Near Infrared Transmission Spectra of Barley of Malting Grade Represent a Physical-Chemical Fingerprint of the Sample That Is Able to Predict Germinative Vigour in a Multivariate Data Evaluation Model

Birthe Møller

ABSTRACT

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The physiological and physical-chemical basis of barley germination was studied. Vigour was defined as germination percentage after 24 h and viability as that of 72 h. The barley samples were analysed under germination capacity and energy conditions after harvest and after long time cold storage at 7°C three–six years. These parameters were each correlated by Partial Least Squares Regression (PLSR) to two separate multivariate data sets: a set of ten physical-chemical parameters and to Near Infrared Transmission (NIT) spectra (850–1050 nm). Surprisingly high correlation coefficients for each of these two data sets were obtained especially with vigour, extract (%) and β-glucan in wort (mg/L) when outliers with viability below 92% were removed. Hard, slowly germinating seeds were more resistant to decay in vigour and viability storage than soft seeds. This change could be predicted by PLSR correlations to the two physical-chemical multivariate methods. Vigour was a more sensitive indicator for the ability to store than viability. The steep criterion was also found to have a physical-chemical basis. The results indicate that NIT calibrations can be used to predict vigour in malting grade barley.

Key words: Malt quality, Near Infrared Transmission spectroscopy, physical-chemical properties, seed imaging analysis, seed viability, seed vigour.

INTRODUCTION

The malting barley quality complex[5,6,14,20,21,28–30] consists of a wide range of physical and chemical criteria that are manifest already in the intact barley grain as well as indirect parameters, which are first developed during malting and brewing. To overview such an elaborate quality complex, with a minimum of hard assumptions constitutes a great challenge to the brewing chemist. However, thanks to multivariate pattern recognition data analysis[14–16,20,21] also called chemometrics, it is now possible to identify the more and less unique analytical pattern of each individual barley sample for classification and correlation and to trace the influence of important variables as visualised on a graphic display. Now an extended sequential data dissection and component synthesis and identification is possible. Then new hypotheses can be generated and verified to prior knowledge and to further cycles of data analyses and experiments.

Multivariate data analyses have been rather sporadically used for analysing data in brewing research[10,11,14,19–21,28,29,31].

Instrumental screening methods for quality *at-line* and *on-line* have been exploited in the production chain of the brewing industry throughout the last 15 years. Examples are Near Infrared Transmission (NIT) spectroscopy[17] and seed imaging and hardness[29] determination instruments, which will be further utilised in this experimental multivariate approach to elucidate the relations between germinative properties and physical-chemical properties, in order to predict malt quality.

Our research group[25–27,38] has focused on finding a simple and relatively fast criterion for seed vigour. We demonstrated[25], that the barley samples could be classified according to malt quality in a plot with estimated "vigour" (g%1) as abscissa and "viability" (g%3) as ordinate.

This paper is an extended in depth analysis of the barley material harvested in 1993–1999 used by Munck and Møller[25] for germinative classification here also including the storage and steep aspects.

Vigour and viability of seeds are the most important quality criteria in malting barley[6,25,32,33,35]. Because the germination parameters are highly influenced by weather conditions, it is difficult to get a reliable estimation of malting barley quality by testing varieties for one year in one environment only. In Northern Europe there will typically be one to two extreme years with unacceptable vigour and viability during a 10-year period.

It is therefore important to make seed collections in years yielding low vigour seeds, because each such year is unique and cannot be simulated by artificial treatments. The chemical-physical composition[2,18,19,37] can be influenced by temperature, precipitation, fertilisers[1,7] and

The Royal Veterinary and Agricultural University, Department of Dairy and Food Science, Food Technology, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark.

Corresponding author. E-mail: bm@kvl.dk

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Table I. Sample plan with sample identification and analysis of "vigour" g%1 GC conditions shortly after harvest (germination capacity method). A1, A2, A3 (Alexis), B1 (Blenheim), L1 (Lysimax), M1, M2 (Meltan) were delivered by farmers (0th generation) in 1993 and propagated in 1994–1999. Samples grown on Zealand (z), Funen (f) and in Scania (s). Sample identification example: A8^{57} = Alexis grown in 1998 with sample number 57.

		1993	1994	1995	1996	1997	1998	1999
0 growing generation	**A1**	77,0 (f) ^{1}	37,3 (z) ^{8}	85,8 (z) ^{18}	83,1 (z) ^{30}	45,5 (z) ^{50}	72,5 (z) ^{56}	-
			19,0 (s) ^{9}	35,5 (s) ^{19}				
			74,0 (f) ^{10}	46,3 (f) ^{20}				
	A2	68,3 (s) ^{2}	42,8 (z) ^{11}	-	97,6 (z) ^{31}	-	-	-
			85,5 (f) ^{12}					
	A3	2,8 (s) ^{3}	22,5 (s) ^{13}	-	98,4 (z) ^{32}	-	-	-
	B1	81,0 (s) ^{4}	33,3 (z) ^{14}	37,8 (f) ^{21}	93,8 (z) ^{33}	-	-	-
	L1	85,8 (z) ^{5}	55,3 (z) ^{15}	72,8 (z) ^{22}	-	-	-	-
	M1	32,3 (s) ^{6}	58,5 (f) ^{16}	77,8 (s) ^{23}	85,3 (z) ^{34}	-	-	-
	M2	12,0 (s) ^{7}	15,0 (s) ^{17}	75,3 (s) ^{24}	81,9 (z) ^{35}	-	-	-
1st growing generation			**A1z**	80,5 (z) ^{25}	86,6 (z) ^{36}	52,5 (z) ^{51}	79,3 (z) ^{57}	-
				52,5 (s) ^{26}				
				40,3 (f) ^{27}				
			A1s	-	94,6 (z) ^{37}	-	-	-
			A2z	-	95,2 (z) ^{38}	88,1 (z) ^{52}	74,5 (z) ^{58}	-
			A2f	-	95,4 (z) ^{39}	-	-	-
			A3s	-	97,3 (z) ^{40}	-	-	-
			B1z	61,5 (f) ^{28}	89,3 (z) ^{41}	-	-	-
			L1z	72,5 (z) ^{29}	96,9 (z) ^{42}	75,5 (z) ^{53}	75,0 (z) ^{59}	-
			M1f	-	84,9 (z) ^{43}	-	-	-
			M2s	-	79,0 (z) ^{44}	-	-	-
2nd growing generation				**A1zz**	86,3 (z) ^{45}	-	-	-
				A1zf	96,3 (z) ^{46}	-	-	-
				A1oz	90,0 (z) ^{47}	-	-	-
				A1of	90,6 (z) ^{48}	-	-	-
				L1oz	94,0 (z) ^{49}	-	-	-
3rd growing generation					**A1ozz**	50,3 (z) ^{54}	-	-
					A2zoz	76,5 (z) ^{55}	83,0 (z) ^{60}	-
					L1ozz	-	77,3 (z) ^{61}	-
4th growing generation							**A2zoooz**	85,3 (z) ^{62}
							L1zoooz	80,8 (z) ^{63}
							L1ozzoz	90,5 (z) ^{64}

weathering including microorganisms[12,24,36], which in malting may influence steeping conditions[12,13,35,36], water sensitivity[6] and dormancy[6]. The climate also affects levels of pre-germination[7].

In order to obtain a sample set, which is suitable for over viewing and comparing the different quality control methods and for demonstrating the advantage of multivariate data analysis, we have grown the same batch of seeds of different samples in a range of years with widely different climatic conditions (Table I). In order to preserve vigour the material was stored dry under refrigerated conditions.

MATERIALS AND METHODS

Materials

A rare extreme season with a difficult and wet harvest occurred in 1993 in Denmark and Southern Sweden producing a great variation in seed vigour. Table I gives an overview of the origin of the samples and their "vigour" (defined as g%1) measured by EBC 3.5.2[3] (GC method) shortly after harvest. The samples are marked with numbers 1–64. Seeds from the seven original batches from 1993 listed to the left in Table I were planted in 1994–1998 as the 0 growing generation of seeds. The harvested seeds from 1994, called first growing generation, were planted in 1995–98. Harvested samples from 1995, called second growing generation were planted in 1996 and so on until the year 1999. The sample numbers are used to facilitate identification of specific samples in the figures. All three locations were good soils (JB 5-6) representing a geographical area of approximately 200 km^2 in Southern Scandinavia. In all, 64 samples were collected, 63 of which were subjected to analysis of malt quality (See Table I for sample identification and Tables IIA–B, IIIA–B, IVA–B and VA–B for results). Seed size was very low in 1998 and 1999 presumably due to a high level of weeds in the field those years, a condition that did not affect germination properties. All samples were stored at 7°C with 13.5 ± 0.8% moisture. There was no remaining dormancy in the samples in 1999 when the samples were micromalted.

The mean 3-day (72 h) germination energy (g%3) of the material fell from 97.1% to 94.7% during the storage period of six years from 1993 to1999.

Alexis and Blenheim are categorised as malting varieties. The reason for including the varieties for feed, Meltan and Lysimax, is that it is necessary to include extreme barley varieties in order to expand the range of important parameters for an improved multivariate evaluation of the efficiency of the instrumental screening methods. Lysimax has an extreme mutant gene (*lys3a*), which causes a deviating amino acid composition high in lysine[22,23]. This variety is included for theoretical reasons because of its high level of hydrophilic proteins, fast germination characteristics and low β-glucan content.

Analytical methods

A. Germination analyses (Tables I–III)

Two different germination analyses were carried out with 100 seeds in four replicates. In both methods percentages of germinated kernels (*n*) were calculated and the germinated kernels were removed after 24, 48 and 72 h.

(1) Determination of total germinative capacity (GC) in percentage (Tables I and IIA–B). Less than two months after harvest (1993–1999) seeds ($n = 64$) were immersed in a 0.75% H_2O_2 solution to remove dormancy, according to EBC standard method 3.5.2[3]. Every day the H_2O_2 solution was changed and new added to each sample.

(2) Determination of the germinative energy (GE) percentage (EBC 3.6.2)[3] (Table IIIA–B) was performed for samples grown 1993–1996 in the year 1999 after storage three–six years at 7°C (47 samples in total). Kernels were germinated in 90 mm petri dishes with two layers of filter paper (Whatman No. 1) and 4 mL H_2O.

In both analyses (GC and GE) the samples were placed in a dark Refritherm incubator at 20°C. The standard deviation between the replicates was less than 5%.

Germination Index (GI) and Germination Homogeneity (GH) were determined for both GC and GE analyses[32,33] according to EBC 3.7[3].

(3) Estimation of vigour and viability (Tables I–III). Vigour was defined as 24 h germination percentage or g%1(here denoted as "vigour") in the application of both EBC methods 3.5.2[3] (GC) and 3.6.2[3] (GE) as suggested by Munck and Møller[25]. Viability is defined as 72 h germination percentage or g%3 (here denoted as "viability")[25].

B. Physical kernel parameters (Tables IVA–B and VIA)

These analyses were made in 1999 together with the chemical analyses, NIT analyses and the pilot malting.

Thousand-kernel weight: 1000 kernels of every sample were weighed in three replicates.

Seed imaging measurements: A GrainCheck™ 310 instrument (Foss Tecator, Höganas, Sweden) was used to determine the kernel size and light reflectance parameters and their distribution: width, length, roundness, area, volume and total reflected light intensity. The instrument uses digital image analysis to determine the parameters for every single kernel measured in bulk and to calculate the average and standard deviation values for the sample which consists of 300 kernels. In total 60 samples were measured.

Hardness of barley seeds: Kernel hardness (Hardness Index – HI) was determined by the Perten SKCS 4100 (Single Kernel Characterisation System, Perten North America, Reno, NV, USA). The hardness index refers to the American wheat classification system defined by the United States Department of Agriculture, Technical Service Division of the Grain Inspection, Packers and Stockyard Administration. The Perten SKCS 4100 determines individual kernel weight, moisture content, diameter and crushing force profiles described as hardness index[29]. HI was determined as an average of the 300 kernels. Samples with $HI < 33$ are characterised as soft, $33 < HI < 46$ as semi-soft, $46 < HI < 59$ as semi-hard, and samples with $HI > 59$ as hard. The instrument is optimised for wheat and accepts only kernels with a width of more than 2.2 mm and a round wheat-like seed form. Analysing barley there are a great number of rejections (a mean of 464 to obtain 300 kernels). Number of rejected kernels is therefore considered in the investigation. It can be concluded that even if HI is dependent on rejected kernels it gives valuable, specific information. The instrument should be optimised for barley. HI was determined on 58 samples.

C. Chemical analyses (Tables IIA–B and VA–B)

The chemical composition was measured after harvest (1993–1999) according to the standard methods: dry matter (ICC 110/1)[4], protein content (EBC methods 3.2)[3] and content of β-glucan (EBC method 3.11.2)[3]. The α-amylase activity in barley was determined according to ICC 108[4] to study pre-germination.

D. Steeping properties (Table IIA–B)

Steeping characteristics were determined in a separate experiment by weighing 5.2 grams of whole grains before and after steeping in 15 mL 2.5% H_2O_2 for 24 h. The weight enhancement (%) was calculated. This procedure was developed in 2002 in order to facilitate NMR measurements of water uptake (not reported here), allowing the seeds to germinate in immersed state for a long time without change of fluid while still obtaining enough oxygen for germination. In total 62 samples were measured.

E. Malt analysis (Table VA–B)

Micromalting and malt analyses were performed on 63 samples in 1999 with the cold stored grains on a pilot plant system developed and built by the Pajbjerg Plant Breeding Station (The Pajbjerg Foundation, DK-8300 Odder, Denmark). The standardised steeping programme was 8 h with water, 16 h air-break, 9 h with water. Steeping and germination were performed at 16°C and 100% RH. Germination was performed in 87 h. The kilning programme was 16 h at 45°C, 2 h at 65°C and 6 h at 85°C. A sample of Alexis was used as a standard. Extract was determined with a refractometer and β-glucan in wort was determined using an in-house colour-binding method by which β-glucan and Congo red develop a colour complex, which is measured with a spectrophotometer.

F. Near infrared transmission (NIT) spectroscopy (Table VIB)

In 1999 spectra were obtained for intact whole kernels stored 1993–1999 ($n = 62$) when measuring bulk samples of 60.0 g using an Infratec 1225 Food and Feed Analyzer (Foss Tecator, Höganäs, Sweden). The spectrophotometer measures spectra in the wavelength area of 850–1050 nm, collecting data every second nm yielding 100 data points for each of the 62 samples measured.

G. Multivariate data analysis

Data analysis was performed according to Martens and Næs[15] using the software "Unscrambler" version 7.6 SR-1 from CAMO A/S, Trondheim, Norway for Principal Component Analysis (PCA) and Partial Least Squares Regression (PLSR)[16] where Jack-knife validation calculates the important variables[17]. Different combinations of principal components (PC's) are tested, and the combination showing the most interesting results are shown (primarily PC1: PC2). The principal components indicated as PC's in PLSR analysis with the 0“Unscrambler” software are mathematically not identical with the PC's denoted in PCA analysis. Data processing was performed by scaling the physical-chemical and physiological analyses (1/std.dev.). The NIT spectra were transformed to the first derivate. The performance of the regression models is evaluated by its prediction error in terms of root mean square error of cross-validation (RMSECV). The relative error (RE) in percentage is calculated[28] as

$$(\mathrm{RMSECV}/(\mathbf{y}_{\mathrm{max}} - \mathbf{y}_{\mathrm{min}})) * 100$$

where $\mathbf{y}_{\mathrm{max}}$ is the highest reference value and $\mathbf{y}_{\mathrm{min}}$ the lowest reference value of the **y** parameter in question.

Abbreviations

A	Alexis
AREA	Area of kernel (mm^2)
B	Blenheim
BG	(1-3,1-4)-β-glucan in barley
BGwort	(1-3,1-4)-β-glucan in wort (mg/L)
C	Wort colour
EBC	European Brewery Convention
EXTRACT	Extract yield (%)
g%1–3	Germination percentage day 1–3
GC	Germination Capacity Method EBC 3.5.2[4]
GE	Germination Energy Method EBC 3.6.2[4]
GH	Germination Homogeneity[32,33]
GI	Germination Index
HI	Hardness Index
INTENSITY	Total intensity
L	Lysimax
LENGTH	Kernel length (mm)
M	Meltan
NIR	Near Infrared Reflection
NIT	Near Infrared Transmission
P	Protein
PC	Principal Component in PCA and PLSR, see Materials and Methods **G**
PCA	Principal Component Analysis
RE	Relative Error in percentage
PLSR	Partial Least Squares Regression
RMSECV	Root Mean Square Error of Cross-Validation
ROUND	Kernel roundness
STEEP	% Water uptake after 24 h of steep
TKW	Thousand Kernel Weight
VOLUME	Volume of kernel (mm^3)
VP	Vigour Potential
WIDTH	Kernel width (mm)

RESULTS AND DISCUSSION

An overview of the experimental material with means, ranges and standard deviations (Tables I–V)

The germination profiles under GC conditions for years and varieties are displayed in Fig. 1A and Fig. 1B respectively. Mean “vigour” (Table IIA) for the years 1993 and 1994 (51.3 and 44.3%) are especially low in contrast to the best malt quality years 1996 and 1999 (90.8 and 85.5%). However, when estimating “viability” as g%3 the mean results in 1995 (93.6%) are even lower than in 1993 (94.9%) and in 1994 (96.3%) compared to 99.1% in 1996. These differences are reflected in the germination profiles in Fig. 1A and may be explained by the available meteorological data from Zealand.

As discussed in the following, the year 1995 gave rise to exceptionally hard seeds (HI) (Table IVA) with the lowest steep value (Table IIA). Precipitation was exceptionally low during July and August (51 mm) of 1995 compared to the wet years 1993 (181 mm) and 1994 (169 mm). The corresponding values are for 1996 (87 mm), 1997 (108 mm), 1998 (168 mm) and for 1999 (143 mm). The years 1993 and 1995 were regarded as difficult barley quality years for the malting industry, but for different reasons: too much precipitation in 1993 and too little in 1995. The other years were acceptable to excellent (1996).

There was a pregermination tendency in 1999 (three samples only) (Table IIA) including the malting variety Alexis, which, however, did not seem to influence germination (“viability”g%3 = 98.9%).

With respect to germination properties (GC conditions) of the four varieties (Fig. 1B, Table IIB), Lysimax is the fastest germinator (“vigour” g%1 = 79.7%). It is the only variety which displays field germination in addition to the one sample of Alexis harvested in 1999 (α-amylase in samples L9[63]: 41.6 units, A9[62]: 53.7 units).

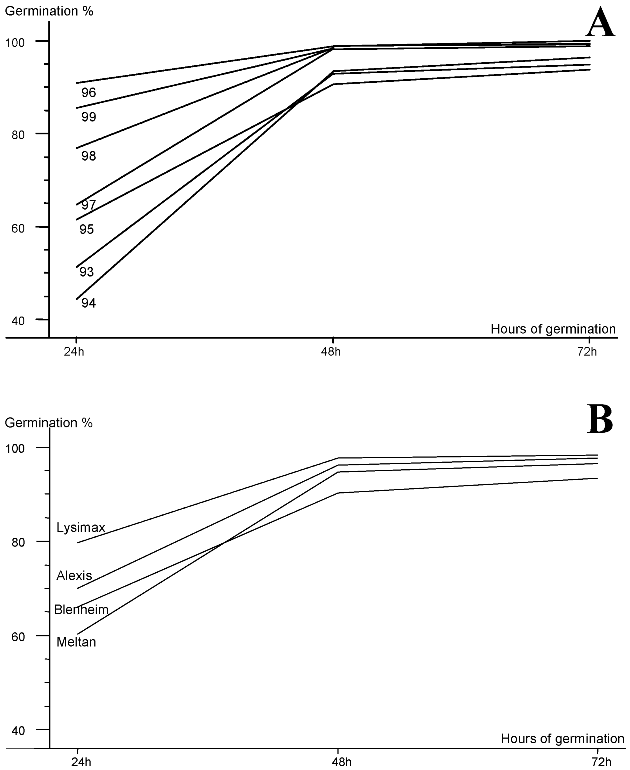

Fig. 1A. Average germination percentage at 24, 48 and 72 hours according to the germination capacity (GC) method after harvest according to harvest year (data Table IIA). **B.** Average germination percentage at 24, 48 and 72 hours according to the germination capacity (GC) method after harvest according to variety (data from Table IIB).

Table IIA. Average and std. dev. of germination capacity, α-amylase (barley) and steeping parameters divided according to harvest year.

	1993	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**Total**
n	7	10	12	20	6	6	3	64
g%1 (GC)	51.3 ± 34.8	44.3 ± 23.7	61.5 ± 18.2	90.8 ± 5.9	64.7 ± 17.5	76.9 ± 3.8	85.5 ± 4.9	77.5 ± 15.8
“Vigour”	2.8–85.8	15.0–85.5	35.5–85.8	81.9–98.4	72.5–83.0	80.8–90.5	80.8–90.5	2.8–98.4
g%2 (GC)	92.8 ± 7.8	93.3 ± 4.5	90.6 ± 5.7	98.7 ± 0.5	98.3 ± 1.2	98.4 ± 0.8	98.3 ± 1.0	96.8 ± 2.8
	75.8–98.8	85.3–98.6	78.3–96.3	97.4–99.4	96.8–99.9	97.5–99.6	97.6–99.5	75.8–99.9
g%3 (GC)	94.9 ± 6.6	96.3 ± 3.5	93.6 ± 4.4	99.1 ± 0.3	99.5 ± 0.4	98.9 ± 1.0	98.9 ± 0.6	97.9 ± 2.0
“Viability”	80.5–99.3	88.3–99.3	84.5–98.5	98.5–99.6	99.3–100.0	97.5–99.8	98.3–99.5	80.5–100.0
GI (GC)	7.1 ± 1.7	6.6 ± 1.2	7.4 ± 1.0	9.3 ± 0.5	7.5 ± 1.0	8.1 ± 0.3	8.8 ± 0.4	8.4 ± 0.9
	4.9–8.8	5.4–8.9	6.1–8.8	8.3–9.9	6.4–9.0	7.8–8.5	8.4–9.2	4.9–9.9
GH (GC)	58.8 ± 6.4	50.3 ± 7.9	49.9 ± 7.9	71.9 ± 9.0	52.7 ± 8.5	56.3 ± 2.7	63.8 ± 6.6	59.34 ± 11.9
	50.2–69.6	42.7–64.0	38.2–62.4	59.0–85.9	44.4–66.5	53.1–59.1	59.2–71.3	38.2–85.9
α-Amylase	0.43 ± 0.53	0.18 ± 0.08	0.11 ± 0.03	0.12 ± 0.04	0.2 ± 0.2	1.0 ± 1.2	47.7 ± 8.6	4.1 ± 13.2
		(*n* = 9)		(*n* = 19)		(*n* = 3)	(*n* = 2)	(*n* = 58)
	0.1–1.5	0.1–0.3	0.1–0.2	0.1–0.2	0.1–0.6	0.1–3.1	41.6–53.7	0.1–53.7
Steep	38.1 ± 1.6	36.9 ± 2.7	35.6 ± 2.0	37.3 ± 2.3	37.8 ± 2.1	42.6 ± 1.6	41.5 ± 1.9	38.0 ± 2.4
	(*n* = 5)							(*n* = 62)
	36.4–40.3	31.4–40.3	32.6–38.7	33.2–43.2	35.5–41.1	40.7–44.8	39.3–42.9	31.4–44.8

Table IIB. Average and std. dev. of germination capacity, α-amylase (barley) and steeping parameters divided according to variety.

	Alexis	**Blenheim**	**Lysimax**	**Meltan**
n	37	6	11	10
g%1 “Vigour” (GC)	70.0 ± 25.5	66.1 ± 26.2	79.7 ± 11.8	60.2 ± 29.3
g%2 (GC)	96.1 ± 4.8	90.2 ± 7.6	97.6 ± 2.0	94.7 ± 4.9
g%3 “Viability” (GC)	97.5 ± 3.7	93.4 ± 5.4	98.7 ± 0.8	96.5 ± 3.8
GI (GC)	8.0 ± 1.4	7.8 ± 1.6	8.4 ± 0.8	7.4 ± 1.4
GH (GC)	60.1 ± 13.0	54.9 ± 14.7	61.7 ± 10.8	56.5 ± 6.5
α-Amylase	0.2 ± 0.1	0.3 ± 0.5	10.1 ± 20.0	0.1 ± 0.1
	(*n* = 33)		(*n* = 9)	
Steep	37.3 ± 2.7	37.3 ± 1.3	41.0 ± 3.0	36.4 ± 2.1
	(*n* = 36)		(*n* = 10)	

The feed barley Meltan (mean estimated “vigour” g%1 = 60.2%) is the slowest in germination compared to the malting barley varieties Alexis (70.0%) and Blenheim (66.1%). Blenheim, however, shows the lowest mean estimated “viability” with a mean value of 93.4% compared to 96.5% for Meltan, 97.5% for Alexis and 98.7% for Lysimax.

The germination results (GE conditions) after cold storage in Tables IIIA–B as well as the physical-chemical and malt parameters given in Tables IVA–B and VA–B are elaborated upon in the discussion together with the multivariate evaluation.

Evaluating physical-chemical and germinative malting parameters of the barley material by PCA

In this study the PCA biplot in Fig. 2A summarises the 19 variables of which 11 are classified as “manifest” on the barley raw material level and eight as “indirect” characteristics, only to be attained through germination and malting. The PCA biplot presents a convenient overview over the relations between the 63 barley and malt sample (for identification see Table I) and the 19 analytical parameters (Tables IIA–B, IVA–B and VA–B) where the sample symbols marked according to variety, year and number lying near to each other represent similar quality profiles. Variable symbols lying near to each other are positively correlated

It is seen that the variables describing the abscissa – PC1 (Fig. 2A, 34% of the variance) are the physical and germinative parameters HI, Rej (rejected grains during HI determination), Round, Width, Volume, TKW and the GC germination parameters g%1, g%3, GI and GH as well as Extract while the ordinate PC2 (25% of the variance) is mainly described by Steep, Intensity, Length, BGwort and Area. The sign Extract is adjacent to the germination parameters g%1, g%3, GI and GH in a cluster. They are thus positively correlated.

The light reflection intensity value registered with the RGB camera in the GrainCheck instrument gives a low value with darker weathered seeds as for the rainy harvest year 1993 (marked 3), while the dry year in 1995 (5) gives

Table IIIA. Average and std. dev. of germination energy and moisture parameters after 3–6 years of storage according to harvest year.

	1993	1994	1995	1996
n	5	10	12	20
g%1 “Vigour” (GE)	36.1 ± 24.1	43.9 ± 29.7	58.4 ± 18.3	78.3 ± 12.7
g%2 (GE)	76.6 ± 35.2	82.8 ± 29.2	95.7 ± 2.2	98.2 ± 2.0
g%3 “Viability” (GE)	85.2 ± 22.1	87.4 ± 26.5	97.1 ± 1.4	99.3 ± 0.6
GI (GE)	5.9 ± 1.4	6.5 ± 1.5	7.2 ± 1.0	8.3 ± 0.9
GH (GE)	47.8 ± 4.7	49.6 ± 8.3	51.7 ± 4.7	59.4 ± 10.5
Moisture, min-max	13.3–14.5	13.3–15.4	12.9–14.5	11.7–13.8

Table IIIB. Average and std. dev. of germination energy and moisture parameters after 3–6 years of storage according to variety.

	A93–96	B93–96	L93–96	M93–96
n	26	6	5	10
g%1 “Vigour” (GE)	67.1 ± 22.0	64.5 ± 18.3	70.3 ± 24.9	36.2 ± 25.9
g%2 (GE)	93.9 ± 17.4	94.8 ± 6.4	97.7 ± 1.4	80.8 ± 28.5
g%3 “Viability” (GE)	95.2 ± 16.4	96.2 ± 4.6	98.4 ± 0.9	89.8 ± 18.0
GI (GE)	7.7 ± 1.2	7.5 ± 1.0	8.0 ± 1.5	6.0 ± 1.4
GH (GE)	55.4 ± 8.7	52.9 ± 7.0	60.4 ± 12.3	47.2 ± 7.6
Moisture, min-max	12.4–15.3	11.7–14.1	12.9–13.7	12.7–15.4

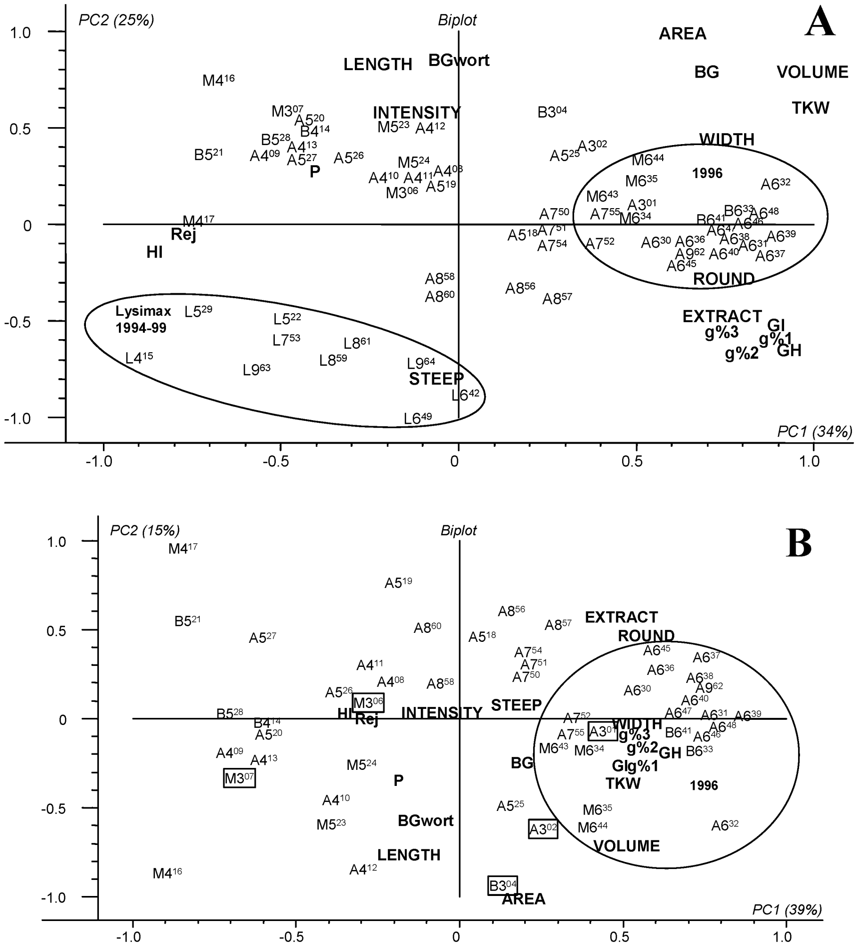

Fig. 2A. PCA biplot (PC1:2) overviewing all samples and physical-chemical malting parameters. For sample identification see Table I. Outliers A3^{03} and L3^{05} are removed. Germination method is GC^{4}. **B.** PCA biplot (PC1:2) for all samples without the extreme variety Lysimax. Outlier A^{03} is removed. Germination method is GC^{4}. See abbreviations in the Materials and Methods section.

Table IVA. Average and std. dev. of physical and morphological parameters according to harvest year.

	1993	1994	1995	1996	1997	1998	1999	Total
n	5	10	12	20	6	6	3	62
TKW	41.8 ± 1.5	38.1 ± 2.6	38.0 ± 2.6	43.8 ± 3.1	40.3 ± 3.5	31.9 ± 2.7	33.6 ± 7.8	39.9 ± 5.3
Round	0.29 ± 0.08	0.25 ± 0.02	0.28 ± 0.01 (*n* = 11)	0.30 ± 0.02 (*n* = 19)	0.30 ± 0.02	0.29 ± 0.02	0.28 ± 0.03	0.29 ± 0.03 (*n* = 60)
Length	8.80 ± 0.16	9.08 ± 0.34	8.76 ± 0.27 (*n* = 11)	8.56 ± 0.22 (*n* = 19)	8.60 ± 0.11	8.58 ± 0.18	8.58 ± 0.03	8.71 ± 0.29 (*n* = 60)
Width	3.65 ± 0.08	3.44 ± 0.08	3.51 ± 0.09 (*n* = 11)	3.60 ± 0.10 (*n* = 19)	3.62 ± 0.12	3.58 ± 0.10	3.48 ± 0.19	3.56 ± 0.12 (*n* = 60)
Area	22.51 ± 0.73	22.16 ± 0.89	21.87 ± 0.82 (*n* = 11)	22.19 ± 0.80 (*n* = 19)	21.86 ± 0.53	21.34 ± 0.52	21.32 ± 0.91	21.99 ± 0.81 (*n* = 60)
Volume	51.57 ± 2.68	48.17 ± 2.58	48.46 ± 2.54 (*n* = 11)	50.91 ± 2.78 (*n* = 19)	49.55 ± 2.42	47.47 ± 1.94	46.97 ± 4.33	49.38 ± 2.94 (*n* = 60)
Intensity	65.43 ± 1.33	74.83 ± 5.36	75.67 ± 2.00 (*n* = 11)	69.25 ± 1.91 (*n* = 19)	65.37 ± 2.10	65.46 ± 2.29	68.51 ± 2.40	70.23 ± 4.85 (*n* = 60)
HI	56.7 ± 8.9 (*n* = 4)	57.8 ± 9.3	65.8 ± 9.7 (*n* = 11)	41.7 ± 4.9	60.5 ± 11.4	58.1 ± 9.4 (*n* = 4)	45.5 ± 17.6	49.5 ± 11.9 (*n* = 58)
Rej. kernels HI	405 ± 102 (*n* = 4)	575 ± 171	567 ± 138 (*n* = 11)	356 ± 131	356 ± 131	536 ± 58 (*n* = 4)	448 ± 199	464 ± 163 (*n* = 58)

Table IVB. Average and std. dev. of physical and morphological parameters according to variety.

	Alexis	Blenheim	Lysimax	Meltan
n	36	6	10	10
TKW	40.8 ± 3.8	41.4 ± 3.5	32.2 ± 3.3	41.6 ± 3.4
Round	0.29 ± 0.02 (*n* = 34)	0.29 ± 0.01	0.27 ± 0.01	0.27 ± 0.02
Length	8.70 ± 0.31 (*n* = 34)	8.81 ± 0.20	8.51 ± 0.16	8.89 ± 0.28
Width	3.61 ± 0.08 (*n* = 34)	3.58 ± 0.09	3.37 ± 0.07	3.53 ± 0.08
Area	22.18 ± 0.50 (*n* = 34)	22.52 ± 0.56	20.64 ± 0.27	22.40 ± 0.77
Volume	50.38 ± 1.65 (*n* = 34)	51.16 ± 2.58	44.25 ± 1.02	50.07 ± 2.51
Intensity	70.92 ± 4.90 (*n* = 34)	71.61 ± 5.55	67.45 ± 5.09 (*n* = 10)	69.84 ± 3.38
HI	48.3 ± 9.7 (*n* = 33)	56.9 ± 13.2	68.6 ± 14.5 (*n* = 9)	54.2 ± 8.3
Rej. kernel HI	418 ± 130 (*n* = 33)	518 ± 245	584 ± 186 (*n* = 9)	454 ± 127

a light-coloured seed with high reflectance value (Table IVA). The Intensity sign on the PCA plot in Fig. 2A is consequently located near samples grown in 1995 (5).

Extract (Fig. 2A) is associated to Round kernels, while BGwort is related to Length, as in the study of Nielsen[29]. BGwort is negatively correlated to the variable Steep because it is located in the opposite direction along PC2 – the ordinate. Looking for patterns between samples from harvest years the samples of 1996 (6), which have by far the best malt quality, are all located very close to each other to the right. They are more closely connected to Extract and germination parameters compared to samples from the other harvest years, which are widely distributed, indicating a smaller variance in malting quality between the 1996 samples.

The PCA plot in Fig. 2A is influenced by the extreme variety Lysimax (marked L), of which nearly all samples are located in the bottom left corner near the Steep variable.

The Lysimax is an outlier barley variety because of its high steep percentage, low β-glucan content and fast germinations in spite of a hard kernel. It has been included to test the sensitivity of the instrumental methods.

When the samples of Lysimax are excluded (Fig. 2B), the associations between Extract and Round as well as

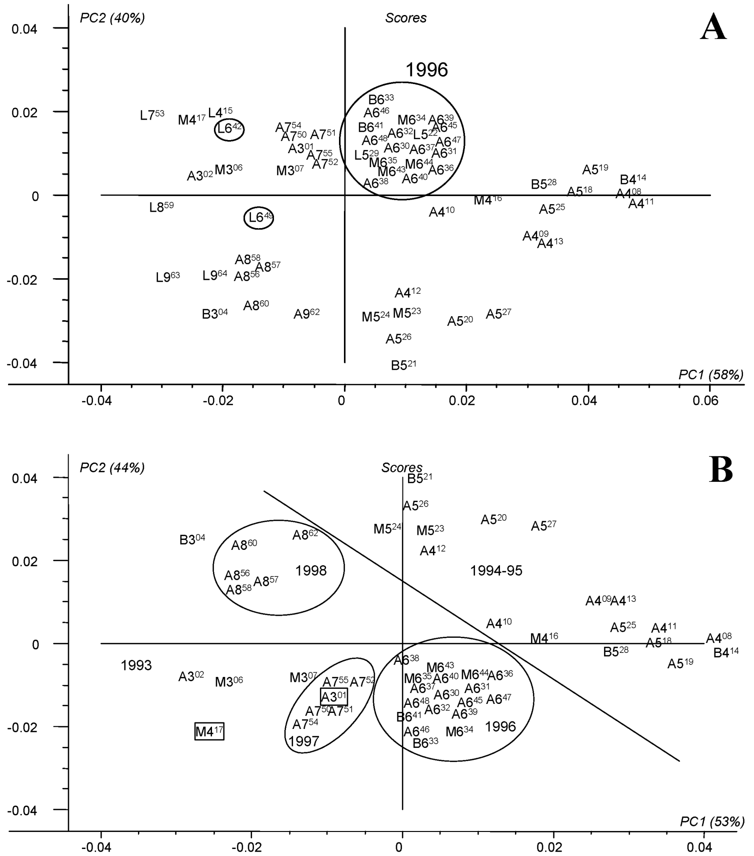

Fig. 3A. PCA score plot (PC1:2) for NIT-spectra (1.der.) for all samples. A3^{03} and L3^{05} not measured due to lack of material. **B.** PCA score plot (PC1:2) for NIT-spectra (1.der.) excluding samples of Lysimax.

BGwort and Length from Fig. 2A are confirmed. Now the samples do not divide according to variety as much as to harvest year. As in Fig. 2A, the samples harvested in 1996 (6) (encircled) are located together to the right of the PCA plot highly influenced of Extract and germination properties. Samples from 1997 (7) and 1998 (8) are placed in the upper half in the middle of the plot, whereas samples harvested in 1994 (4) and 1995 (5) are located to the left, indicating low malting quality. The original samples from the difficult harvest year in 1993 (3) are marked with a square in Fig. 2B. These samples were originally selected to describe a large variation in germination characteristics and are consequently located all over the plot.

We can thus conclude that there are tendencies towards a PCA classification according to harvest year in this material with 19 parameters.

The barley material in this investigation is far from a complete design with equal number of samples and varieties each year, which makes it difficult to evaluate with classic statistics based on means and variances of classes which each, should consist of several samples. It is important to note that this is not a problem in multivariate analysis based on pattern recognition where each sample is individually defined as a more and less unique pattern[8] in this case by 19 variables which all are complete. It is, however, clear that a small number of samples as for 1999 ($n = 3$) with only two varieties makes it difficult to draw general conclusions about that year. The conclusions, which can be drawn on the individual level for these three samples, however, still hold. The multivariate advantage is further discussed by Munck and Møller[23,25].

Classification by PCA of NIT spectra from barley samples

NIT spectra[17] facilitate in principle a global non-destructive physical-chemical fingerprint[23] of the barley samples, where samples are measured in the wavelength area 850–1050 nm obtaining every second measurement, resulting in 100 data points for every sample. This can be used for classification in PCA models and for PLSR calibrations[16,25] where the spectra can be used to predict physical-chemical values (e.g. protein).

NIT spectra were measured on the barley material (Table I) on 62 samples (two samples missing due to lack of material). A PCA plot reflecting the pattern of the whole spectra is displayed in Fig. 3A. Samples with similar spectral patterns are situated adjacent to each other in the plot. The samples divide into different groups, where the encircled samples to the right are harvested in 1996 (6). Two samples of Lysimax (L) (encircled) from 1996 (6) are located to the left outside the 1996 group, but closer to the other Lysimax samples. There is a tendency that the samples located in bottom right quadrant were

Table VA. Average and std. dev. of chemical and malt parameters according to harvest year.

	1993	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**Total**
n	6	10	12	20	6	6	3	63
Protein	12.6 ± 1.6 (*n* = 5)	10.3 ± 0.8	10.5 ± 1.2	9.1 ± 0.4	10.8 ± 0.5	11.2 ± 0.4	10.8 ± 1.3	10.0 ± 1.1 (*n* = 62)
(barley)	10.2–14.5	9.0–11.4	9.2–12.9	8.6–9.8	10.2–11.6	10.7–11.6	9.3–11.8	8.6–14.5
BG	4.0 ± 0.3	3.7 ± 0.5	3.9 ± 0.6	3.9 ± 0.4	3.8 ± 0.4	3.4 ± 0.2	3.3 ± 0.5	3.8 ± 0.4
(barley)	3.6–4.3	2.5–4.5	2.5–4.5	2.9–4.5	3.2–4.2	3.2–3.6	2.9–3.8	2.5–4.5
Extract (%)	71.9 ± 12.9	77.5 ± 7.0	80.1 ± 3.7	82.5 ± 5.2	81.5 ± 2.3	82.4 ± 0.8	81.6 ± 2.1	80.7 ± 5.0
	49.2–83.0	61.2–84.0	70.3–84.1	81.1–85.6	78.2–84.0	81.7–83.7	80.1–83.9	49.2–85.6
BGw (mg/ml)	360.3 ± 121.3	231.1 ± 102.4	204.0 ± 67.5	173.1 ± 52.9	174.5 ± 28.7	174.4 ± 28.1	66.9 ± 7.1	181.2 ± 73.3
	163.2–532.8	61.3–321.3	111.9–328.0	66.3–244.9	153.6–229.3	141.5–210.0	58.7–71.2	58.7–532.8

Table VB. Average and std. dev. of chemical and malt parameters according to variety.

	Alexis	Blenheim	Lysimax	Meltan
n	37	6	10	10
Protein	10.1 ± 1.1 (*n* = 36)	10.5 ± 2.1	10.4 ± 1.2	10.8 ± 1.6
BG	4.0 ± 0.3	4.1 ± 0.5	3.0 ± 0.3	3.8 ± 0.1
Extract	81.1 ± 6.5	81.3 ± 1.9	81.2 ± 1.2	74.7 ± 8.8
BGwort	195.7 ± 74.2	233.6 ± 69.7	104.7 ± 44.3	302.9 ± 95.8

harvested in 1994 (4) and 1995 (5), most of the samples found in the top left quadrant were harvested in 1997 (7) and samples harvested in 1998 (8) and 1999 (9) are seen in the bottom left quadrant.

The PCA pattern obtained from the NIT analyses in Fig. 3A is related more to harvest year than to the pattern in the PCA plot of physical and chemical parameters in Fig. 2A, although all the samples of Alexis (A), Blenheim (B) and Meltan (M) harvested in 1996 (6) are located close together on both plots. There is a stronger tendency for a clear classification of Lysimax (L) in the analytical PCA plot in Fig. 2A than in that of the NIT in Fig. 3A.

When the extreme Lysimax samples are excluded from Fig. 3A, a much clearer division according to harvest years is seen (Fig. 3B). This is comparable to the change in the PCA classification plots between Fig. 2A and Fig. 2B with and without Lysimax respectively.

All samples harvested in 1998 (8) are located in the top left quadrant (Fig. 3B), all 1997 (7) samples in the bottom left quadrant, and the samples harvested in 1996 (6) are found in the bottom right quadrant. In the top right quadrant the samples from 1994 (4) and 1995 (5) are located close together. One exception is M4[17] (low "vigour" and normal "viability"), which is located in the opposite direction of the 1994 samples (4) as an outlier. Samples from 1993 (3) are located "all over" to the left side of the PCA plot (Fig. 3B), indicating larger variation in physical-chemical structure compared to e.g. 1997 (7) samples because the 1993 samples (3) were selected to obtain large variation.

In conclusion the resemblance of the sample classifications with the two different analytical methods in Fig. 2A–B and Fig. 3A–B indicates a firm physical-chemical basis for the NIT spectral analysis, which will be further supported by the high prediction values in the following discussion.

Prediction of barley germination and malt quality parameters from two different physical-chemical data sets by PLSR

It is of great interest for the plant breeding, malt- and brewing industry to be able to predict indirect parameters (e.g. germination parameters, extract, BGwort as **y**) from parameters manifest in the barley raw material e.g. by the fast non-destructive NIT spectroscopy measurements now used *on-line* by the grain industry to predict water and protein[17]. The precision of such measurements should be indicative enough to be able to classify barley samples *on-line* into two classes: High and low malting quality where only the first is accepted for further analytical scrutiny *at-line*[25]. Here two separate screening methods for manifest physical-chemical properties are compared: the set of ten parameters (six seed imaging measurements plus TKW, hardness, protein, BGbarley) and the NIT dataset in making such predictions by PLSR. This is done for two reasons. First to increase the validity of the predictions in a limited material and second to better understand how the NIT technology works. A great number of PLSR predictions are shown in Table VIA (a01–a20) using the set of ten variables and in Table VB (b01–b28) where NIT measurements are used (**X**). Outlier samples have been detected and removed stepwise by consulting the influence plots of the PLSR software as described by Munck and Møller in the adjacent paper in this issue[25]. It is a general tendency that the prediction of indirect parameters, after storage in both materials, produces outliers with a "viability" (g%3, GE) below 92%(Tables VIA–B). This observation is valid for 16 cases. Exceptions are a05, a06, b15 and b25. There are no such outliers in the g%1 and g%3 predictions (a01–a03, b01–b07) under GC conditions because of the low number of samples with viability below 92% before storage.

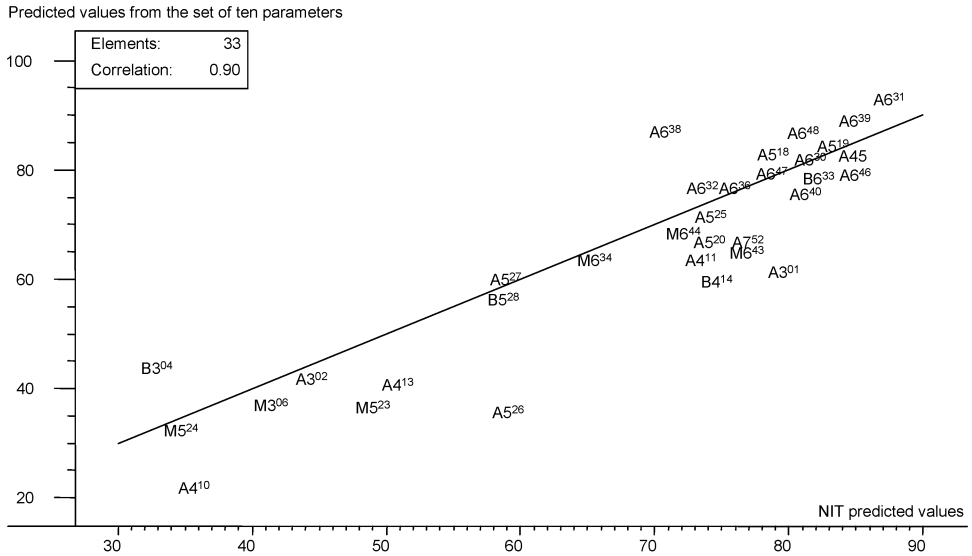

Fig. 4. NIT prediction of "vigour" g%1 GE correlated to the same prediction by the set of ten manifest physical-chemical variables

In order to obtain models, which are more relevant in practice, we have excluded the extreme variety Lysimax in the predictions except for a18–a20 (Steep, Extract and BGwort) from the ten physical-chemical parameters.

Germination parameters

Surprisingly high correlation coefficients are obtained for both "vigour" (g%1) and "viability" (g%3) before and after outlier removal. When comparing the datasets in Table VA and Table VB it is seen for both GC and GE conditions that g%1 (a01 and b01 for GC, a04–a06 and b08–b10 for GE) is better predicted r = 0.73–0.94 with higher correlation coefficients than for g%3 r = 0.39–0.80 (a02 and b02–b03 for GC and a07–a09 and b11–b13 for GE).

A high frequency of low viability among outliers (*GE-conditions*) *was identified after long-term storage with regard to the* g%1 (b10) *and especially with the* g%3 (a08–a09, b12–b13) *predictions. This is in accordance with our previous assumption*[25] *that the physiological condition of the germ should not be able to be predicted by the two physical-chemical screening methods. Removing the low viability outliers improves both types of correlations indicating a firm physical-chemical basis especially for "vigour"* g%1 *of seed samples with reasonable viability* (<92%).

With the set of ten physical-chemical parameters in Table VIA it is possible by Jack-knife validation to register the important variables in each correlation. These variables are ordered in sequence after falling importance in Table VIA. It is seen that two different patterns of these variables arise:

GC: TKW, HI, Volume, Width, Round, Intensity, BG, P (a01–a02),

GE: P, Round, Length, Width, Volume, Intensity (a04–a09).

GE g%3 (a07–a09) has fewer outliers than GE g%1(a04–a06). Germination homogeneity (GH) can be predicted by the set of ten variables (r = 0.70–0.77; a03, a10) with a similar pattern of important variables for GC as given above. It is clear that these differences in importance of the ten different variables as **X** for GC and GE predictions as **y** rests with the germination parameters because the set of ten variables has only been measured once in 1999 together with the malting analyses. We therefore presume that the six seed imaging parameters as well as TKW, protein, β-glucan and Hardness (HI) were not affected by storage at 7°C 13.5% water in three–six years.

This seems reasonable for at least the eight first parameters but should be checked in a future experiment.

To evaluate if the predicted values of "viability" (g%1) by the best models from the two sets of measurements correspond, a diagram is shown where the predicted values for each sample from NIT are plotted as abscissa against those from the ten variables as ordinate. *As is seen from Fig. 4 the correlation coefficient between the two prediction methods is* r = 0.90. *This result strongly supports our hypothesis*[25] *that "vigour"* (g%1) *in malting grade barley can be predicted from the physical-chemical measurements, because the predicted values obtained from two independent measurements correlates.*

Predicting the effect of storage on vigour and viability

It is again surprising to note that both the effect of three–six years storage at 7°C on "vigour" Δg%1 (GCg%1 – GEg%1) and "viability" Δg%3 (GCg%3 – GEg%3) have a profound physical-chemical basis. This is demonstrated by the significant predictions for both the set of ten parameters in Table VIA (a11; r = 0.76 respectively a12; r = 0.71) as well as for NIT VIB (b16; r = 0.80 respectively b17 r = 0.89). It is interesting to note that the parameter hardness (HI) is characterised as an important variable in the predictions a11 and a12.

It should be emphasised that the GC (peroxide treatment method EBC 3.5.2[3]) and the GE comparison (BRF method EBC 3.6.1[3]) is relative and that the respective

Table VIA. PLSR correlations with jack-knife validation between the ten physical-chemical parameters (TKW, HI, P, BG, width, length, area, volume, round, intensity) as **X** and indirect germination and malting variables as **y**. Material 1993–1996 without Lysimax samples (n = 42) in the top of the table. Samples with low viability GE (<92%) = underlined, medium viability GE (92–98%) = **bold**, high viability GE (>98%) = normal.

y	No.	Step*	*r*	RMSECV	RE	PC**	*n*	Total outlier samples removed	Significant variables***
g%1 GC	a01	0	0.78	16.33	18.9	1	42		TKW, HI, Volume, Width, Round, Intensity, BG, P
g%3 GC	a02	0	0.63	2.79	18.6	1	42		TKW, HI, P, Width, Round, Volume
GH GC	a03	0	0.77	8.17	17.1	1	42		HI, TKW, Round, Width, Volume, Intensity, Length, P
g%1 GE	a04	0	0.73	17.09	18.1	1	42		P, Round, Length, Width, Volume, Intensity
g%1 GE	a05	I	0.84	12.41	14.5	1	35	M07, A08, A09, A12, **B21**, A37, B41	P, Round, Length, Width, Volume
g%1 GE	a06	I	0.94	7.77	9.1	4	35	M07, A08, A09, A12, **B21**, A37, B41	P, Width, Round, Length, Volume
g%3 GE	a07	0	0.39	13.98	16.5	1	42		P, TKW
	a08	I	0.56	3.60	14.5	1	40	M07, A12	P, HI, TKW
	a09	II	0.73	1.74	14.5	2	39	M07, A12, M16	P, INT, TKW
GH GE	a10	0	0.70	6.17	14.9	2	42		Length, Round, P, Area
Δg%1	a11	I	0.76	14.59	16.3	1	36	A03, A10, A12, M16, **M23**, **M24**	Intensity, TKW, Width, Volume, HI, Round
Δg%3	a12	II	0.71	2.22	14.6	2	37	A03, M07, A10, A12, M16	Volume, Area, TKW, HI
Steep	a13	0	0.60	1.57	11.7	3	42		Intensity
Extract	a14	0	0.62	3.79	15.5	1	52	A03	Round, Length, P, HI, TKW, Area
Extract	a15	I	0.81	1.33	14.3	2	48	A03, M07, A12, M16, **A20**	P, Length, Round, HI, Area, Width
BGwort	a16	0	0.61	65.30	14.1	1	52	A03	P, Width, Intensity
BGwort	a17	I	0.78	39.90	11.4	4	50	A03, M07, A12	P, Width, Intensity
Predictions including the Lysimax samples									
Steep	a18	0	0.82	1.65	12.3	4	62		Intensity, TKW, HI
Extract	a19	0	0.82	1.24	13.3	3	58	A03, M07, A12, M16, **A20**	P, Length, Round, HI, Area, Intensity
BGwort	a20	0	0.69	65.57	23.4	2	63		P, Length, Area, BG, Round, Volume

*Step of outlier selection from influence plot

**Minimum value of residual validation variance

***Variables ordered after degree of importance

g%1 and g%3 values are not fully comparable. However, we have in an unpublished experiment compared GC and GE measurements side by side during another storage experiment with normal and heat damaged barley where dormancy was completely removed after four months. After four–six months of storage there were no significant differences in "viability" (g%3) between the GC and GE conditions while it seemed that the peroxide condition in GC increased "vigour" (g%1) compared to GE, but only for the untreated seeds. There was thus no negative effect on "vigour" (g%1) by the peroxide. The differences (Δ) between the GC and GE conditions given above should thus make sense and merit a more detailed study of the effect of storage on individual samples.

This is done in Fig. 5A and Fig. 5B for "vigour" (g%1) and for "viability" (g%3) respectively where the GC values at harvest are plotted as the abscissa against the GE values as the ordinate. A line drawn on these plots marks unchanged parameters. In Fig. 5A, all 1996 samples (6) are situated in the circle to the right marked 1996 together with a few samples from other years.

It is seen that samples of Blenheim (B) and Alexis (A) from 1994 (4) and 1995 (5) actually increase in "vigour" (Fig. 5A) when stored to 1999, whereas especially samples of Meltan (M) irrespectively of production year are unchanged or decrease during the same period. The cause of the recorded increase in vigour after storage could tentatively be related to a protective storage effect by a high HI of these samples from 1994 (4) and 1995 (5) (Table IVA). Remarkably, the samples from 1996 (6) are all placed below the line, indicating a significant loss in "vigour" after storage from this premium harvest, which also could be seen in Table IIIA. These samples are all very soft (Table IVA).

It is concluded from the results in this experiment that hard seeds store better than soft.

In contrast to our findings regarding "vigour" (g%1), estimated "viability" (g%3) (Fig. 5B) indicates less effect of harvest year on "viability" due to cold storage. There are, however, large differences due to storage of individual samples. The samples of Meltan seem to be more sensitive for decrease in "viability" after storage ($M3^{07}$, $M4^{16}$, $M5^{24}$, $M5^{23}$, $M6^{44}$), whereas a range of samples of Blenheim (B), Alexis (A) and Lysimax (L) from l994 (4) and 1995 (5) actually increase moderately as was registered with the "vigour" comparison. Cold storage of malting barley on a large scale is considered safe[8]. Our finding suggests that storage-sensitive barley batches should be considered before long-term cold storage.

The difference in germination behaviour is expected to be associated with moisture content. When moisture content data are used as ID in Fig. 5B (not displayed here), there is a weak trend showing a few samples where viability is affected by storage ($M3^{07}$, $A4^{12}$, $M4^{16}$, $A4^{10}$, $B3^{04}$) with a higher moisture content in the moderate range of 13.3–15.4% and a decrease in "vigour" after storage. The majority of samples were stored with water content of 11.7–14.0% (13.5 ± 0.8%). It is unlikely that these relatively small increases in water content can fully explain the drastic loss of "viability" of these samples.

The samples that have the largest reduction in "vigour" and "viability" have the lowest malting quality: $A4^{12}$ (Ex-

Table VIB. NIT (1.der.) prediction of germination, malting data and chemical-physical data for samples (1993–1999) of Alexis, Blenheim and Meltan (Lysimax excluded) (*n* = 52). GE was not available at 97–99 samples. Samples with low viability GE (<92%) = underlined, medium viability GE (92–98%) = **bold**, high viability GE (>98%) = normal.

y	No.	Step*	*r*	RMSECV	RE	PC**	*n*	Outliers***
g%1 (GC) 1993–99	b01	0	0.86	12.43	16.4	10	52	
g%3 (GC) 1993–99	b02	0	0.50	2.96	31.2	1	52	
	b03	I	0.76	1.50	15.8	4	49	M17, **B21**, **B28**
g%1 (GC) 1993–96	b04	0	0.92	10.41	12.1	10	42	
g%3 (GC) 1993–96	b05	0	0.59	2.89	19.3	1	42	
	b06	I	0.77	1.56	17.3	2	39	M17, **B21**, **B28**
GH (GC) 1993–96	b07	0	0.78	8.14	17.1	5	42	
g%1 (GE) 1993–96	b08	0	0.74	16.81	17.8	4	42	
	b09	I	0.77	14.94	15.7	4	41	A12
	b10	II	0.80	13.55	14.3	3	38	A12, M16, **A20, A27**
g%3 (GE) 1993–96	b11	0	0.31	14.42	17.0	1	42	
	b12	I	0.68	1.88	15.7	1	39	M07, A12, M16
	b13	II	0.80	0.89	3.4	3	37	B04, M07, A10, A12, M16
GH (GE) 1993–96	b14	0	0.59	6.94	24.4	4	42	
	b15	I	0.75	4.40	17.1	4	37	**B21**, A31, A37, A39, M44
Δg%1	b16	0	0.80	16.64	13.1	8	42	
Δg%3	b17	I	0.89	2.08	7.8	9	40	M07, A12
Steep	b18	0	0.61	1.91	14.2	1	52	
Extract	b19	0	0.73	3.33	13.6	5	52	
BGwort	b20	0	0.77	52.06	12.7	9	52	
TKW	b21	0	0.92	1.43	9.9	10	52	
HI	b22	0	0.94	3.33	7.4	10	49	
P	b23	0	0.97	0.31	5.2	10	52	
Width	b24	0	0.63	0.07	18.4	3	50	
	b25	I	0.74	0.05	13.2	3	49	M17
Length	b26	0	0.77	0.19	16.5	7	50	
Round	b27	0	0.77	0.01	12.5	5	50	
Intensity	b28	0	0.96	1.24	7.4	2	50	

*Step of outlier selection from influence plot

**Minimum value of residual validation variance

***Total outlier samples removed from correlation BGbarley, Area and Volume below *r* = 0.60

tract: 70.03%, BGwort: 417.33 mg/L), M3^{07} (Extract: 64.11%, BGwort: 532.83 mg/L) and M4^{16} (Extract: 61.23%, BGwort: 321.27 mg/L).

Cold storage can be seen as a stress treatment, which is likely to affect vigour as does accelerated ageing by short-term heat treatment[26,27,34], however, to a much lesser extent. Estimated "vigour" (g%1) has a much greater response than GI (Tables IIA–B and IIIA–B). For example, the relative decrease in mean "vigour" by cool storage at six years for the barley samples in 1993 was 30% (from 51.3 to 36.1%) compared to 16% for GI (from 7.1 to 5.9).

It is concluded by comparing Fig. 5A with Fig. 5B that the "vigour" (g%1) *criterion is a more sensitive indicator for effects due to storage than the "viability"* (g%3) *criterion.*

There are marked relative germinative improvements comparing initial GC with GE after storage regarding "vigour" (g%1) *and "viability"* (g%3) *for many of the samples grown in 1994* (*4*) *and 1995* (*5*) *featuring hard seeds with relatively low steep figures. These effects should also be significant in a long-time GE to GE storage comparison.*

The storage sensitivity of the variety Meltan (M) could not be explained by the physical-chemical criteria alone. A slow negative microbial effect on the ability to germinate at these conditions cannot be excluded where a harder more closed structure could have a guarding effect on the germ. Anti-microbial proteins[24] have been discovered in the endosperm e.g. (1→3)-β-glucanase and chitinase that are able to dissolve (1→3)-β-glucan and chitin, which constitutes the cell wall of the fungal hyphae. There are differences between barley varieties in this respect e.g. Lysimax used in this experiment is overproducing the above-mentioned antifungal proteins in large amounts[24].

Investigating the nature of the steep criterion

In this experiment it has only been possible to obtain a low correlation coefficient of *r* = 0.60–0.61 to predict Steep from the NIT measurements (Table VIB, b18) as well as from the ten physical-chemical parameters (Table VIA, a13). When the samples of Lysimax are included in the model with the ten physical-chemical variables, the correlation coefficient is increased to *r* = 0.82 (Table VIA, a18). This is not surprising because the samples of Lysimax have a higher average steep compared to the other varieties (Table IIB), so the number of samples with high values will be more balanced to the rest of the samples in this model. However the total range of Steep in the samples (31.4–44.8 in Table IIA) is the same with or without Lysimax, because one sample of Alexis deviates from the rest having a Steep value of 44.8%.

As for Ulonska and Bauner[36], significant correlations were not found between Steep and germination speed g%1 for either GE or GC analyses in this experiment. The significant variables in the steep correlations with the set of the ten parameters (a13 and a18) were Intensity, TKW,

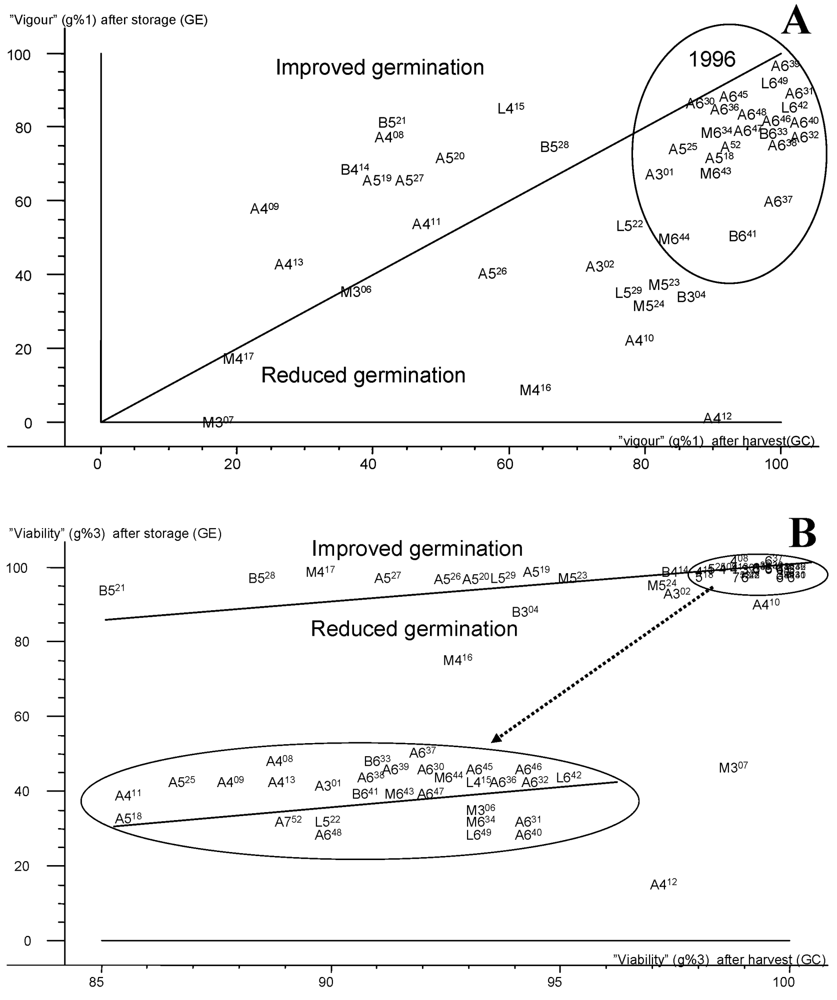

Fig. 5A. "Vigour" g%1 GC at harvest (abscissa) related to "vigour" g%1 GE after storage (ordinate) for samples grown in 1993–1996. **B.** "Viability" g%3 GC at harvest (abscissa) related to "viability" g%3 GE after storage (ordinate) for samples grown in 1993–1996. Abbreviations as mentioned in the Materials and Methods section.

and hardness (HI). The last two were expected[6,9] while the involvement of the intensity (light reflection) parameter could be indicative of a connection to weathering (indicator for dark seeds) related to softening of seeds.

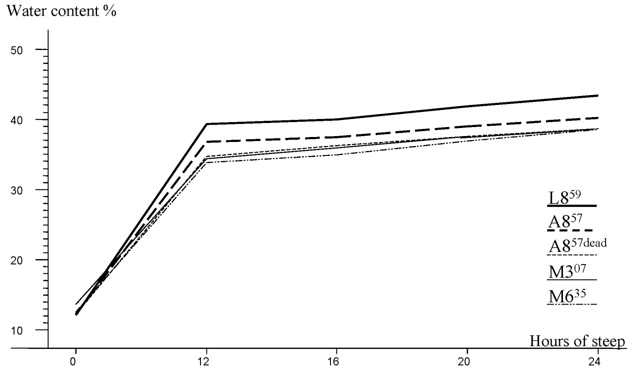

Fig. 6. Water uptake during 24 hours of steep for five samples of Lysimax, Alexis and Meltan. Germination values (g%1, g%3) for GC method: $L8^{59}$ (75.0, 97.5) $A8^{57}$ (79.3, 99.8), $A8^{57dead}$ (0, 0), $M3^{07}$ (12.0, 98.0), $M6^{35}$ (82.0, 99.0).

Optimal steeping conditions are essential if barley's full malting potential is to be realised[6]. Water uptake rates vary between the year of harvest, and between different varieties of barley as seen in Tables IIA–B. The steep character has been found to be influenced by kernel size[6], endosperm structure and composition[18,35] including the amount of mealy or steely grains[6,9]. Ulonska and Bauner[36] concluded that barley varieties require specific combinations of steeping and germination time to reach optimal values of modification and malt quality. These conditions have not been possible to optimize in the present experiment.

In order to make a focused study on the steep parameter and its dependence on the physical-chemical structure of the seed in relation to viability we have therefore taken out five samples from Table I for a separate standardised experiment. The "vigour" (g%1) and "viability" (g%3) values for the five samples are given in the text of Fig. 6.

A sub-sample of Alexis $A8^{57}$ (Table I) was heated to 100°C at low water content (12%) to produce a dead sample for comparison with the untreated one. In Fig. 6 the increase in water uptake (%) from 0 to 24 h of steep for the five samples is shown involving immersion in dilute peroxide solution (as for steep values in Table IIA–B). It is seen that all samples have a similar water uptake curve form although reaching different steep levels. Lysimax ($L8^{59}$) has the fastest water uptake (ending at 43%) compared to the other samples, confirming the 24 h steeping results given in an overview for all samples in Table IIB. This effect is likely to be partly due to a higher amount of hydrophilic proteins in this mutant, but is also caused by smaller thousand kernel weight (TKW 28.6 g for $L8^{59}$) compared to the other varieties that show decreasing steep percentage with increasing TKW ($A8^{57}$: 33.6 g, $M3^{07}$: 40.8 g and $M6^{35}$: 44.9 g).

The living Alexis sample ($A8^{57}$) had the second largest water uptake and reached water content after 24 h of steep at 40%. This sample had a slightly higher water uptake already after 12 h of steep compared to the same heat-treated sample. This was as expected. It is interesting, however, that the dead sample $A8^{57}$ actually had a surprisingly high water uptake ending at 38% at 24 h, which is comparable with the living Meltan samples.

With respect to the germination properties of the samples (Fig. 6) $L8^{59}$, $A8^{57}$ and $M6^{35}$, all have a "vigour" (g%1) larger than 75% (Table II), whereas $M3^{07}$ has a g%1 at 12% and the dead $A8^{57}$ sample has a g%1 at 0%. The large difference in vigour between the Meltan samples is not influencing their steep profiles, which are almost identical (the small difference could be due to different TKW).

In the present material it is noteworthy (Table IVA) that the dry harvest year 1995 that produced very hard kernels (HI = 65.8) compared to the favourable year 1996 (HI = 41.7) also had the lowest mean steep value (35.6%) compared to 37.3% in 1996 (Table IIA). Likewise 1995 had lower germination homogeneity (GH = 49.9) than 1996 (GH = 71.9). The evidence given above indicates that differences between varieties in the physical-chemical properties of the seed endosperm are important for determining the steep (water uptake) which in the start is a passive process rather independent of "vigour" and "viability" as indicated in the special experiment in Fig. 6. However, the variation in the steep parameter kept at constant steeping time in the main material in this investigation is rather low if one removes the extreme non malting variety Lysimax, which is an exceptional outlier combining hard seeds with high steep. It is concluded that the multivariate aspects of the steep parameter should be studied with a barley material with variable hardness where the steep level was optimized for each sample under more realistic experimental conditions compared to the above experiment.

Malting parameters

Using the ten physical-chemical parameters to predict Extract and BGwort it is seen, that by removing up to five outliers (most with a low "viability") it is possible to obtain a correlation coefficient of $r = 0.81$ for Extract (two PC's) and $r = 0.78$ for BGwort (four PC's) (Table VIA, a14–a17). These are slightly better predictions than using NIT measurements ($r = 0.73$ and $r = 0.77$ respectively, b19–b20), however, with no outliers removed (Table VIB). The number of PC's (five–nine) were much higher for the NIT correlation indicating a higher level of complexity. The significant parameters in Table VIA for Extract were P, Length, Round, HI and Area confirming the relationships between these parameters which was discussed in the PCA classification in Fig. 2A–B demonstrating the usefulness of the multivariate stepwise approach in visualising data to get indications for providing predictions.

Short-circuiting the two data sets by PLSR predictions of each of the set of ten variables to NIT spectra

As indicated by comparing the classifications of the PCA's of the separate datasets in Fig. 2A–B and Fig. 3A–B there are resemblances, which are further, strengthened in the PLSR predictions in Tables VIA–B. The final proof that NIT spectroscopy represents a non-destructive fingerprint of the whole barley seed sample is demonstrated by the high NIT predictions of each of the set of the ten physical-chemical parameters in Table VIB. Especially Protein ($r = 0.97$), Intensity ($r = 0.96$), HI ($r = 0.94$) and TKW ($r = 0.92$, 10 PC's indicating high complexity) show high correlation coefficients (while the seed imaging parameters Round, Length, Width and Intensity vary from $r = 0.74$ to $r = 0.96$ with a lower number of PC's (two–seven). It is interesting to note that the secondary parameters Area and Volume calculated from the three other primary parameters (Length, Width, Round) by the software in the GrainCheck instrument are not significantly correlated to NIT data. The β-glucan parameter cannot be predicted by NIT spectroscopy or by the set of ten parameters in this investigation when Lysimax is excluded because of the low variation of β-glucan in this limited material (Table VB). It can therefore be concluded that a large NIT calibration sample set has to be collected which shows a broad diversity in every parameter of importance, if the *on-line* NIT technology should be able to be extended in practice from protein and water to predict other parameters such as g%1, BGbarley, HI, Extract and BGwort.

CONCLUSIONS

Multivariate data analysis classification with the algorithm PCA is a strong tool for obtaining a preliminary overview of associations between different variables and individual samples (Fig. 2A–B and Fig. 3A–B). For further verification and focusing, PLSR multivariate prediction of specific parameters is essential (Table VI). The results from the two independent screening analyses (The set of ten variables and NIT) to estimate the physical-chemical status of the germinative and malting parameters support our earlier conclusions[25] regarding the usefulness of germinative classification plots with g% as abscissa and g%3 as ordinate for malt quality prediction. Thus the indirect parameters such as germination and malting properties are heavily dependent on the manifest multivariate physical-chemical properties in barley which may be used for prediction. This is only possible by multivariate pattern recognition analysis.

The surprisingly high correlation coefficients obtained using NIT can obviously be explained by high prediction values by NIT of each of the set of the ten physical-chemical variables discussed above.

It is concluded that germination characteristics and malt quality are influenced by two major functional factors – the physiological viability of the germ and the physical-chemical structure of the endosperm. The first factor is assessed by removing the low vigour outliers in the correlations by the PLSR influence plot[16,25]. The second factor is related to "vigour" (g%1) by limiting the required substrate to the germ for germination and growth of the plantlet in live seeds as suggested by our research group[25].

It should be emphasised that the prediction models in Table VIA–B only consider the physical-chemical endosperm properties underlying "vigour" (g%1) and "viability" (g%3) and cannot predict the physiological state of the germ in a test set of barleys with unknown germination properties. Thus "vigour" (g%1) cannot be used alone in order to judge germinative quality but is dependent on g%3 or the tetrazolium test for viability as outlined in the two-dimensional germinative classification plot[25]. A single score value combining the g%1, g%2 and g%3 parameters such as in GI is not sufficient[25]. The results from this investigation explain in more detail the advantage of the multivariate basis of the germinative classification. It is clear that the physical-chemical structure in malting barley of normal malt quality is the fundamental and dominating factor for predicting germination speed "vigour" (g%1) by NIT. Therefore NIT spectroscopy is feasible for a preliminary screening *on-line* for potential vigour of samples which should be checked for viability.

The vigour criterion is a more sensitive indicator for storage effects than viability. The same conclusion applies to modelling critical criteria such as Extract and BGwort where vigour plays a central role.

This study suggests that the physical-chemical seed structure such as hardness/softness also plays a major role in regulating the water uptake (steep) and in protecting the germ during long time storage. These results should be further verified by an experimental multivariate data analytical approach.

The "viability" (g%3) trait besides information on the physiological dimension also carries a substantial physical-chemical component as demonstrated by the significant correlation coefficients for NIT in Table VIB, however, with lower values than those for "vigour" (g%1) and with a greater number of outliers. Besides the tetrazolium test, g%8 should be selected in future basic research as a more clean parameter for viability better reflecting the physiological status of the germ than g%3.

It is expected that a barley data bank[25] covering a large variation in physical-chemical composition, with a wide range of varieties grown in different years and climatic regions, will be able to reduce the relative high errors of the statistically significant prediction models obtained in this investigation made with a limited number of samples.

The establishment of an extensive source of semi-artificial intelligence by a range of representative calibration models is the prerequisite for employing non-destructive NIT spectroscopy as a first *on-line* selection criterion[25] for "vigour" and malting quality of barley, where the best deliveries with regard to "vigour" (g%1) can be checked for viability by the tetrazolium test *at-line* as a second selection within two hours.

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