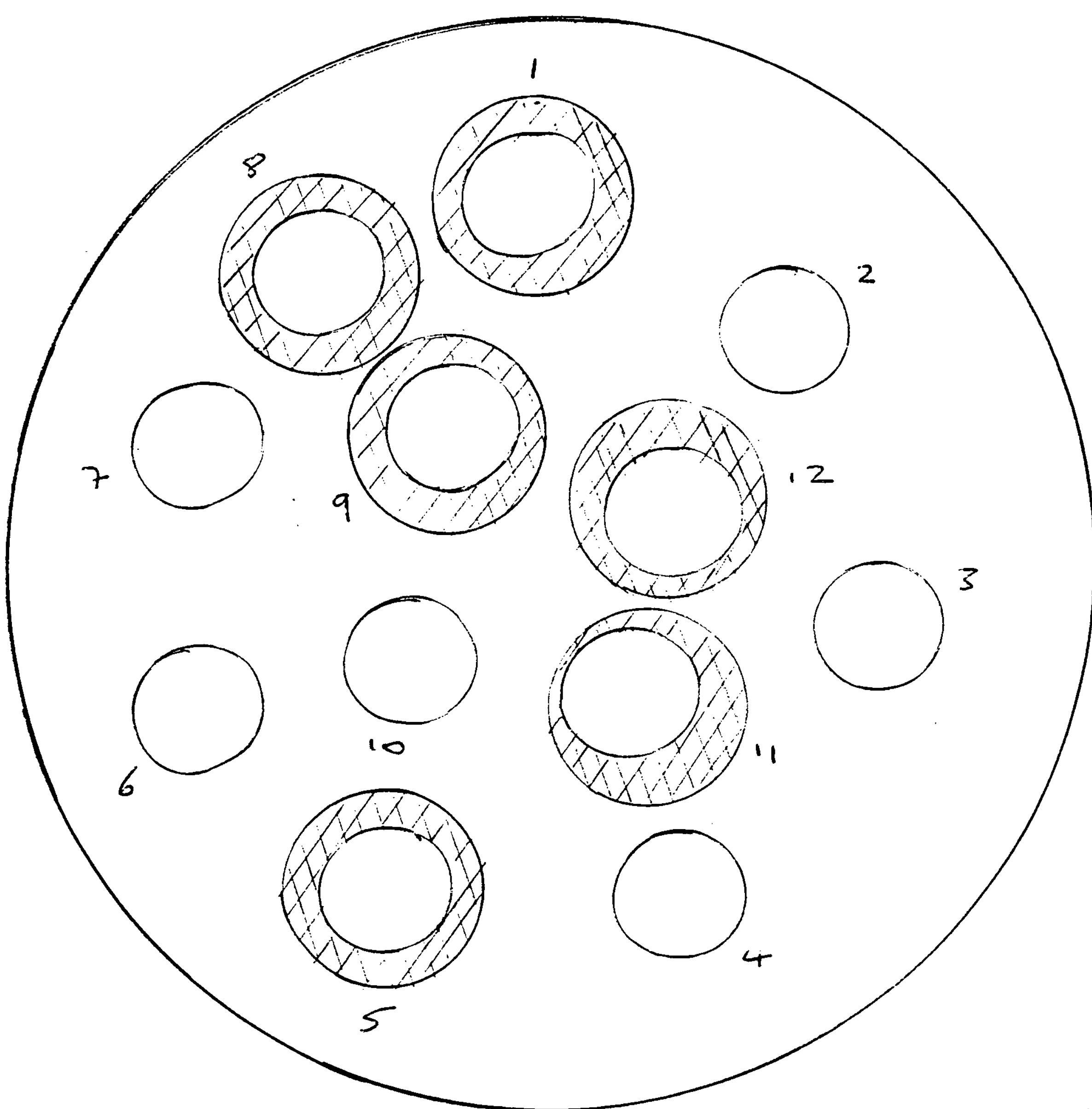
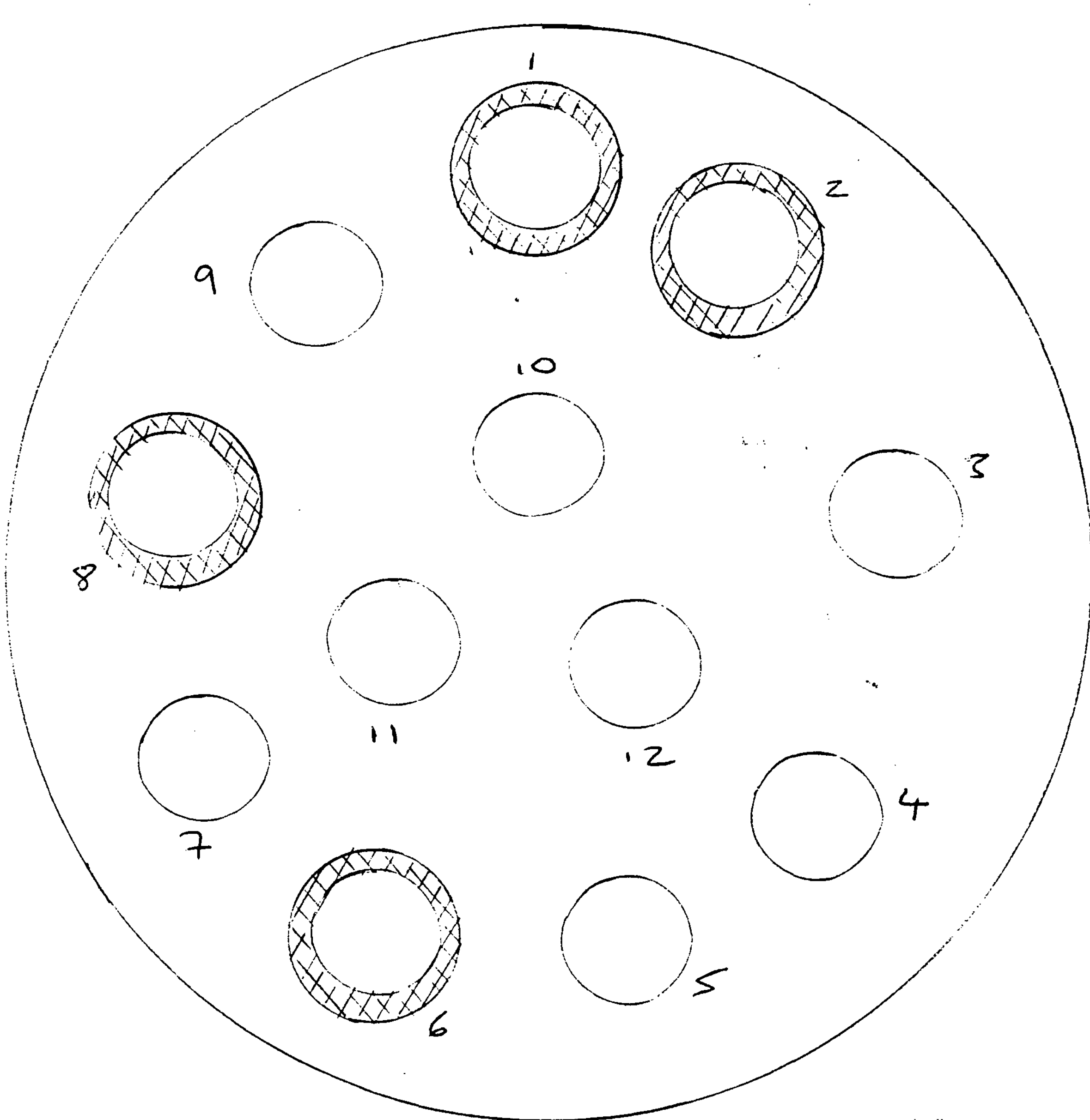


Figure 15 Phosphatase leakage from live corns



1 - No embryo
2 - intact corn
3 - intact corn
4 - intact corn
5 - half-corn
6 - intact corn
7 - intact corn
8 - husk peeled from corn
9 - husk peeled from corn
10 - intact corn
11 - half-corn
12 - half-corn

Figure 16 Phosphatase leakage from dead corns



1 - No embryo

2 - half-corn

3 - intact corn

4 - intact corn

5 - intact corn

6 - husk peeled from embryo

7 - intact corn

8 - husk peeled from embryo

9 - intact corn

10 - intact corn

11 - intact corn

12 - intact corn