A New Two Dimensional Germinative Classification of Malting Barley Quality Based on Separate Estimates of Vigour and Viability

Birthe MØLLER and Lars MUNCK

Department of Food Science, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark

Abstract: It is surprising that not even today do germination data seem fully integrated with malting data in barley quality evaluation. In order to implement such an integration, pattern recognition multivariate data analysis (chemometrics) is essential. Inspired by the results from chemometric analyses of whole germination curves we tested a two-dimensional classification plot of barley samples based on separate estimates for "vigour" (g%1) germination energy (GE) as abscissa with limits at 70% and 30% and "viability" (g%3) as ordinate with limits at 98% and 92%. The seven barley classes obtained visualise the quality differences in a consistent and instructive way clearly differencing and ordering malting barleys with falling extract% and increasing wort β-glucan (mg/l) according to a subsequent validation analysis. "Vigour" g%1 could surprisingly be predicted by Partial Least Squares Regression (PLSR) correlation by Near Infrared Transmission (NIT) and by a separate set of ten physical-chemical analyses. Samples with "viability" g%3 lower than 92% were outliers. It was concluded that germination speed is connected with the structure of the seed, which regulates the availability of substrate for germ growth near connected to the speed of malt modification. It is suggested that a NIT PLSR prediction model for "vigour" can be used directly "on-line" for quality control in the grain industry and by plant breeders. A fast germinative classification plot can be established with NIT spectroscopy for "vigour" and the Tetrazolium germ-staining test for "viability" within two hours.

Keywords: germination classification; vigour; viability; Near Infrared Transmission; physical-chemical properties

Optimal germination performance is undoubtedly the most important quality criterion for malting barley. The industry and trade are dependent on reproducible and representative analyses as expressed in European Brewery Convention (EBC) Analytica (3.5–3.7) (Anonymous, 1998) regarding germinative energy (GE) % (in 3 and 5 days) and capacity (GC) % as well as germination index (GI) and homogeneity (GH). Quality classification indices based on elaborate pilot malting analyses and expert evaluation without the germinative analyses were developed by Molina-Cano (1987) for EBC and recently by our brewing research group (Nielsen *et al*. 2002) using fuzzy logic analyses. The latter index could be predicted (Nielsen et al. 2002) by NIT spectroscopy in a PLSR chemometric model. Monnez (1987) found it difficult to embrace the quality complex in one figure and suggested a more complex classification obtaining two hierarchical indices based on malting and brewing parameters by multivariate analysis yet not including germinative data in the analysis. Therefore we (Munck & Møller 2004a, b; Møller 2004) decided to combine germinative, chemical, malting and brewing data in a multivariate data analysis starting with investigating the pattern of germination response curves by Principal Component Analysis (PCA).

MATERIAL AND METHODS

Two barley materials are used:

I. 17 samples of the malting barley variety Alexis grown all over Europe in 1994 collected and analysed for malting quality at UdL-IRTA Centre, Spain, by Dr. José Luis Molina-Cano (Møller *et al*. 2002).

II. 42 barley samples of the varieties Alexis, Blenheim and Meltan grown in Southern Scandinavia collected in 1993–1996 were analysed for germination energy (GE), seed physical-chemical parameters and malt quality after cold storage (7°C, 13.5% water) in 1999. Percentage of germination on day1–day3 was determined according to EBC Analytica 3:6 (Anonymous 1998).

The "Unscrambler" chemometric software (Camo A/S, Norway) was used.

RESULTS AND DISCUSSION

Germinative classification

The germination curves (percentage of germinated grains for days 1–8 (g%1–g%8) (Figure 1A) were used in an unsupervised PCA calculation for the 17 samples of the variety Alexis grown in different localities all over Europe (Figure 1B). The PCA biplot shows that three samples grown in Spain (E) are located to the left in the plot, whereas all the samples from Finland (SU) are located in the bottom right corner. In the top right corner there are samples from Germany (D), Czech Republic (CZ), The Netherlands (NE) and two of the samples grown in Denmark (DK). The variables germination% from days 1 to 8 are shown in the plot as loadings (1, 2, 3, 4, 5, 6, 7, 8). It is seen that day 1 percentages are located in the top, day 2 closer to the rest of the loadings, and days 3–8 are located near each other in a group. This can also be seen from the germination curves in Figure 1A, while the curve shape after 3–8 days is more levelled for the 17 samples.

To investigate in more detail why the samples are located as they are in the PCA plot in Figure 1B, the notation refers to germination percentage after one day (bold) and after three days. The 3-day germination percentage is taken as the most conveniently measured representative for the close loading cluster 3–8. From this it is seen that there is a clear gradient in germination percentage after three days along the abscissa from left to right, and for germination percentage after one day along the ordinate from below to above. Now it is possible to ascertain the meaning of the "hidden" principal components PC1 and PC2 in the plot. PC1 mainly describes the variation due to germination percentage day 3. This axis can approximately be described to represent "viability" or germination energy (GE). The germination percentage after three days does not increase very much to day 8 (Figure 1A), and this factor can therefore give an estimate of living grains. PC2 mainly describes the variation due to germination percentage day 1. Concluding from earlier investigations where GI and g%1 correlate well,

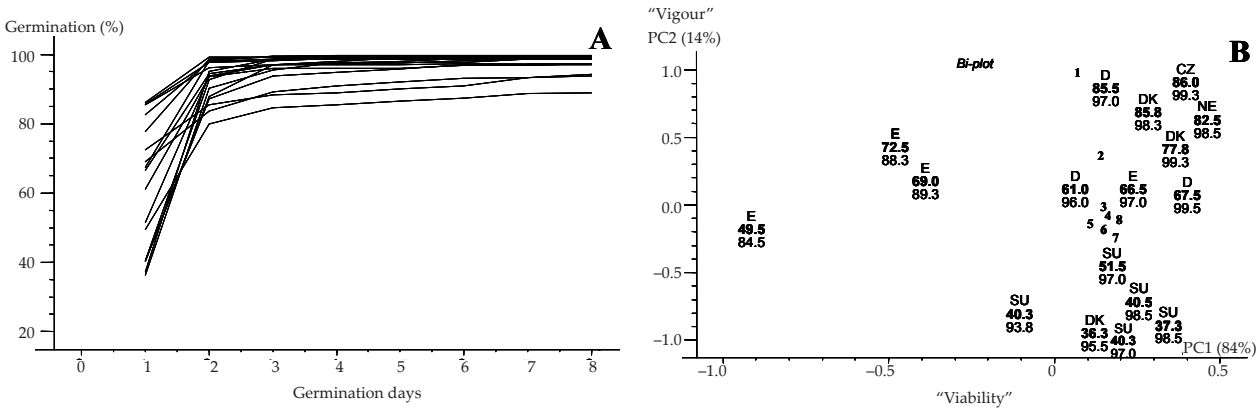

Figure 1. Multivariate evaluation of germination profiles g%1 – g%8 for 17 untreated Alexis barley seed samples grown in EBC trials in Europe in 1994. A. Germination profiles for the 17 samples. B. PCA biplot (PC1:2) of the germination profiles for the 17 samples with identification of each sample position by figures for g%1 in bold and g%3 in normal font. Letters denote country symbols. Figures in large and bold are loadings g%1(1)–g%8 (8)

PC2 can be described as an expression of germination speed "vigour".

From the PCA in Figure 1 a hypothesis can be generated that in a simple two-dimensional germinative abscissa-ordinate plot g%1 and g%3 could be used for malting quality classification.

This hypothesis was further confirmed by studying another data set where 42 micro malted barley samples with a large variation in "vigour" (abscissa) and "viability" (ordinate) are plotted as seen in Figure 2. Two quality levels of both "viability" (92 and 98%) and "vigour" (30 and 70%) are introduced in the plot. This leads to a division of the barley samples into 7 classes: 1.1, 1.2, 1.3, 2.1, 2.2, 2.3 and 3.0.

It is clearly seen that the "vigour" component is complementary to the germinative energy component "viability" g%3 in differentiating the whole material with regard to extract % and to an even greater degree with regard to the critical quality criteria (β-glucan in wort), revealing the dependence of cytolytic activity in the malt on a swift and complete germination. The mean values of the malting barley classes in Figure 2 reveal clear gradients in these important quality criteria. The feed barley Class 3 is clearly unsatisfactory for malting with mean figures of 70.1% for extract and 382.2 mg/l for β-glucan in wort. The germination index has a high correlation of $r = 0.99$ in this material with g%1 and can thus be used as an indication for vigour. g%1 should however be preferred because it is faster to measure and is much more responsive.

It is concluded that the proposed two-dimensional classification system with the barley material tested here is highly sensitive for predicting and discriminating the levels of extract (%) and β-glucan in wort (mg/l) which are central parameters in the barley malt quality complex. It is suggested that further malting barley quality research should be directed to utilise vigour and viability information from germination curves for quality classification.

Predicting the germinative parameters by instrumental analyses and chemometrics in order to develop screening methods for plant breeders and industry

Prediction by ten physical-chemical parameters

It would be advantageous if it were possible to predict the indirect parameters such as germination properties from manifest physical-chemical parameters from ungerminated barley. Here we use a set of ten physical-chemical parameters: Protein (P), β-glucan (BG), single seed hardness (HI, Perten SKCS 4100) and six morphological imaging barley data (Graincheck Foss A/S) as well as thousand kernel weight (TKW). The supervised chemometric algorithm PLSR can test this. The prediction of the germination properties is shown in Table 1.

With respect to GE determined after three to six years of storage, the PLSR correlation to the set of the ten physical-chemical variables is $r = 0.73$ (one PC, RE = 18.1) with a characteristic sequence of significant variables (Protein, Round, Length, Width, Volume, Intensity). Seven outliers are detected

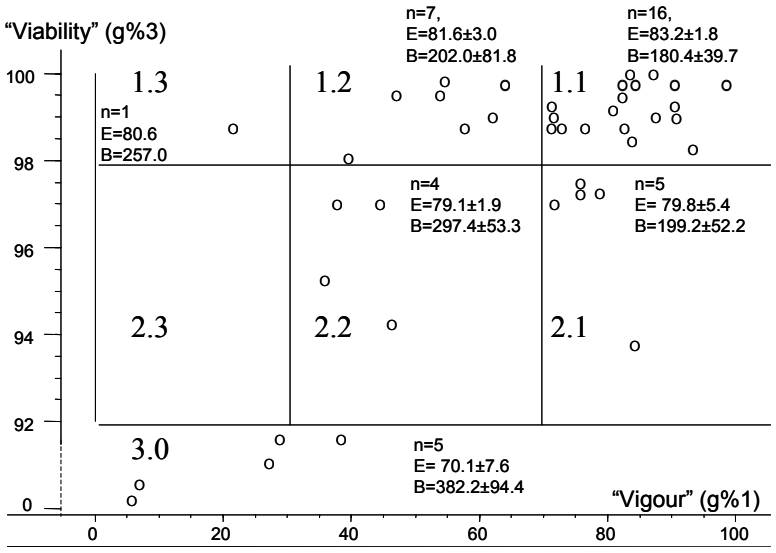

Figure 2. Germinative energy classification for a malting barley material ($n = 42$). "Vigour" g%1 (abscissa) and "viability" g%3 (ordinate). Enlargement of classification plot with "viability" ≥ 92%. See text for discussion. E = Extract %, B = mg/l (1→3, 1→4)-β-glucan in wort

Table 1. PLSR Jack-knife correlations between the ten manifest parameters (TKW, HI, P, BG, Width, Length, Area, Volume, Round, Intensity) as (*X*) and hidden germination and malting variables (*y*). Samples with low viability (< 92%) = underlined, medium viability (92–98%) = bold, high viability (> 98%) = normal

y (GE)	No.	Step*	*r*	RE	PC**	*n*	Total outlier samples removed	Significant variables***
	a01	0	0.73	18.1	1	42		P, Round, Length, Width, Volume, Intensity
g%1	a02	I	0.84	14.5	1	35	M07, A08, A09, A12, **B21**, A37, B41	P, Round, Length, Width, Volume
	a03	I	0.94	9.1	4	35	M07, A08, A09, A12, **B21**, A37, B41	P, Width, Round, Length, Volume
	a04	0	0.39	16.5	1	42		P, TKW
g%3	a05	I	0.56	14.5	1	40	M07, A12	P, HI, TKW
	a06	II	0.73	14.5	2	39	M07, A12, M16	P, INT, TKW
GH	a07	0	0.70	14.9	2	42		Length, Round, P, Area

*step of outlier selection from influence plot; **minimum value of residual validation variance; ***variables ordered according to degree of importance

(removed in two steps, Table 1), two of which have extremely low "viability" g%3 (M07 and A12) and B21 with reduced "viability" g%3.

When the seven outliers are removed, the correlation improves to $r = 0.84$ (one PC, RE = 14.5) with $r = 0.94$ for four PC's (RE = 9.1). The pattern of importance is unchanged. There is a clear tendency for low "viability" outliers in the correlation models given in Table 1. This is especially apparent in the "viability" g%3 GE prediction in Table 1 where the correlation coefficient is improved from $r = 0.39$ (one PC, RE = 16.5) to $r = 0.73$ (two PC, RE = 14.5) when removing three outliers which all have low "viability" (g%3). It is also concluded that the prediction of "viability" g%3 from the ten parameters has a significantly lower correlation coefficient than that of "vigour" g%1.

We can thus conclude that "vigour" g%1 can be predicted by the set of the ten physical-chemical parameters to a surprisingly great extent. From this information a new hypothesis can be generated stating the physical-chemical nature of "vigour" (Munck & Møller 2004a,b).

Prediction by Near Infrared Transmission Spectroscopy

It is seen that the ten physical-chemical barley parameters were able to roughly predict germination properties of a sample. Using NIT spectroscopy a physical-chemical fingerprint is likewise obtained. The ten physical-chemical manifest parameters are here expanded to 100 variables. Therefore it is expected that NIT will also be able to predict germination.

In Table 2 a relatively high correlation of $r = 0.80$ for "vigour" g%1 and for "viability" g%3 GC (three PC's) is seen using the first derivate of NIT spectra. As with the prediction of indirect germination variables using the ten manifest parameters in Table 1 there is a clear tendency that g%3 gives lower predictions with NIT than g%1 and that outliers have a low viability. The low vigour outliers in y in the NIT correlations cannot obviously contribute to the prediction of germination speed or "vigour" g%1 on the basis of analysing ungerminated kernels.

The most important variable for the prediction of "vigour" in Table 1 is Protein, followed by Round, Length, Width and Volume. Most of these parameters are obtainable with NIT with e.g. correlations of $r = 0.97$ for Protein, $r = 0.77$ for Round and $r = 0.94$ for HI. A rather good prediction for "vigour" from NIT measurements is therefore expected to stem from the physical-chemical properties manifest in the grains, which are essential for access of nutrients to the embryo influencing germination speed (g%1) such as endosperm cell wall thickness.

With the strategy of focusing on the structural factor by PLSR and identifying the physiological (viability) nature of the outliers in y the surprising conclusion is drawn that germination speed

Table 2. NIT (1st derivate) prediction of germination, malting data and chemical-physical data for samples of Alexis, Blenheim and Meltan. Samples with low viability (< 92%) = underlined, medium viability (92–98%) = bold, high viability (> 98%) = normal

y (GE)	No.	Step*	r	RE	PC**	*n*	Outliers***
g%1 (GE)	b01	0	0.74	17.8	4	42	
	b02	I	0.77	15.7	4	41	A12
	b03	II	0.80	14.3	3	38	A12, M16, **A20**, **A27**
g%3 (GE)	b04	0	0.31	17.0	1	42	
	b05	I	0.68	15.7	1	39	M07, A12, M16
	b06	II	0.80	3.4	3	37	B04, M07, A10, A12, M16
GH (GE)	b07	0	0.59	24.4	4	42	
	b08	I	0.75	17.1	4	37	**B21**, A31, A37, A39, M44

*step of outlier selection from influence plot; **minimum value of residual validation variance; ***total outlier samples removed from correlation

"vigour" in this investigation has a much more pronounced structural component than the physiological one within the range of viability which is characteristic of malting barley (above g%3, 98%). The g%3 variable also reflects seed structure to some degree but with a much lower correlation to the structural parameters than g%1. It is therefore concluded that the structural physical-chemical factor is the main determinator for "vigour" g%1, defined as the early growth rate of the emerging plantlet in barley of malting grade. As in Table 1 with the ten physical-chemical barley parameters, NIT predictions of GE g%1 and GE g%3 are improved by removing the outliers which in NIT spectroscopy all were found to be low in "viability" g%3.

These preliminary results can be interpreted as follows (Munck & Møller 2004): 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 (a low malt modification resistance) in dissolving cell walls and in enzyme spreading in the endosperm. Fast germination, i.e. high "vigour", should therefore be operative for the maltsters as an indicator of efficient malt modification representing the structural functional factor related to physics and chemistry. Thus, by securing a high "viability" above g%3: 92% the structural functional factor becomes limiting in malting and brewing performance (Munck & Møller 2004). Thus "vigour" g%1 can be estimated directly "online" by a NIT calibration.

The outliers with low "viability" (g%3) that have been found in the models in Tables 1 and 2 are deviates in *y* (g%3) and not in *X* (NIT or the ten physical-chemical parameters). When removal of outliers determined in *X* no improvements in correlation coefficients are found. The detected outliers in *X* do not show low "viability". This indicates that neither NIT nor the ten physical-chemical parameters can be used for predictions of "viability" in unknown samples (Møller 2004). This is in accordance with the initial hypothesis that physical-chemical analyses should not be able to trace the physiological properties (low viability) dependent on an ungerminated embryo only contributing less than 5% of the intact barley seed. A separate method for "viability" is thus needed as a supplement to "vigour" (g%1) to remove low "viability" outliers. The germination percentage after 3 days (or theoretically more correctly 8 days germination) or the Tetrazolium embryo staining test could be used for that purpose. A complete germinative classification could thus be done within one or two hours.

Alternatively to NIT measurements the maltsters could germinate samples in 24 hours as well as determine the percentage of living grains with the fast Tetrazolium test (approximately one hour

analysis time), and from here obtain a 24 hours germinative classification plot where samples will cluster according to malt quality. The g%1–g%3 germinative classification as such or based on data derived from the Tetrazolium test and/or NIT calibrations should be developed to a convenient tool in classification of barley for malt quality (Munck & Møller 2004; Møller 2004).

CONCLUSION

The advantages and possibilities for plant breeders and industry of using multivariate data analyses such as PCA for early prediction of malting barley quality (extract % and wort β-glucan mg/l) in a germinative classification plot with separate estimates for "vigour"(g%1) as abscissa and "viability" (g%3) as ordinate has thus been shown. In another contribution to IBGS-IX we have further exemplified the great advantage of using multivariate analysis in plant breeding, genetics and biotechnology (Munck & Møller 2004b). The physical-chemical basis of germination speed "vigour" g%1 revealed in this paper and by Munck and Møller (2004a); Møller (2004) can be used to develop PLSR multivariate predictions of "vigour" by fast non-destructive instrumental methods (grain image analysis /hardness or NIT spectroscopy) to be used "at-line" or "on-line".

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Abstrakt

Møller B., Munck L. (2004): **Nová metoda hodnocení jakosti sladovnického ječmene pomocí dvourozměrné klasifikace klíčení založené na stanovení klíčivosti a energie klíčení.** Czech. J. Genet. Plant Breed., **40**: 102–108.

Pomocí chemometrické analýzy úplných křivek klíčivosti jsme ověřovali možnosti použití diagramu dvourozměrné klasifikace založené na odděleném stanovení jednak procenta vyklíčených zrn po 1 dnu klíčení (vigour, g%l) resp. energie klíčení (GE) na svislé ose v rozmezí 30 % až 70 %, jednak procenta vyklíčených zrn po 3 dnech klíčení (viability, g%3) na vodorovné ose v rozmezí 92 % až 98 %. Mezi sedmi třídami ječmene, které bylo možné rozlišit, byly zřetelně viditelné a spolehlivě prokazatelné rozdíly v jakosti. Jak potvrdily následné ověřovací analýzy, metoda jasně diferencuje sladovnické ječmeny podle procenta extraktu a obsahu β-glukanu (mg/l). Překvapivě lze vigour g%l odhadnout pomocí korelace (vypočtené na základě regresní analýzy metodou parciálních nejmenších čtverců – Partial Least Squares Regression, PLSR) hodnot propustnosti v oblasti blízkého infračerveného záření (Near Infrared Transmission, NIT) s nezávislým souborem dat 10 fyzikálně-chemických rozborů. Vzorky s viabilitou g%3 pod 92 % jsou považovány za extrémní odchylky. Byl učiněn závěr, že rychlost klíčení má souvislost se strukturou zrna ječmene, na které závisí dostupnost substrátu pro růst zárodku a která má velký vliv na rychlost

modifikace endospermu. Proto jsme toho názoru, že odhad „vigour" pomocí předpovědního modelu NIT PLSR může být používán přímo on line pro kontrolu jakosti v semenářství a sladovnictví i pro účely šlechtění. Rychlé vyhodnocení klíčení může být provedeno pomocí NIT spektroskopie (vigour) a pomocí tetrazoliového testu (viability) během 2 hodin.

Klíčová slova: hodnocení jakosti; klíčivost; energie klíčení; Near Infrared Transmission; fyzikálně-chemické vlastnosti

Corresponding author:

Dr. Brithe Møller, The Royal Veterinary and Agricultural University, Rolighedsvej 30, Frederiksberg C, DK-1958 Denmark
tel: + 45 352 835 07, fax: + 45 352 832 65, e-mail: bm@kvl.dk