

A New Germinative Classification Model of Barley for Prediction of Malt Quality Amplified by a Near Infrared Transmission Spectroscopy Calibration for Vigour “On Line” Both Implemented by Multivariate Data Analysis

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ABSTRACT

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A new germinative two-dimensional classification plot fully compatible to the current EBC analyses (EBC methods 3.5–3.7) is proposed for malting barley based on separate estimates for “vigour” (24 h germination) as abscissa with limits at 70% and 30% and for “viability” (72 h germination) as ordinate with limits at 98% and 92%. Early detection of germination by image analysis was improved by utilising the auto fluorescence of the root cap. The seven hierarchical germinative classes visualise the quality differences in a consistent way, ordering classes according to falling extract % and increasing wort β -glucan (mg/L).

It was surprising to discover that significant barley Near Infrared Transmission (NIT) spectroscopy based Partial Least Squares Regression prediction models for “vigour” and “viability” were obtained after removing the PLSR outliers. The majority of these were found to be low in vigour.

It was concluded after experimental validation that the physical-chemical structure of the seed, reflected by the correlation of the barley NIT spectral fingerprints to germination speed, is connected to the availability of substrate for germ growth. This is another aspect of the speed of malt modification.

An automated combination instrument for measuring physical-chemical and seed germination parameters is suggested for quality control and to establish an *on-line* NIT calibration network for integrated germinative and malting quality classification.

Key words: Germinative classification, malting barley, seed vigour, seed viability, malt quality, near infrared transmission spectroscopy.

INTRODUCTION

The need for more informative germinative malting barley analyses

Optimal germination performance is without a doubt the most important quality criterion for malting barley^{5,11,14,16,40,41}. The industry and trade are dependent on reproducible and representative laboratory analyses for germinative capacity (GC%) and energy³⁷ (GE%) and for germination speed (GI-germination index^{38,39}) as expressed in the methods 3.5–3.7 in EBC Analytica³.

It is surprising that even today germination data do not seem fully integrated with malting data in barley quality evaluation, but are rather used as univariate pre-qualification criteria with respect to live (viable) seeds at 3–5 days germination with GC% and GE% methods. In the following we will, inspired by the results from multivariate pattern recognition data analysis (also called chemometrics^{17,18,44}) of germination profiles, define the optimal practical criteria for vigour and viability. We will find that these criteria should basically represent two different dimensions in malting barley analysis, analogous to those of acceleration and mass in physics. This paper focuses on how to utilise germination information more effectively for prediction of malt quality by a new two way dimension germinative classification. It also aims at prediction of germinative data by calibration to automated instrumental analytical methods calibrated by chemometric models to speed up these very slow germination analyses.

MATERIALS AND METHODS

Two barley materials (I–II) are utilised, the results of which are published elsewhere^{22,24} but are here recalculated and presented in a completely new form to support our case. The materials were:

I. The 17 samples of the malting barley Alexis²⁴ grown in different places in Europe in 1994 were collected and analysed for malting quality at Centre UdL-IRTA, Spain²⁴ by Dr. José Luis Molina-Cano. The same collection was also heat treated at our institute²⁴ to study artificial ageing^{6,7} as a measure for vigour and to calculate the vigour potential (VP) according to Aastrup *et al.*¹.

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II. A barley material of 42 samples²² of the varieties Alexis, Blenheim and Meltan grown in Southern Scandinavia, collected in 1993–1996, was analysed for GE, seed physical-chemical analyses and malt quality after cold storage (7°C, 13.5% water) in 1999. Møller²² publishes a detailed study on this material in this issue. The barley material²² was collected under a number of years with different growing conditions in order to obtain a representative material spanning a wide variation in quality parameters.

Methods

Germination analyses

a. Germinative energy (GE) % was determined using the BRF method (EBC method 3.6.2)³. This method was used on 4 × 100 grains. GI and GH were calculated for all methods according to Riis and Bang Olsen³⁸ and Analytica-EBC (EBC method 3.7)³.

b. Heat-treatment to determine vigour potential. This was determined for Material I by Møller *et al.*²⁴ according to Aastrup *et al.*¹.

c. A new method for detection of early germination by inspection under fluorescent light. In screening for vigour, reproducible early detection of chitting is fundamental. We have found that inspection under fluorescent light (366 nm) is useful, because the newly developed root caps display a characteristic blue fluorescence (Fig. 1), making them stand out against the seed background. The fluorescence is tentatively assigned to ferulic acid, which binds to the cell walls²⁹.

The image from each single kernel is degraded in transversal lines for an area in the middle and for both ends of the grain, and the average intensity in these areas is determined. The intensities are compared, and the image analysis concludes whether the grain is germinated or not, based on the average intensities.

We have preliminary tested two germination methods for determination of g%1; germination with hydrogen peroxide (GC³) and the BRF-method³ (GE). The correlation for manually counting with and without using the light table (visual inspection) is quite good (H₂O₂: $r = 0.99$; BRF: $r = 0.98$). Comparing manual inspection with digital image analysis the GE BRF-method³ ($r = 0.99$) is as precise as for visual inspection according to the illumination method by the light table, whereas for germination using H₂O₂, the correlation is much lower ($r = 0.89$). It appears that the hydrogen peroxide bleaches the root head, so the separation with image analysis between the emerging root cap and the rest of the grain is not as good as when using BRF (GE) germination.

It can be concluded that it is possible to use fluorescent light to detect the early chitted grains by image analysis in a GE screening. The image analysis should be optimised to detect the sprouts after 24 hours automatically. The experiments are preliminary. It should be possible to develop the method further to a prototype, e.g. combined in an apparatus, which also determines single seed form properties, light reflectance intensity and hardness as suggested by the work of Møller²².

d. Physical, chemical and malt quality analyses. Thousand Kernel Weight (TKW), seed form parameters,

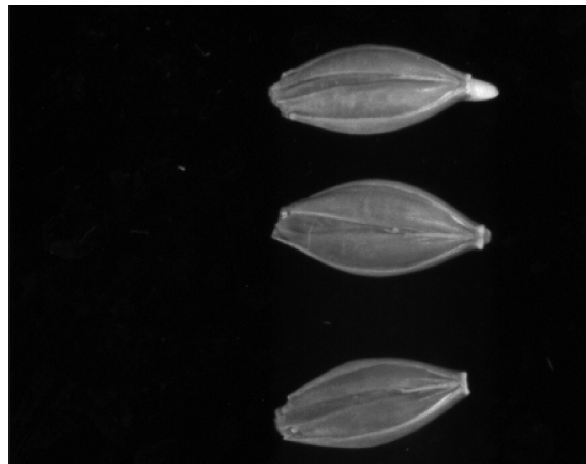


Fig. 1. Image of three kernels illuminated by fluorescence light after 24 hours of germination. The grain above is germinated; the two grains below are not germinated.

light reflection intensity, NIT-spectroscopy and hardness were analysed according to Møller²².

Extract and wort colour were determined on Material I at Centre UdL-IRTA²⁴. The (1 → 3, 1 → 4)-β-glucan in barley and wort was analysed by the calcofluor method^{2,30}.

e. Multivariate data analysis. Principal Component Analysis (PCA) and Partial Least Squares regression (PLSR) were performed as described by Martens and Næs¹⁷ and Martens and Martens¹⁸ using the “Unscrambler” software from CAMO A/S, Trondheim, Norway. The principal components indicated as PC’s in the PLSR analyses with the “Unscrambler” software are mathematically not identical with the PC’s denoted in the PCA analysis. The importance of the X variables is evaluated using Jack-knife validation proposed by Martens and Martens¹⁸.

Abbreviations

| | |
|--------|--|
| BG | (1 → 3, 1 → 4)-β-glucan in barley |
| BGwort | (1 → 3, 1 → 4)-β-glucan in wort |
| C | Wort colour |
| EBC | European Brewery Convention |
| EXT | Extract |
| g%1–8 | Germination percentage day 1–8 |
| GC | Germination Capacity |
| GE | Germination Energy |
| GH | Germination Homogeneity |
| GI | Germination Index |
| HI | Hardness Index |
| NIT | Near Infrared Transmission |
| P | Protein |
| PC | Principal Component see comment in Materials and Methods e |
| PCA | Principal Component Analysis |
| PLSR | Partial Least Squares Regression |
| RE | Relative Error in percentage |
| RMSECV | Root Mean Square Error of Cross-Validation |
| ROUND | Kernel roundness |
| STEEP | % Water uptake after 24 hours of steep |
| TKW | Thousands Kernel Weight |
| VP | Vigour Potential |
| WIDTH | Width of kernels |

RESULTS

Vigour and viability identified by multivariate data analysis as independent principal components from a set of barley germination data²⁴ (Material I)

In contrast to probability statistics used on germination data^{6,7} that is based on distributional assumptions, it is possible by multivariate data analysis to classify the form of each individual barley germination curve (Fig. 2A) with a minimum of *a priori* assumptions. Initially we are thus leaving Material I (Table I) in the hands of a chemometric model – Principal Component Analysis (PCA) in order to characterise the relationship between samples (1–17) and the germination parameters (g%1–g%8) letting data speak for themselves.

The data (Table I) from 17 germination profiles from samples of the Alexis variety (1–8 days germination, Material I) are visualised in Fig. 2A. The PCA in Fig. 2B is a combined score/loading plot (biplot) featuring the different germination observation days 1–8 (g%1–g%8) as variables (loadings) marked in bold (1–8). Neighbouring sample score points have similar germination profiles. The position of the loadings can be interpreted so that sample scores characterising the whole germination profile for e.g. sample 1DK, 3DK, 7NE, 9CZ and 16D located near to day 1 (**1**) are fast germinating while samples 2DK, 11SU, 13SU and 14SU situated below in the opposite direction along the ordinate are slow germinators. It is obvious that the signs **1** (g%1) and **2** (g%2) above are distributed along the ordinate together with a cluster of **3–8** (g%3–g%8) further below with its members situated close to each other.

One of the foremost tasks of chemometric data analysis besides data reduction is to support or even extend the scientific language in an evaluation dialogue between the graphic data interface and prior knowledge. This is done in continuing the evaluation process of the PCA plot, with an interpretation plot to ascertain the character of the PC's. We may now mark each sample point in Fig. 2B with its values for g%1 (in bold) and g%3 in Fig. 2C.

Three-day germination percentage (g%3) is taken as the most conveniently measured representative for the close loading cluster g%3–g%8. A glance at the thus labelled PCA bi-plot convinces us that there is a clear gradient in g%3 along the abscissa from left to right and for g%1 along the ordinate from below to above. It is now possible to interpret the meaning of the graphic representations PC1 and PC2 (principal components) of the PCA plot as “viability” (abscissa; g%3; germination “mass”) and “vigour” (ordinate; g%1; germination “acceleration”) respectively and to mark the axes accordingly (Fig. 2C). The orthogonal character of the PC's inherent in the PCA algorithm used above depicts their basic independence in variance set by the algorithm. The mean GI's of the samples in the four quadrants displayed in Fig. 2C also supports the idea that there is a vigour gradient along the ordinate. In this material there is a reasonable correlation ($r = 0.92$) between g%1 and GI (as compared to $r = 0.73$ for g%2, and $r = 0.46$ for g%3) thus supporting the notion that germination day 1 (g%1) can be used as a convenient estimate for germination velocity.

A simple two-dimensional plot with “vigour” as abscissa and “viability” as ordinate for the classification of malting barley in germination energy and capacity classes (Material I)

In interpreting the principal components of the previous PCA (Fig. 2B and Fig. 2C) of the EBC Alexis barley material grown in widely different locations in Europe, we generated a hypothesis that the information from the 1–8 day germination curves could be simplified into two parameters one for vigour and one for viability. This leads us to the simple two-dimensional plot in Fig. 3A, which represents a reduction of the original data material of germination profiles in Table I. Here we approximate vigour as g%1 (abscissa) and viability as g%3 (ordinate). In order to avoid confusion we put the designates for vigour and viability in Fig. 3A and in the following figures and text in quotation marks as “vigour” and “viability” to distinguish their character as estimates. In order to visualise effectively the range of measurement, g%1 is chosen as

Table I. Germination profile values 1–8 days (GE conditions EBC 3.6.2) for the 17 Alexis samples in Fig. 2. Germination index (GI) and germination homogeneity (GH) according to EBC 3.7. The letters in the sample notation denotes country. Vigour potential (VP) is calculated after heat treatment according to Aastrup *et al.*¹. Background data from the paper of Møller *et al.*, 2002²⁴ previously unpublished. (Material I).

| | 1 | 2 | 3 | 4 | 7 | 8 | GI | GH | VP |
|------|----------|----------|----------|----------|----------|----------|-----------|-----------|-----------|
| 1DK | 85.8 | 97.8 | 98.3 | 98.8 | 99.3 | 99.3 | 8.8 | 64.6 | 1.5 |
| 2DK | 36.3 | 90.3 | 95.5 | 97.8 | 98.5 | 98.5 | 6.0 | 42.6 | 4.8 |
| 3DK | 77.8 | 98.5 | 99.3 | 99.3 | 99.3 | 99.3 | 8.2 | 56.5 | 1.7 |
| 4E | 72.5 | 85.5 | 88.3 | 89.0 | 93.3 | 94.3 | 8.3 | 52.2 | 1.1 |
| 5E | 69.0 | 83.8 | 89.3 | 91.0 | 93.3 | 93.8 | 7.8 | 42.7 | 1.1 |
| 6E | 49.5 | 80.0 | 84.5 | 85.5 | 88.8 | 89.0 | 6.8 | 40.4 | 2.3 |
| 7NL | 82.5 | 98.0 | 98.5 | 98.5 | 99.0 | 99.0 | 8.6 | 61.3 | 3.3 |
| 8E | 66.5 | 94.5 | 97.0 | 97.3 | 98.5 | 98.5 | 7.5 | 47.5 | 3.3 |
| 9CZ | 86.0 | 99.3 | 99.3 | 99.3 | 99.3 | 99.3 | 8.8 | 66.0 | 2.6 |
| 10D | 67.5 | 98.3 | 99.5 | 99.8 | 99.8 | 99.8 | 7.5 | 50.2 | 1.8 |
| 11SU | 40.5 | 92.8 | 98.5 | 98.5 | 98.8 | 99.0 | 6.1 | 41.3 | 2.2 |
| 12SU | 40.3 | 87.3 | 93.8 | — | 97.0 | 97.0 | 6.1 | 39.2 | 2.6 |
| 13SU | 37.3 | 95.0 | 98.5 | — | 99.0 | 99.0 | 6.0 | 45.6 | 2.8 |
| 14SU | 40.3 | 87.8 | 97.0 | — | 99.3 | 99.3 | 5.9 | 36.1 | 2.3 |
| 15SU | 51.5 | 93.8 | 97.0 | — | 98.8 | 98.8 | 6.6 | 43.7 | 2.0 |
| 16D | 85.5 | 96.3 | 97.0 | 97.0 | 97.3 | 97.3 | 8.9 | 64.5 | 2.0 |
| 17D | 61.0 | 93.5 | 96.0 | 96.0 | 96.8 | 97.3 | 7.2 | 46.1 | 1.8 |

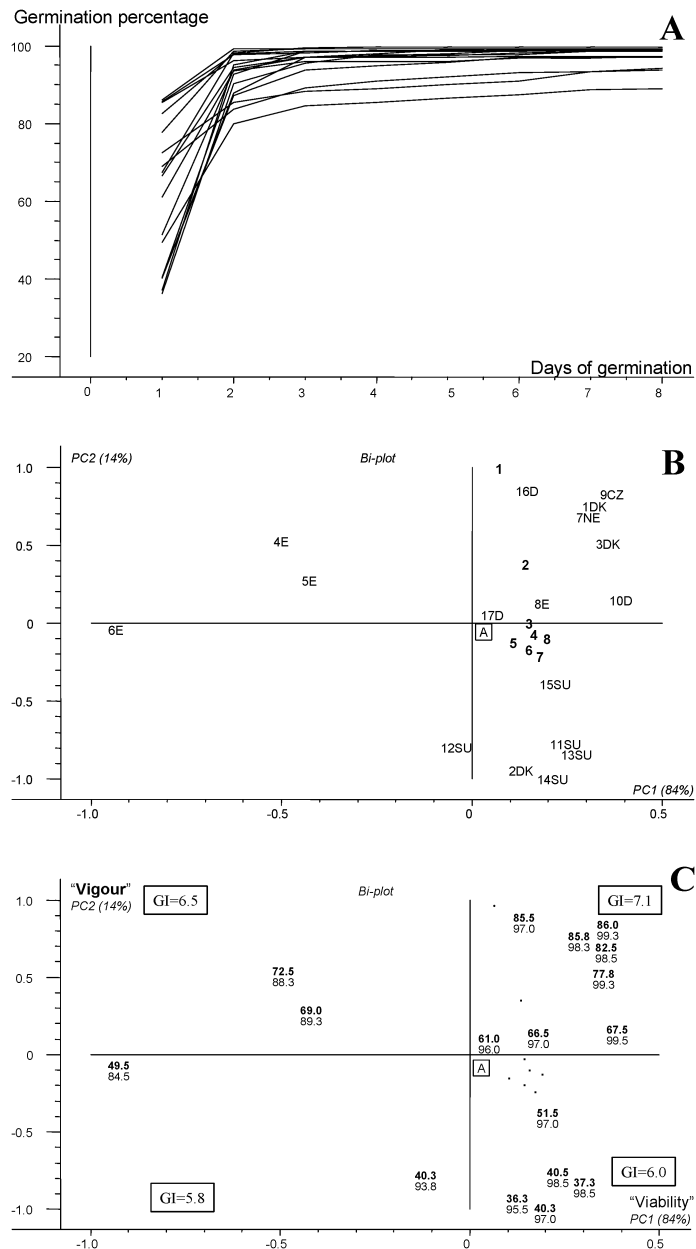


Fig. 2. Multivariate evaluation of germination profiles $g\%1$ – $g\%8$ (see Table I²⁴) for 17 untreated, non-dormant Alexis barley samples grown in EBC trials in Europe in 1994. **A.** Germination profiles of the 17 samples. **B.** Principal component analysis (PC1 (abscissa); PC2 (ordinate)). Biplot of the germination profiles for the 17 samples no 1–17. Letters denote country symbols. Figures in bold are loadings (variables) $g\%1$ (**1**)– $g\%8$ (**8**). **C.** Same PCA as B but with identification of each sample position by figures for $g\%1$ in **bold** (estimate for “vigour”) and $g\%3$ normal text (estimate for “viability”). GI = Germination index 3 days mean for each quadrant. See text for discussion.

the abscissa (x -axis) because it has a wider range compared to the ordinate (y -axis) $g\%3$, keeping in mind that the human vision can more precisely evaluate horizontal patterns compared to vertical. We will thus focus on “vigour”.

As first guidelines for the germinative classification based on GE^3 analysis conditions, we tentatively set the demarcation lines for “viability” at 95% and for “vigour”

70%, as outlined in Fig. 3A. It is clearly seen that the simple classification plot (Fig. 3A) differentiates the samples equally well as the PCA-model with the same material in Fig. 2B. In order to evaluate the classification we display the malt analyses of the quartile classes in Fig. 3A in Table II. There is a clear tendency in reduction of extract and increase in barley β -glucan, protein and wort colour from classes 1:1 and 1:2 to 2:1 and 2:2. Class 1:1

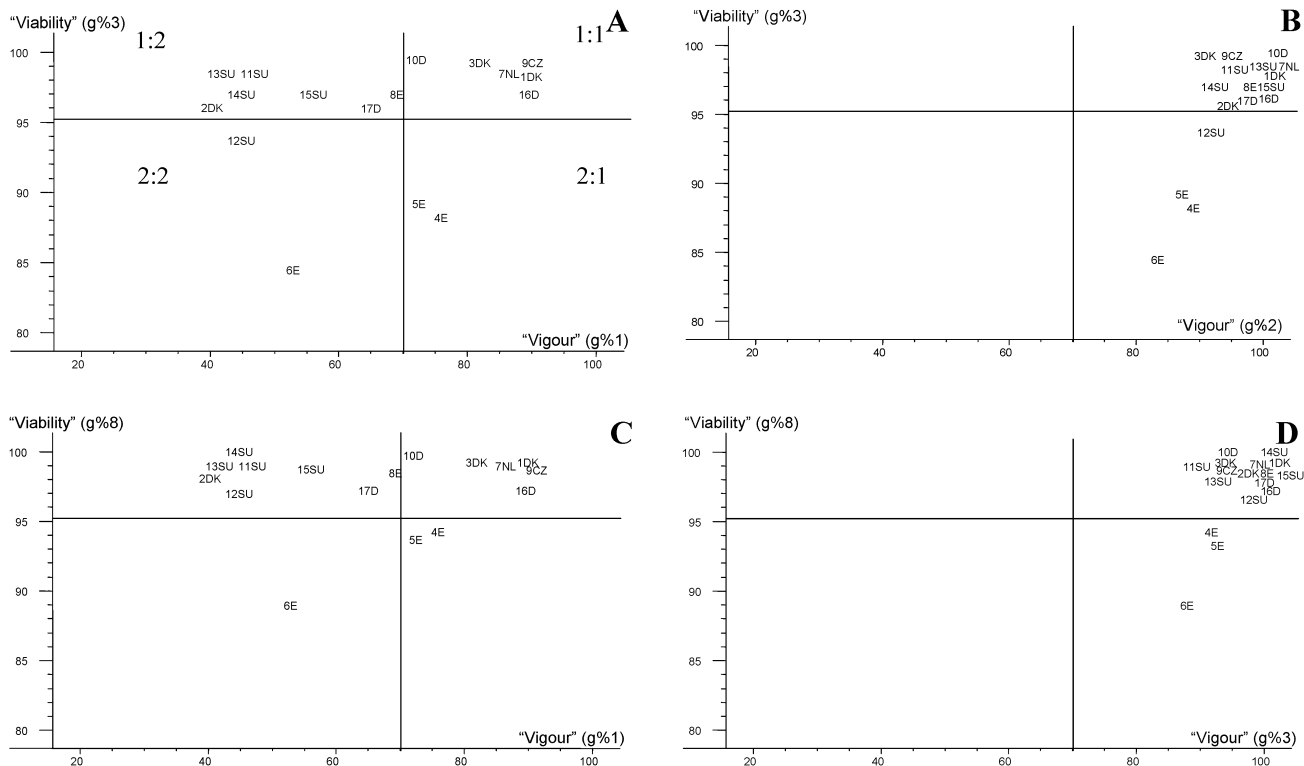


Fig. 3. Germinative classification plots of the Alexis samples²⁴ from Fig. 1 (Material I). **A.** “Vigour”(abscissa) defined as g%1, “viability” (ordinate) as g%3. For barley and malt analyses see Table II. **B.** “Vigour” g%2 (abscissa); “viability”(ordinate) g%3. **C.** “Vigour” g%1 (abscissa); “viability” g%8 (ordinate). **D.** “Vigour” g%3 (abscissa); “viability” g%8 (ordinate). For discussion see text.

Table II. Evaluation of the classes in the germinative classification in Fig. 3A (Material I, $n = 17$)⁴.

| | <i>n</i> | “Vigour” (g%1) | “Viability” (g%3) | GI | GH | TKW | Ext | BGbarley | P | C |
|-----|----------|-------------------|----------------------|-----------|------------|------------|------------|-----------|------------|-----------|
| 1:1 | 6 | 80.8 ± 7.2 | 98.6 ± 0.9 | 8.4 ± 0.5 | 60.5 ± 6.1 | 45.3 ± 5.6 | 81.1 ± 1.7 | 3.6 ± 0.2 | 9.4 ± 0.6 | 3.2 ± 1.9 |
| 1:2 | 7 | 46.0 ± 12.2 | 97.1 ± 1.1 | 6.3 ± 0.6 | 43.3 ± 3.8 | 43.0 ± 8.1 | 80.0 ± 1.5 | 4.0 ± 0.3 | 10.6 ± 1.6 | 2.7 ± 0.7 |
| 2:1 | 2 | 70.8 ± 2.5 | 88.8 ± 0.7 | 6.5 ± 0.2 | 47.5 ± 6.8 | — | 80.1 ± 1.0 | 4.3 ± 0.0 | 14.0 ± 0.1 | 9.2 ± 1.0 |
| 2:2 | 2 | 44.9 ± 6.5 | 89.1 ± 6.5 | 5.8 ± 0.2 | 42.4 ± 0.6 | 42.4 ± 0.6 | 78.6 ± 0.2 | 4.7 ± 0.4 | 13.8 ± 0.1 | 3.3 ± 1.1 |

has the highest level of “vigour”, “viability”, GI as well as germination homogeneity (GH). We will now comment on how different assignments of “vigour” and “viability” influences the resolution of the classification of these samples.

We first judge g%2 as a candidate for “vigour” in Fig. 3B. We note from the PCA biplot in Fig. 2B that its loadings (2) is positioned between the sign for g%1 (1) earlier assigned for “vigour” and the tight cluster (3–8) of g%3–g%8 indicative of “viability”. The g%2 trait has thus confounding information for both “vigour” and “viability”. Using g%2 for “vigour” and g%3 for “viability” in a classification in Fig. 3B thus greatly narrows the span of “vigour” compared to the assignment of g%1 in Fig. 3A.

Using the conservative estimate for “viability” g%8 in combination with g%1 for “vigour” (Fig. 3C) does not substantially change the picture from our earlier comparison of “vigour” g%1 and “viability” g%3 in Fig. 3A, other than being less discriminative for the outlier 12SU. By applying g%8 for “viability” the samples move on the average 2% upwards along the viability axis (ordinate) com-

pared to g%3 (Fig. 3A). Thus g%3 is for analytical time reasons the most practical estimate for “viability”.

The question is now to what extent the g%3 trait carries information regarding vigour when we plot it as an estimate for “vigour” against the conservative viability estimate g%8? Not very much as seen by the tight cluster of the whole material in Fig. 3D compared with the great differentiation power of our comparison g%1 versus g%3 in Fig. 3A. The extreme outlier samples 4E, 5E and 6E are however still classified as outliers below the 95% demarcation line for “viability”.

We thus conclude that the EBC methods³ featuring 3 and 5 days of germination are overwhelmingly indicative for viability. We claim that in a classification plot defined as “viability” g%3 (which almost all in the malting industry seem to agree on), a differentiating estimate for “vigour” such as one-day germination as proposed in the diagram (Fig. 3A) would be a useful tool for the industry. In the following we will further test this option while discussing possibilities of improving the precision of the method.

A proposal for a two-dimensional germinative classification for vigour and viability demonstrated on a barley material with highly diverse germination and malting performance (Material II)

In order to support a proposal for a classification system using g%1 and g%3 as estimates for vigour and viability respectively we now select a part of a material of barley of varying composition and malting quality (Material II), described in detail by Møller²². The 42 barley samples were harvested in 1993–96, cold stored at 7°C and analysed in 1999. The classification plot (GE conditions) appears as an overview in Fig. 4A and is enlarged with regard to the malting barley classes in Fig. 4B. Here we have introduced two levels of both “viability” g%3 (92% and 98%) and “vigour” g%1 (70% and 30%). We thus arrive at a tentative classification system of six malting barley classes 1.1, 1.2, 1.3 and 2.1, 2.2, 2.3 as well as a feed barley class 3.0 (Fig. 4A).

Table III shows mean values and standard deviations for barley and malt quality for these classes. In order to trace possible gradients within the classes, they are divided into sub clusters **a** and **b**, as displayed in Fig. 4B and in Table III. Values for extreme samples are also given. Now we use the germination, chemical and malting analyses from Table III to evaluate the classification of the barley samples in Fig. 4A and Fig. 4B.

The material has a large variation in “vigour”, “viability”, GI and GH due to variety and year of production. It is clearly seen that the “vigour” component is complementary to the germinative energy component “viability” in differentiating the whole material with regard to extract % and to an even greater degree with regard to BGwort, revealing the dependence of cytolytic activity in the malt on a swift and complete germination. The mean values of the malting barley classes reveal clear gradients in these important quality criteria. First, from the right to the left along the “vigour” axis from 79.0 to 17.8 g%1; (class 1:

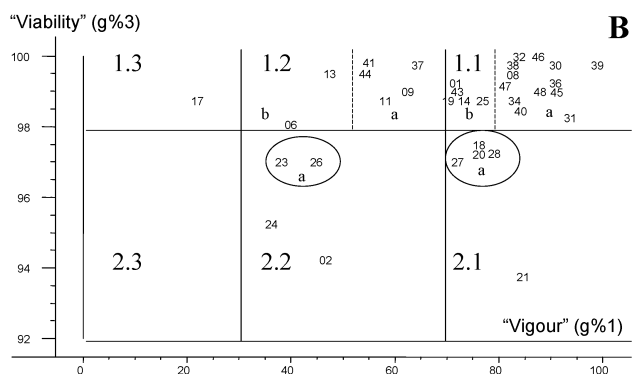
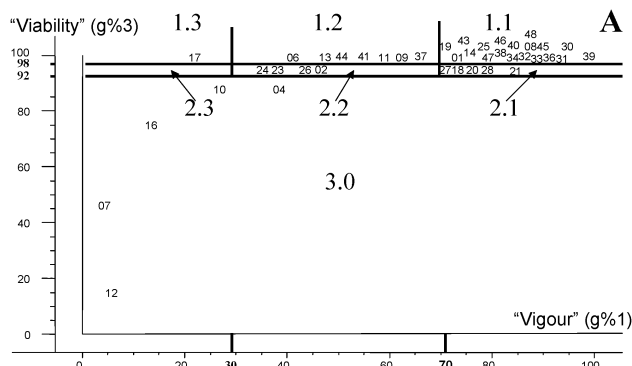


Fig. 4. Germinative classification for the malting barley reference material ($n = 42$) (Material II). “Vigour” g%1 (abscissa); “viability” g%3 (ordinate). For discussion of the limits and classes see text. For barley and malt analyses see Table III. **A.** Overview. **B.** Enlargement of the malting grade classes in the plot with “viability” $\geq 92\%$.

extract 83.2–80.6%, BGwort 180.4–257.0; class 2: extract 79.8–79.1%, BGwort 199.2–297.4) and second, from above to below along the “viability” g%3 axis from 99.2 to 95.9 g%3; (class 1.1 versus 2.1 extract 83.2–79.8%, BGwort 180.4–199.2 and for class 1.2 versus 2.2; extract 81.6–79.1% and BGwort 202.0–297.4).

Table III. Germination and barley and malt analyses for the 42 reference barley samples classified in Fig. 4. Mean and standard deviations of the different classes including values from extreme samples (Material II).

| Class | <i>n</i> | “Vigour” (g%1) | “Viability” (g%3) | GI (g1–g3) | GH (g1–g3) | TKW | Extract (%) | BGwort (mg/l) | BG (%) | P (%) |
|------------|-----------|--------------------|--------------------|------------------|--------------------|-------------------|-------------------|---------------------|------------------|-------------------|
| 1:1 | 16 | 79.0 ± 8.6 | 99.2 ± 0.5 | 8.3 ± 0.6 | 56.3 ± 10.0 | 43.6 ± 2.1 | 83.2 ± 1.8 | 180.4 ± 39.7 | 4.1 ± 0.3 | 9.3 ± 0.5 |
| a | 11 | 83.7 ± 5.5 | 99.4 ± 0.6 | 8.6 ± 0.5 | 59.3 ± 9.3 | 44.3 ± 1.8 | 83.9 ± 1.1 | 171.4 ± 37.1 | 4.0 ± 0.3 | 9.2 ± 0.4 |
| b | 5 | 68.8 ± 3.2 | 98.9 ± 0.2 | 7.6 ± 0.2 | 49.7 ± 8.6 | 42.1 ± 1.9 | 81.6 ± 2.1 | 200.2 ± 42.0 | 4.3 ± 0.3 | 9.5 ± 0.6 |
| 1:2 | 7 | 50.3 ± 8.5 | 99.2 ± 0.7 | 6.6 ± 0.4 | 54.5 ± 7.6 | 41.1 ± 3.9 | 81.6 ± 3.0 | 202.0 ± 81.8 | 3.9 ± 0.3 | 10.0 ± 1.2 |
| a | 5 | 54.7 ± 4.5 | 99.4 ± 0.5 | 6.8 ± 0.3 | 57.3 ± 7.2 | 42.3 ± 3.9 | 82.5 ± 2.9 | 176.2 ± 37.2 | 3.9 ± 0.3 | 9.4 ± 0.7 |
| b | 2 | 39.5 ± 5.3 | 98.8 ± 1.1 | 6.2 ± 0.3 | 47.7 ± 1.9 | 38.2 ± 2.1 | 79.3 ± 2.6 | 266.6 ± 151.6 | 3.7 ± 0.0 | 11.4 ± 1.4 |
| 1:3 | 1 | 17.8 | 98.8 | 5.4 | 54.5 | 35.3 | 80.6 | 257.0 | 3.9 | 10.2 |
| 2:1 | 5 | 73.1 ± 5.3 | 96.6 ± 1.6 | 8.0 ± 0.4 | 54.8 ± 4.1 | 37.1 ± 1.8 | 79.8 ± 5.4 | 199.2 ± 52.2 | 4.0 ± 0.5 | 10.2 ± 0.5 |
| a | 4 | 71.3 ± 3.8 | 97.3 ± 0.2 | 7.8 ± 0.2 | 53.1 ± 2.4 | 37.3 ± 2.0 | 79.1 ± 5.9 | 209.7 ± 53.9 | 4.2 ± 0.3 | 10.0 ± 0.5 |
| no21 | 1 | 80.5 | 93.8 | 8.6 | 61.2 | 36.6 | 82.9 | 157.2 | 3.2 | 10.6 |
| 2:2 | 4 | 37.3 ± 5.1 | 95.9 ± 1.4 | 6.1 ± 0.3 | 46.7 ± 1.1 | 40.7 ± 1.6 | 79.1 ± 1.9 | 297.4 ± 53.3 | 4.1 ± 0.2 | 12.7 ± 0.8 |
| a | 2 | 37.4 ± 4.8 | 97.0 ± 0.0 | 6.1 ± 0.2 | 47.1 ± 1.7 | 40.0 ± 0.5 | 80.1 ± 2.6 | 261.1 ± 55.7 | 4.0 ± 0.1 | 12.1 ± 0.3 |
| no2 | 1 | 42.5 | 94.3 | 6.4 | 45.9 | 43.0 | 78.7 | 341.7 | 4.3 | 13.7 |
| no24 | 1 | 32.0 | 95.3 | 5.9 | 46.4 | 39.9 | 77.5 | 326.0 | 4.0 | 12.9 |
| 3:0 | 5 | 13.6 ± 14.6 | 63.0 ± 31.8 | 4.8 ± 0.9 | 42.6 ± 5.8 | 40.0 ± 1.9 | 70.1 ± 7.6 | 382.2 ± 94.4 | 3.8 ± 0.2 | 12.1 ± 1.5 |
| no4 | 1 | 34.3 | 88.0 | 6.0 | 41.7 | 42.6 | 78.9 | 330.5 | 4.2 | 14.5 |
| no10 | 1 | 22.5 | 90.0 | 5.3 | 39.5 | 37.6 | 76.3 | 309.1 | 3.8 | 11.4 |
| no16 | 1 | 9.3 | 75.3 | 4.6 | 37.3 | 39.8 | 61.2 | 321.3 | 3.6 | 11.0 |
| no7 | 1 | 0.3 | 46.5 | 3.7 | 52.3 | 40.8 | 64.1 | 532.8 | 3.7 | 12.3 |
| no12 | 1 | 1.5 | 15.3 | 4.6 | 42.2 | 39.2 | 70.0 | 417.3 | 3.9 | 11.1 |

Table IVA. PLSR predictions of Extract and BGwort from germination parameters g%1, g%2 and g%3 for Material II ($n = 42$).

| | r | RMSECV | PC* | RE | n | Sign. variables |
|---------|------|--------|-----|-------|-----|-----------------|
| Extract | 0.85 | 2.79 | 3 | 11.43 | 42 | non |
| BGwort | 0.80 | 50.92 | 1 | 12.42 | 42 | g%1, g%2, g%3 |

*Minimum value of residual validation variance

Table IVB. NIT (1. der.) PLSR prediction of germination data for the same sample set as in Table IVA. Samples with low viability g%3 GE conditions (<92%) = underlined, medium viability (92–98%) = **bold**, viability >98% = normal text.

| | Step** | r | RMSECV | PC* | RE | n | Removed outliers in each step |
|--------|--------|------|--------|-----|------|-----|--------------------------------------|
| g%1 GE | 0 | 0.74 | 16.81 | 4 | 17.8 | 42 | |
| | I | 0.77 | 14.94 | 4 | 15.7 | 41 | <u>A12</u> |
| | II | 0.80 | 13.55 | 3 | 14.3 | 38 | <u>M16</u> , A20 , A27 |
| g%3 GE | 0 | 0.31 | 14.42 | 1 | 17.0 | 42 | |
| | I | 0.68 | 1.88 | 1 | 15.7 | 39 | <u>M07</u> , <u>A12</u> , <u>M16</u> |
| | II | 0.80 | 0.89 | 3 | 3.4 | 37 | <u>B04</u> , <u>A10</u> |

*At minimum value of residual validation variance

**Step of outlier selection from an influence plot

As seen from Table III, there are also clear gradients within the classes in extract and BGwort. An exception is subclass 1.2a containing five samples which have slightly higher extract and lower wort β -glucan values than the preceding higher subclass 1:1b. These differences are, however, not significant. Sample 21, which is a negative outlier in class 2.1, has in spite of its low 93.8% “viability” a much better malting quality than its position would indicate. This is partly due to its low β -glucan content in the barley (3.2% compared to the mean of 4.2% of subclass 2a ($n = 4$)) leading to low BGwort (157.2) and to its high “vigour” (80.5%) leading to high extract (82.5%). Note that the samples 02 and 24 from the inferior subclass 2.2 with higher “viability” (94.2 and 95.3%) than sample 21, but with lower “vigour” (42.5 and 32.0%), are much lower compared to sample 21 in extract (78.7 and 77.5%) and much higher in BGwort (341.7 and 326.0). The “vigour” component thus brings an essential differentiating element into the germinative classification plot.

The feed barley class 3.0 is clearly unsatisfactory for malting, as seen from the figures from the individual samples with mean figures of 70.1% for extract and 382.2 for BGwort. GH (Table III) has, as in the example discussed previously (Table II), a tendency to be positively correlated to “vigour” (g%1). GI has a high correlation of $r = 0.99$ in this material with g%1. It is concluded that the proposed germinative classification system with the barley material tested here is highly sensitive for predicting and discriminating the levels of extract (%) and BGwort (mg/L), which are the central parameters in the barley malt quality complex.

Defining the theoretical basis for the vigour and viability concepts by multivariate analysis explaining why vigour can be predicted by NIT spectroscopy (Material II)

Multivariate correlation between the germination profile and malt quality variables. We will now briefly explore the multivariate connections between the variables in our previous classification data set (Table III, Fig. 4A and Fig. 4B) with the aim to generate new hypotheses

inspired by finding structures in data, interpreted by experience in brewing science. Partial Least Squares Regression (PLSR) analysis^{12,17} is a two-way matrix (\mathbf{X} , \mathbf{y}) correlation model based on a decomposition principle comparable to the one block (\mathbf{X}) method PCA. It is here possible to judge the significance of the variables and their contribution in the total correlation¹⁸.

In understanding how the germinative classification system works we first asked ourselves how the germination profile (g%1, g%2, g%3) as \mathbf{X} is related to each of the parameters extract% and BGwort (mg/L) as \mathbf{y} . Table IVA shows a significant positive PLSR correlation between extract% and the whole germination profile of $r = 0.85$, however, none of the individual germination profile parameters are significant. An analogous PLSR correlation with BGwort (mg/L) displays a significant negative correlation coefficient of $r = 0.80$ where a high BGwort (mg/L) implies low germination values. Here all three germination parameters give significant individual contributions. It is concluded as expected from the evaluation of the germinative classification plot in Fig. 4 and Table III that the germination profile seen as a multivariate whole is highly informative regarding important malt quality parameters.

Obviously there are two major functional factors, which have to be taken into consideration when breaking down the endosperm to extract and BGwort:

1. The physiological factor of germ viability, i.e. plant hormone and enzyme dissemination into the endosperm for enzyme induction through the aleurone tissue necessary for malt modification.
2. The structural factor, i.e. the physical-chemical endosperm structure and composition of importance for resistance to malt modification and for the remaining β -glucan in wort.

Predicting germination variables from NIT spectroscopy profiles by PLSR. We did not expect that our two physical-chemical sets of analyses would be able directly to sense physiological-biochemical changes related to viability in the germ constituting less than 5% of the entire seed weight. At an early stage in our investigation

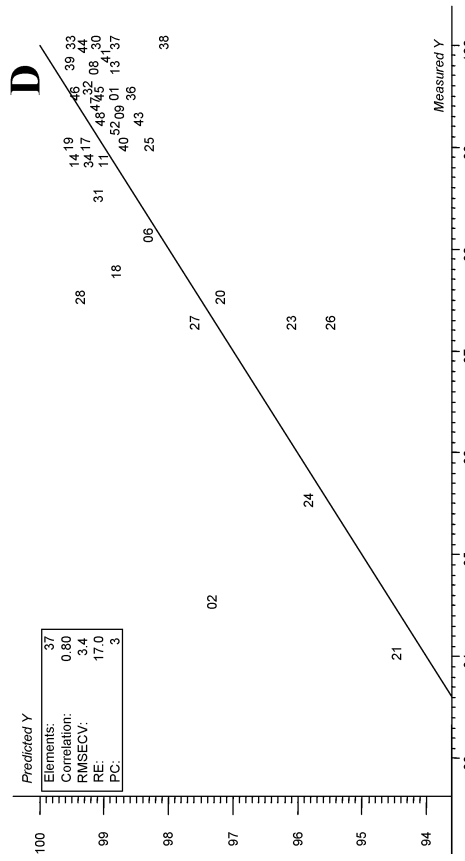
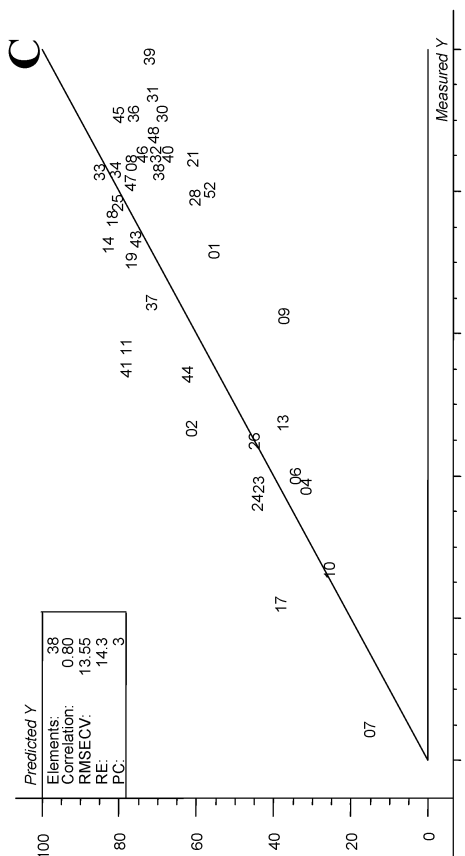
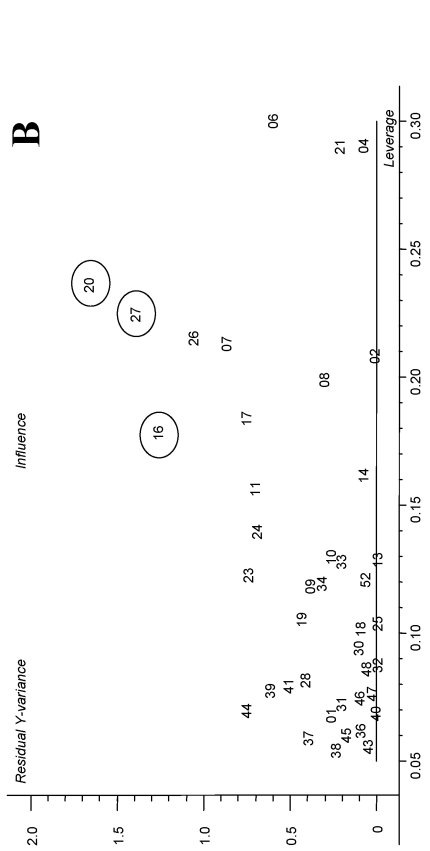
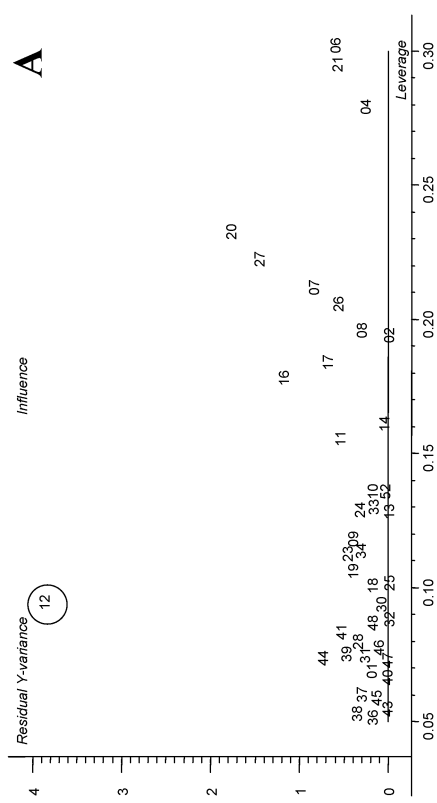


Fig. 5. NIT spectroscopy predictions for “vigour” g%1 and “viability” g%3 of the barley material ($n = 42$) classified in Fig. 4. Letters and numbers denote samples. Outliers are encircled and removed in later step. See discussion in text. **A.** Influence plot (step 0) for outliers in prediction of g%1 from NIT. One outlier will be removed. **B.** Influence plot (step 1) for outliers in prediction of g%1 from NIT. Three outliers will be removed. **C.** g%1 PLSR correlation plot, step II ($n = 38$). Three PC's for g%1. Four outliers in all are removed. See discussion in text. **D.** g%3 PLSR correlation plot, step II ($n = 37$). Seven outliers in all are removed. See discussion in text.

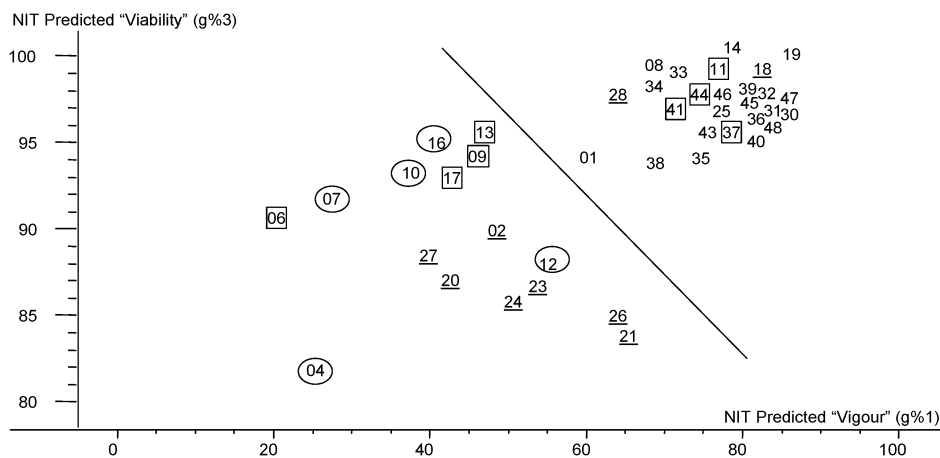


Fig. 6. Germinative classification plot g%1 “vigour” (abscissa) and g%3 “viability” (ordinate) values predicted by PLSR correlations with NIT. See discussion in text.

with material II²² we were surprised that the non-destructive NIT analysis on whole kernels, which should reflect the physics and chemistry of the seed (functional factor 2), could make reasonable PLSR predictions of “vigour” g%1 – a character which we first thought belonged to category 1 (the physiological factor) defined above. Thus we could get reasonable predictions of “vigour” of $r = 0.74$ (four PC’s, RE = 17.8) with all samples ($n = 42$) as seen in Table IVB.

In multivariate analysis it is important to select and define the nature of outliers. This is done by consulting a PLSR influence plot^{17,18} of the g%1 PLSR correlation to NIT data above as shown in Fig. 5A. Here sample 12 is clearly identified as an outlier. Removing this sample the correlation coefficient is improved to $r = 0.77$ (Table IVB, four PC’s, RE = 15.7) giving a new influence plot (Fig. 5B) where three new outliers were found 16, 20 and 27. A recalculation (Table IVB) improves the correlation to $r = 0.80$ (Table IVB, three PC’s, RE = 14.3) for the 38 samples as seen in the correlation plot in Fig. 5C.

A similar stepwise procedure of outlier identification by influence plots is presented for g%3 “vigour” PLSR correlation to NIT data in Table IVB. Here outlier removal leads to a high increase in the correlation coefficient from $r = 0.31$ (one PC, RE = 17.0) to $r = 0.80$ (three PC’s, RE = 14.3) in two steps. The last correlation is presented as a plot in Fig. 5D. Five outliers 07, 12, 16, 04 and 10 were identified. When consulting the germinative classification plot in Fig. 4A; 07, 12, 16, 04 and 10 are classified as class 3.0 feed barley while the outlier samples 20 and 27 from Fig. 5C belong to class 2:1 medium grade malting barley.

It is concluded that samples with low “viability” are outliers in the PLSR NIT correlations for prediction of “vigour” and even more marked in those for “viability”.

It should be pointed out that the outliers with low “viability” g%3 identified by the PLSR influence plots above are deviates in y (g%3) and not in X (NIT). We have checked that there are no improvements in the correlation coefficient by removal of X outliers. These do not show low “viability”. This implies that “viability” cannot be predicted in unknown samples by NIT spectroscopy. This

is in accordance with our initial hypothesis that physical-chemical analyses like NIT should not be able directly to trace physiological factors represented by low germ viability (e.g. dead and slowly germinating kernels). If information for “viability” had been carried in NIT data – this would have been possible. A separate method for “viability” such as g%3 (GE) or theoretically more correct g%8 or by the tetrazolium staining test for live germs (GC³) is thus needed as a supplement to “vigour” g%1 to obtain a reliable germinative classification plot. If one wants to evaluate the theoretical aspects of germ “viability” comparing different kernels under identical substrate conditions, one has probably to excise the germ and resort to embryo culture *in vitro*.

In Fig. 6 we surprisingly see that we can, to some extent, repeat our germination classification plot from Fig. 4A by using the original NIT PLSR predictions mentioned above (no outliers removed) with “vigour” g%1 and “viability” g%3 for each of all 42 samples. The demarcation line between the two classes – high quality above to the right and low quality below to the left – was tentatively drawn to fit in between the main clusters in the plot without any *a priori* judgement, regarding individual sample data. The NIT germinative classification plot thus obtained (Fig. 6) is successful in making a complete separation of the extreme classes 3.0 (feed quality, encircled) and 1.1 (highest malt quality, normal figures). The low malting barley classes 2.1–2.3 are divided with seven samples (underlined) to the left in the plot and two outliers in the high germination quality cluster to the right. The low vigour part of the premium class 1, – classes 1.2 and 1.3 (squared) are divided with four samples in each of the two clusters. If the dividing line between good and low malting quality is drawn between classes 1.3 and 2.1, six samples out of 42 were thus wrongly classified. This preliminary coarse NIT classification with a limited material is a promising indication and demonstrates that further investigations to develop a germinative screening method based on NIT with a much larger calibration material should be profitable.

Checking the results from NIT prediction of germinative parameters by a separate set of data. In our

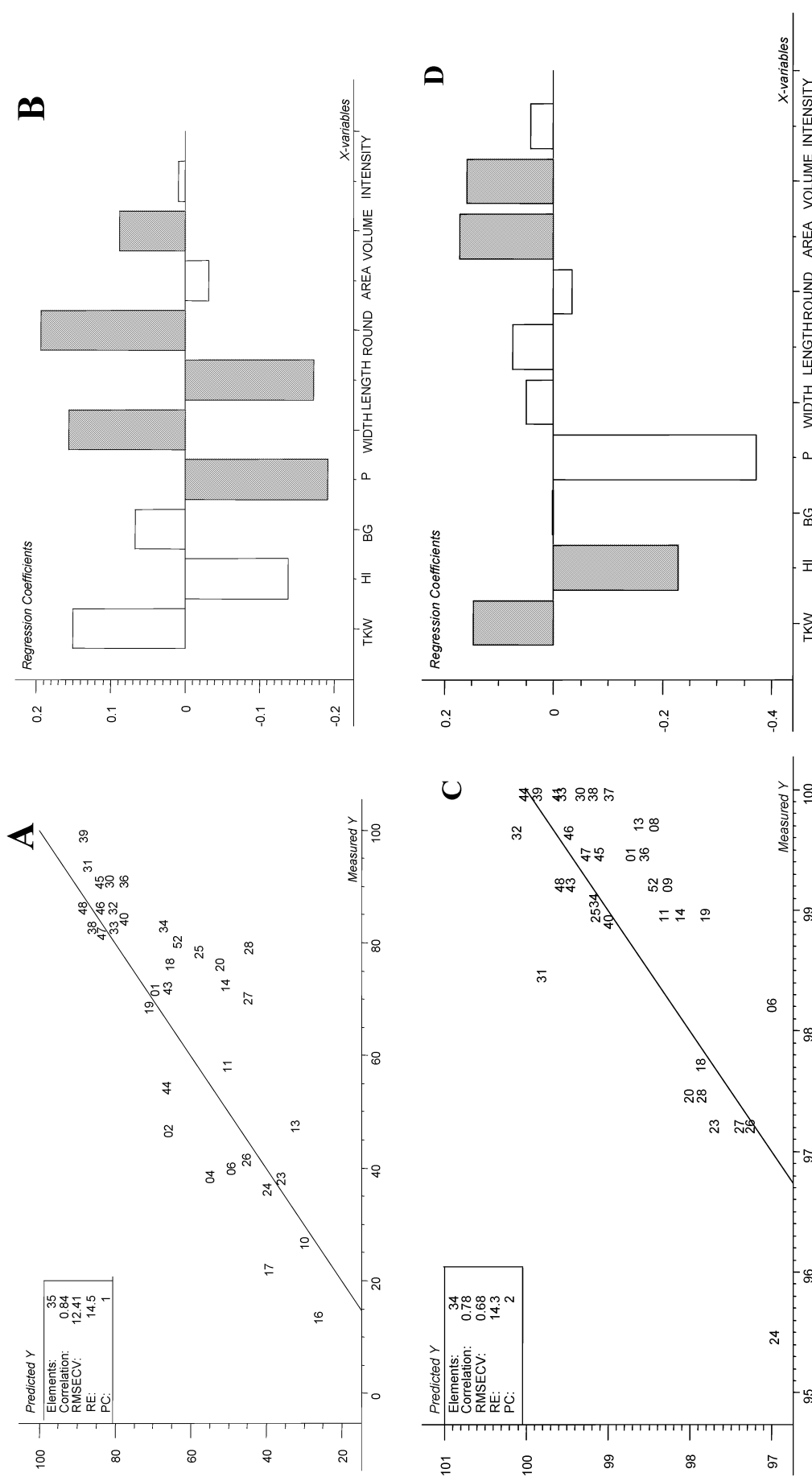


Fig. 7. PLSR correlations and Jackknife regression coefficient plots for the barley material in Fig. 4 and Table III. X = 10 physical-chemical barley analyses (see abbreviations, text and figures). **A.** PLSR correlation “vigour” g%1(GE conditions) = y. **B.** Regression coefficient

plots for 7A. Significant variables shadowed. **C.** PLSR correlation “viability” g%3 (GE conditions) = y. **D.** Regression coefficient plot for 7C. Significant variables shadowed.

multivariate approach to confirm the hypothesis of a major structural impact on “vigour” founded on our NIT PLSR predictions discussed above, we made a separate analysis on the same barley samples using a separate set of ten physical-chemical analyses²² (Fig. 7).

In Fig. 7A–D we have included the ten physical-chemical criteria manifest in the intact ungerminated seed as a representation for structure which is correlated in a PLSR analysis with “vigour” g%1 and “viability” g%3 (GE conditions) with Jack-knife validation. The ten analyses are two chemical analyses (BGbarley and protein analysed on bulk) and eight physical analyses: hardness, seed weight and six automatic seed analyses from the automatic imaging instrument: width, length, round, area, volume and light reflectance intensity. The destructive seed hardness index (HI) is analysed on a separate instrument. In order to eliminate range and numerical differences in parameters, which highly influence the correlation coefficients, we regularly employ scaling as a pre-treatment to data. We obtain, as with NIT spectroscopy, a significant PLSR correlation ($r = 0.73$, one PC, RE = 18.1) with g%1 (“vigour”) as y and the ten physical-chemical parameters as X . When inspecting the influence plots in two cycles we note two “viability” g%3 outlier samples 07 and 12, which are below 92% (Fig. 7A) and one outlier 21 in the medium viability category below 98%. Four outliers; samples 08, 09, 37 and 41 cannot be explained by deviating “viability” properties. In Fig. 7A we have displayed the correlation plots of the barley material when we have eliminated the seven outliers. The correlation coefficient has increased to $r = 0.84$ (one PC, RE = 14.5) with five significant variables; P, round, length, width and volume (Fig. 7B). Using four PC’s in this outlier-corrected material (influence plot not presented here) we obtain a correlation coefficient of $r = 0.94$ and an error of RE of 9.1.

The correlation of “viability” g%3 to the physical-chemical parameters is much lower than for “vigour” in the whole material ($r = 0.39$ versus $r = 0.73$, one PC) as with the NIT analysis. When inspecting the influence plot (not presented here) we identify eight outliers, six having low and one medium low “viability” and the last outlier with medium “viability” and high “vigour” (samples 02, 04, 07, 10, 12, 16, 17 and 21). The correlation plot without the eight outliers is presented in Fig. 7C. It has a distinctly lower correlation coefficient of $r = 0.66$ (RE = 22.7) compared to $r = 0.84$ (RE = 14.5) for “vigour” in Fig. 7A. The four significant variables (Fig. 7D) are HI, area, volume and TKW.

Connecting information to explain the physical-chemical basis of germination. Møller²² explained the ability of NIT to predict “vigour” g%1 by the high individual correlation predictions of NIT to each of the majority of the ten physical-chemical parameters in this barley material. Extract% and BGwort could also be predicted by NIT and by the ten physical-chemical parameters²². Most of the outliers in the latter correlations were also found to be samples with low viability. The tight connection between the malt parameters and the germination profile is demonstrated in Table IVA.

It is clear that the multivariate correlation’s demonstrated above to a great extent explain the predictive qualities of the “vigour” g%1–“viability” g%3 germina-

tive classification plots, how they relate to critical quality criteria and why “vigour” g%1 together with germinative energy “viability” g%3, should be integrated in malting barley evaluation, as an essential complement to the barley and malt analyses.

With our strategy of focusing on the structural factor by PLSR correlation’s to NIT and to the ten physical-chemical variables, identifying the physiological (“viability”) nature of the outliers, we reach the surprising conclusion that germination speed “vigour”, g%1 in this investigation, has a much more pronounced structural component than physiological within the range of viability which is characteristic for malting barley. The g%3 characteristic also reflects to some degree seed structure, but with a much lower correlation to the structural parameters than g%1 and with a larger number of low viability outliers. It seems that the NIT correlations are more robust than those of the set of ten physical-chemical parameters indicating that NIT data should represent the most complete physical-chemical fingerprint of the two screening methods.

We thus arrive at the important conclusion that the structural physical-chemical factor is the main determinate for vigour, defined as the early growth rate of the emerging plantlet in barley of malting grade. We interpret these preliminary results as follows. The substrate availability for the germ is of importance for fast sprouting and is related to the function of how to unlock the complex physical and chemical structure of the food store – the endosperm. This function should also be identical with the aims of the maltster to obtain a fast malt modification in dissolving cell walls and in enzyme spreading in the endosperm. Fast germination, i.e. high “vigour” g%1, should therefore be operative for the maltsters as an indicator of an efficient malt modification representing the structural factor related to physics and chemistry and to extract and BGwort performance respectively.

Thus the structural physical-chemical factor becomes limiting for seed vigour and malting and brewing performance of the barley samples that fulfil the classic germination energy qualification limit of 92%.

DISCUSSION

Classification systems for malting barley quality as tools in trade, industry, plant breeding and research

We have two sources of inspiration for our suggestion to upgrade the germinative energy (EBC 3.6)³ and capacity (EBC 3.5)³ concepts with a supplementary classification system as visualised in Fig. 4A and Fig. 4B. The first is the suggestion given by the PCA algorithm (Fig. 2B) to arrange the material (Table I) into two separate basically independent representations of vigour and viability (Fig. 2C). The second is the fact that maltsters and brewing scientists^{11,19} in practice when considering GI, as an expression for vigour must always check this information against viability expressed as GE% for 3 or 5 days. Fig. 4A and Fig. 4B represent an informative visualisation of this reflective process, which is dramatically simple.

We have found a high correlation between GI and g%1 in the two barley materials presented here. GI could well be used instead of g%1 as an estimate for vigour. We suggest, however, that g%1 should be preferred. First, because it is much more responsive than GI with regard to the vigour trait. Secondly, because day 1 germination information from either the germinative capacity (EBC 3.5.2)²² or energy (EBC 3.6) methods³ could be combined with the fast tetrazolium (EBC 3.5.1) screening method for viability in a germinative classification plot for a diagnosis within 24 hours according to the layout in Fig. 4. Further studies should be made to check the reproducibility of the g%1 estimation for “vigour” in comparison with GI (EBC 3.7) testing the fluorescence tool in non-hydrogen peroxide germination methods for improvement of automated counting at an early germination stage.

The “vigour” and “viability” classification test may also be used in micro and pilot maltings and in full-scale trials as an essential description of germinative “acceleration” and “mass” inherent in the germination quality trait of fundamental importance in industrial malting barley utilisation and economical evaluation. The promising use of NIT screening for an indicative germinative classification (Fig. 6) *on-line* alone or in combination with the Tetrazolium test for “viability” will be discussed in the Conclusions.

The necessary mathematical tools for evaluation of complex covariate data sets

Pattern recognition multivariate analysis is indispensable when evaluating large horizontal data sets with many strongly correlated (covariate) variables such as germination profiles and NIT spectra. If such data has high quality, the fingerprint of variables will in a unique way characterise each sample. Such a characterisation is not possible in classic statistics^{17,18,26}. This property makes it also possible for chemometrics to handle incomplete and unbalanced data sets, which are more difficult to handle with classic methods^{17,18}.

The graphic interface of PCA and PLSR score plots facilitates an interactive overview of the complex data set combined with an in depth scrutiny of the detailed connections between samples and variables as demonstrated in our examples. The chemometric analysis is hypothesis generating letting the data set at first speak for itself with a minimum of *a priori* hypotheses. Validation by prior knowledge and further chemical analyses are dynamically included after the first evaluation. Statistical validation of errors in PLSR is made by data experimentation within the data set by cross validation or by comparing a calibration set with a test set sampled from the same population. The conclusions are supported by evaluating in parallel two separate materials e.g. Material I and II and by comparing separate sets of analyses e.g. the PLSR correlation of g%1 “vigour” to NIT-spectroscopy on one hand and to the set of ten seed physical-chemical analyses on the other as demonstrated above.

Seed scientists such as Ellis and Roberts^{6,7,10} used classic probability calculus and curve fitting when evaluating germination profiles and seed deaths with storage time by accelerated ageing by heat. A seed sample, which could

resist heat, was supposed to have a high vigour⁴. Because of the hard assumptions of their statistical models they could only use one germination variable (e.g. g%8) at a time to study resistance in the decay of viability as a marker for vigour.

In malting research Favier⁸ and Woods *et al.*⁴³ used curve fitting to model the germination rate of dormant seeds during storage and Aastrup *et al.*¹ utilised Ellis and Roberts^{6,7} heat stress probit model for calculating vigour potential (VP). These scientists analysed a limited number of samples and did not test their models by a separate test set. The VP's calculated by probit analysis could not predict germination vigour in Material I, Table I (VP to GI = -0.37)²⁴. The accelerating ageing theory using heat stress is thus far from predicting the vigour of the brewing malt either as GI or as g%1²². In malting technology other form of stresses i.e. from oxygen depletion and water should be more adequate to study.

We do not claim a global calibration for “vigour” g%1 by NIT-spectroscopy but rather that our preliminary results are indicative for the feasibility of a new direction in future malting barley research. This is demonstrated by our validated hypothesis on the importance of seed structure for vigour, first presented in this publication. We can also conclude that in order to achieve our results we have to address the sensitivity (range) as well as the reproducibility of the analyses. Thus, BGwort (Table III) is a much more suitable parameter than wort viscosity and BGbarley for spanning the functional factor of modification resistance^{2,30} just as g%1 is more sensitive than GI as a marker for “vigour”.

Compressing malting barley quality data into information – quality index versus hierarchical classification

It is in this context important to realise that there is nothing such as a combined index for vigour⁴ and viability⁴ because both give unique and complementary information. We have found that their information is best expressed as classes in two-dimensional graphic representations. The seed agronomist Heydecker¹⁰ thus solemnly concludes (p. 225): “Attempts to express germination speed and percentage in one combined index are well meant but confusing”. Hampton and Coolbear in 1990⁹ agree (p. 225) “It seems clear to us . . . that it is most unlikely that any one aspect of behaviour, whether germinative, physiological or biochemical will be a universally reliable index of all aspects of seed vigour”.

However, in brewing science the idea of a malting barley quality index has for a long time been in focus, as discussed and presented by Monnez *et al.*²⁵ and Molinacano²¹ in 1987, the latter publication resulting in an EBC index²¹. Most brewing scientists throughout the years^{15,21,25,31}, in their efforts to classify or to construct a malting quality index for barley, seem to have forgotten to include standardised parameters under GE or GC conditions with regard to viability and vigour. Our results and the above-cited literature indicate that germination parameters for “viability” (g%3) and especially for “vigour” (g%1) are informative enough to be included in malt quality data.

Molina-Cano²¹ constructed the Q malting barley index ranging from 1 (feed barley) to 9 (highest malting quality) in three steps. First, five critical characteristics – extract yield, Kolbach index, apparent final attenuation, viscosity and diastatic power – were selected. The reference value was defined as the overall mean for all varieties, locations and years. In the second step an index of quality was defined for each characteristic ranging from 1–9 based on the reference value and the position of the actual sample value in a normal distribution curve. In the third step an overall index was obtained by a weighted linear combination of the indices of quality for each characteristic where the coefficients were based on the judgement of an expert committee. The Q index was tested as a tool for malting barley quality classification for trials in the years 1982–1985 under variable conditions²¹. Our brewing research group³¹ has recently suggested a refinement of the EBC-method²¹. An acceptance/rejection profile (membership curve) with scores between 0–1 is worked out by experts and related to the range of values for each parameter. Instead of a constant coefficient for each characteristic, fuzzy logic was used to calculate an overall quality index (OQI) value from the memberships curves. This method was able to adequately rank a limited material of malt analyses from 50 spring and winter barley samples. There was surprisingly a reasonable PLSR correlation between the OQI value and NIT spectroscopy³¹, which confirms the conclusions regarding NIT predictions of malt quality parameters in this barley material and by Møller²².

In evaluating malting barley quality, the importance of different parameters indicative for the practical use of the barley cultivars will change in different years^{15,16}. A univariate quality index is not likely to function optimally in such a dynamic reality. Monnez *et al.*²⁵ (Fig. 9, p. 484) therefore used multivariate pattern recognition analysis to obtain two hierarchical indices, one for the suitability of malting and one for the suitability of brewing for use at two and three levels respectively combining expert validation with barley and malt analyses. The indices are displayed in an abscissa-ordinate plot, divided into classes resembling our germinative energy classification plot for “vigour” and “viability”(Fig. 4A and B) but with a much more complex primary data set.

A quality ranking must be able to recognise the pattern of analysis characteristics of each individual barley sample in a larger calibration context of feed and malting barleys. This is only possible by a multivariate chemometric approach. This could be done, if a large jointly used database could be set up for the benefit of brewers, maltsters and plant breeders as a source of artificial intelligence to visualise the position of each new barley sample in a PCA for classification. Van Lonkhuijsen *et al.*¹⁵ have demonstrated the feasibility of such a strategy in a limited scale where it was possible to reduce a considerable number of analyses without losing information.

In a PCA with 186 commercial malt samples Munck²⁷ was able to reduce the 11 quality analyses to three “functional combination factors”, obtained by identifying the nature of the first three principal components. These factors (PC’s) were:

1. “Chemistry” (extract plus a range of enzyme influenced analyses)
2. “Physics” (malt hardness, cell wall thickness (β -glucans), resistance to malt modification²)
3. “Protein”

In another trial from our research group with a smaller material of 50 spring and winter barley samples only the “physics” functional factor prevailed in a clear cut³⁵ manner.

The “functional factor” scores (PC’s) or function specific indices could be used in classification plots analogous to those for germination classification in Fig. 4A and Fig. 4B in a hierarchical way in different combinations, such as 1, 2, 2:3 and 1:3, as suggested by Monnez *et al.*²⁵. They could alternatively be used in a three dimensional classification. We are convinced that if sensitive and reproducible analyses are developed and selected and a broad calibration material is obtained, two to three composite factors covering malting barley functionality should suffice. Such a strategy would be more adaptive to different environmental conditions and much more informative than a simple univariate malting barley quality score and would not increase the analytic workload compared to the present situations. Instead the number of analyses should be able to decrease considerably. The germinative parameters “vigour” and “viability” have their obvious place in such a malting barley quality classification system.

CONCLUSIONS

The role of germinative analyses in developing international malting barley quality control systems based on NIT spectroscopy and image analysis

The simple germinative classification for malting barley presented here could be directly tested and used in practice today with marginal additional costs. Our findings that the major component of the “vigour” parameter is related to the structure of the endosperm makes instrumental analyses such as NIT spectroscopy and image analysis attractive. These parameters are important for the accessibility of the endosperm food store to the embryo and for the speed of malt modification. The non-destructive NIT spectroscopy methods calibrated to protein% and water% are used routinely today for on-line grading of barley by seed elevators and maltsters. The positive experience with global calibrations using NIT with regard to water and protein²⁰ demonstrates that it is possible to calibrate the spectrometers via the Internet. Reproducibility of the reference analyses is often a greater problem than the predictive results obtained from the spectra.

An extended, semi-intelligent, updated database in service for the malting barley industry and trade analyses is a gigantic, far-sighted task which requires international co-operation on a large scale between transnational communities such as EU, industrial branch collaborations like EBC, instrument companies and a network of industries and universities. Experience from the ring tests in the EBC analytical committee tells us that it is the sensitivity of the reference analyses and the reproducibility between labora-

tories, including the necessary germination methods³⁷ and micro maltings, that are the weak points. The issue now is how the great potential of NIT spectroscopy^{33,36} may be expanded as indicated here to all the physical and chemical parameters in barley, which constitute the physiological²⁸ and technological basis⁵ for germination and malting. In order to overcome the exorbitant costs to obtain global calibrations with NIT and to improve precision in establishing the desired data library we suggest that the analyses in the calibration step should also be automated, preferably as a compounded instrument, combining several analyses.

Maltsters⁵ are interested in fast chitting, but slow development of roots and acrospires, while focusing on the speed of malt modification and enzyme development within the endosperm. An instrument for this purpose should be able to analyse 300 seeds in a few minutes with dry single seed analyses starting with seed form/weight parameters and colour and moisture analyses and finishing with a destructive hardness test³⁶. The instrument should also be able to work with wet seeds automatically to determine germinated seeds at g%1 and g%3 using fluorescence for early identification. Thus, varieties with an early chitting and slow development of roots and acrospires could be selected for the best compromise for obtaining a high yield of malt and a fast modification. The processes inside the barley endosperm should be speeded up by proper counter selection where excessive development of external organs of the seed should be retarded. Such an instrument could be used as stand-alone in the seed laboratories of the seed dealers, maltsters and plant breeders e.g. to study water sensitivity¹³ as well as a calibration instrument for NIT spectroscopy. Tentatively the instrument could even include single seed NIT measurements³⁴ for control of barley and malt seed homogeneity. By combining information regarding vigour from NIT spectroscopy with that of the Tetrazolium test (EBC 3.5.1)³ for viability, a germinative classification could be performed within two hours, if a high-quality “global” calibration for g%1 to NIT can be established. The preliminary results obtained with Material II points out that an indicative instant germinative classification could be possible by NIT prediction of “vigour” g%1 and “viability” g%3 alone (Fig. 6). This finding needs further confirmation and explanation.

We have indicated earlier^{26,32} that it should be possible to select improved malting barley by its NIT spectrum as a total physical-chemical spectral fingerprint of the sample positioned on a PCA plot with an ideal barley spectrum as reference. Translated to the task envisaged here, it should be possible by calibration of NIT to “vigour” g%1, checking lines with “viability” g%3 below 92% to breed for the whole physical-chemical complex of endosperm availability as a substrate for the embryo. This characteristic should be identical to the ease of malt modification. By means of a set of barley and malt standards spanning the whole quality range documented and distributed by a few acknowledged inter-calibrated laboratories all users could, within the limits of the method, preliminarily evaluate their own barley by their NIT spectra in a PCA. It is in this context essential to disseminate the rather simple use of basic chemometrics^{17,18,44} to the whole production chain

from plant breeding to malting and brewing. Such knowledge is now in the high days of information technology hiding in the “black box” software of the NIT instruments currently used. Here it does not contribute to the kind of multivariate thinking which today is necessary for successful complex problem solution. All participants in the barley to beer production chain should be able to make a PCA on their own data and know how to interpret the biplot. Chemometrics⁴⁴ in brewing science and technology has a potentially more direct profitable impact compared to, for example, biotechnology in solving complex problems.

A dynamic international malting barley quality reference data and sample library calibrated to measurements to standardised instruments by multivariate analysis would be able to speed up and rationalise quality grading in industry, plant breeding and trade to reach a new standard of rationalisation and precision. The two-dimensional “vigour” g%1/“viability” g%3 classification plot suggested here could be used directly in practice for convenient quality grading under germinative energy and capacity conditions and should be included in the software of a future malting barley grading network based on NIT spectroscopy.

It can be concluded that problems associated with agro-biological based technology, dependent on natural variation, only can be solved by a moderate but long sighted enduring investment in research in order to build up a representative data base for reliable calibrations and predictions. This implies an acknowledgement of the unique covariate properties that characterise each biological individual^{26,42} such as a homozygotic barley line or variety. These properties can only be understood by an open ended interactive exploratory experimental strategy implemented by multivariate pattern recognition data analysis⁴⁴.

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