Pre-germination in barley using NIR hyperspectral imaging

Paulina Engelbrecht,[a] Marena Manley,[a,*] Paul J Williams,[a] Gerida du Toit[a] and Paul Geladi[a]

[a]*Stellenbosch University, Department of Food Science, Private Bag X1, Matieland, 7602, South Africa. E-mail: mman@sun.ac.za*

[b]*Unit of Biomass Technology and Chemistry, Swedish University of Agricultural Sciences, SE 90183 Umeå, Sweden*

Introduction

Pre-germination is the early or unwanted germination of mature grains with little or no visible indication.[1] This could lead to a reduction in crop yield and grain functionality. Pre-germination is triggered by water absorption of the grain pre- or post-harvest.[2] During pre-germination stored nutrients are hydrolysed by hydrolytic enzymes. This hydrolysis of the stored nutrients decreases the grain starch yield. In barley, destined for malting, pre-germination can lead to the loss of viability. Viability is an important characteristic for malt quality.[3] During steeping, pre-germinated grains absorb water, thus providing a suitable environment for undesirable mould development.

Currently pre-germinated grains are inspected visually by graders at grain delivery sites.[4] These inspections are based subjectively on the knowledge and experience of these graders. Grains are also subjected to other time-consuming and destructive tests such as viscosity analyses.[4] There is a need in industry for a rapid, non-destructive and objective test for detection of pre-germination. The application of near infrared (NIR) spectroscopy is well established in quality control environments. A more recent technique is NIR hyperspectral imaging, that combines spectral information of a sample with the spatial dimensions of an image.[5,6] Hyperspectral images allow one to visualise chemical concentration and distribution within a sample.[6] NIR hyperspectral imaging is a rapid non-destructive technique and although multiple kernels are usually analysed simultaneously, results from single kernels can be obtained. A few studies showed the potential of NIR hyperspectral imaging in the detection of sprouted wheat kernels.[5,7,8] The aim of the present study was to establish the potential of NIR hyperspectral imaging to detect pre-germination in intact barley kernels.

Materials and methods

Samples

Samples (10 randomly selected kernels) from three commercial malting barley cultivars, i.e. S04-11, SSG564 and Puma, were kindly provided by SAB Maltings (Pty) Ltd (Caledon, South

Africa). Six subsets, comprising 25 kernels each, were randomly selected from each of the three respective cultivars. These samples were placed in petri-dishes with two layers of Whatman no.1 filter paper, 3 mL of distilled water were added, and the samples were incubated at 21°C for 6, 9, 12, 18 and 24 hrs. One subset of each cultivar was neither treated with water nor incubated and was held as a control. After incubation all the samples (including the control) were frozen at –80°C for 24 hrs, freeze dried for 72 hrs and vacuum-sealed for safe storage.

Image acquisition

Samples were positioned (crease down) on the sample stage (lined with silicon carbide sandpaper) of the sisuCHEMA short wave infrared (SWIR) hyperspectral imaging system (Specim, Spectral Imaging Ltd, Oulu, Finland). This system consists of an imaging spectrograph coupled to a 2-D array HgCdTe detector with a 50 × 100 mm field of view. Images were acquired from 1000 nm to 2498 nm with 6–7 nm intervals and stacked to form a 3D hypercube with the dimensions 320 × 583 pixels × 239 wavelengths. Internal dark and white reference standards were used for image calibration and conversion of reflectance counts to pseudo-absorbance.

Image analysis

The images acquired using the ChemaDAQ software programme (Specim, Oulu, Finland) were transformed from reflectance to pseudo-absorbance in Evince V2.2.2 (Umbio AB, Umeå, Sweden) multivariate image analysis software. Principal component analysis (PCA) with 6 components was used on mean-centred data to clean the images. This included the identification and removal of background, dead pixels, shading errors and edge effects that would interfere with the image analysis. Standard normal variate (SNV) transformation[9] was applied to the images. PCA was recalculated and score plots and score images were used in the detection of pre-germination.

Moisture analysis and tetrazolium tests

Moisture content of the control and samples incubated for 24 hrs was determined after freeze-drying (AACC method 44-40, 1999). The tetrazolium test for viability (no pre-germination) was performed on all the barley samples to confirm the onset of pre-germination. The kernels were cut longitudinally and subjected to a 1% 2,3,5-triphenyl-tetrazolium chloride solution for 30 minutes at 40°C.

Results and discussion

Similar results for all cultivars were obtained, so only those of Puma will be discussed. The score plot of PC 1 (75.75%) vs PC 5 (1.46%), after removal of the unwanted pixels, showed dissimilarities between pre-germinated and non pre-germinated kernels in the direction described by PC 5 [Figure 1(a)].

These results can also be visualised in the score image due the interactive nature of the software.

Although the subsets were imaged randomly, the rows in the score image were reorganised chronologically for ease of interpretation. From the score image of PC 5 [Figure 1(b)], the control

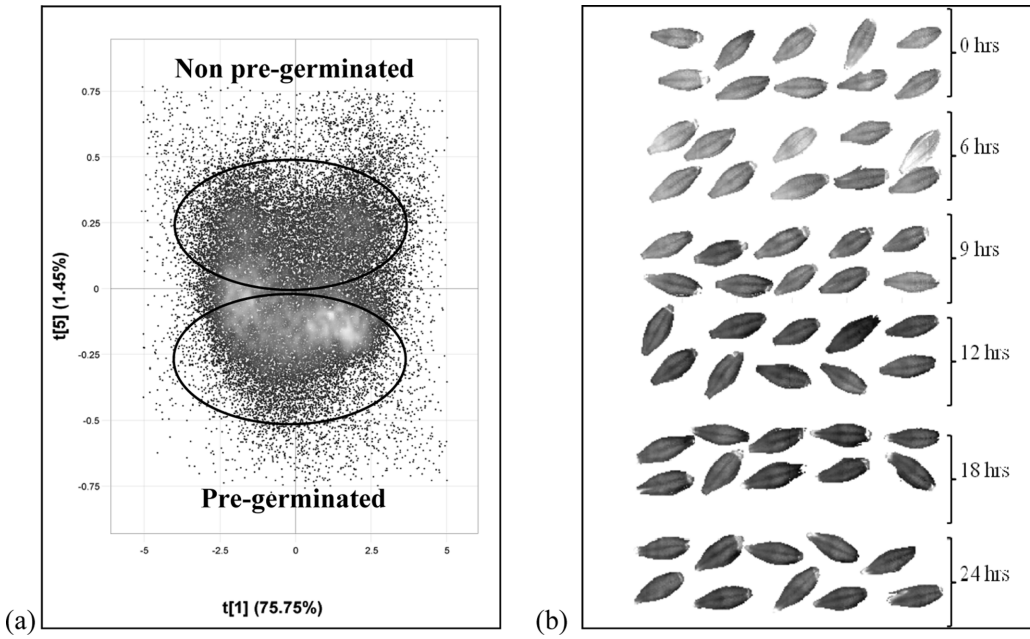


Figure 1. (a) PCA score plot of PC 1 versus PC 5 (b) score image of PC 5 (44 215 pixels).

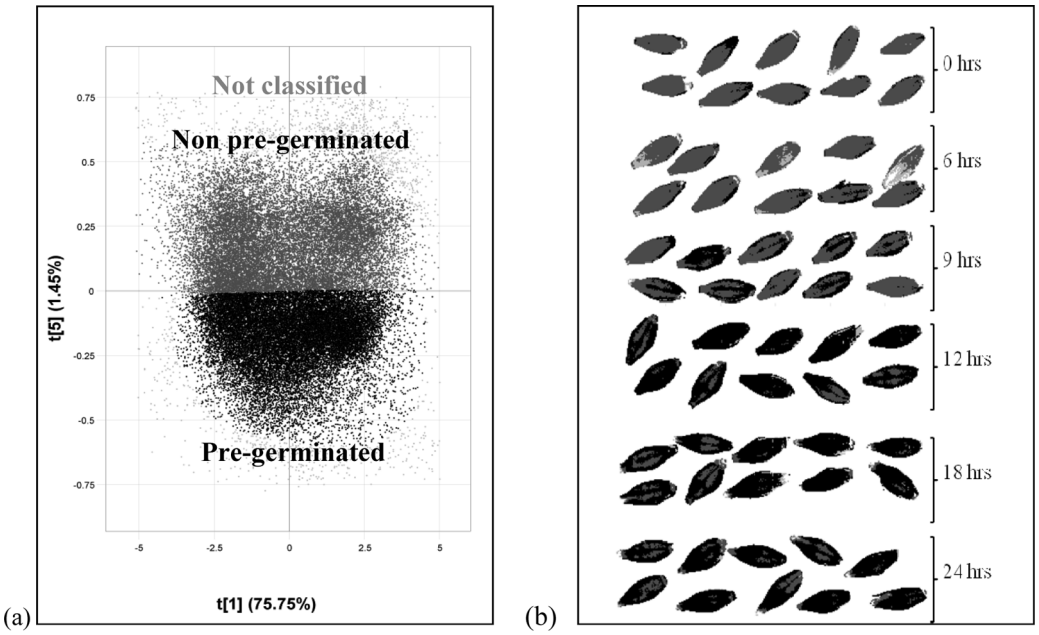
(0 hrs; 100% viable) and some of the kernels incubated for six hours (60% viable) could be distinguished from the remainder of the kernels (difference in grey scale intensity). Pixels with similar score values in the score plot are indicated with related grey scale intensities in the score image. This allowed for identification of single pre-germinated kernels in the score image (light grey). The moisture content of the control and samples incubated for 24 hrs were similar, thus it can be assumed that discrepancies in the score image were not due to moisture content. This, however, needs to be investigated further by interpreting loadings.

By interactive projection of the classes [Figure 2(a)] on the score image, a classification image [Figure 2(b)] was created.

These results could be analysed further by means of partial least squares discriminant analysis (PLS-DA).

Conclusion

The potential to distinguish pre-germinated barley from non pre-germinated kernels using NIR hyperspectral imaging was illustrated. However, this rapid, non-destructive and objective method, needs to be evaluated and validated on barley kernels pre-germinated in a natural environment, i.e. due to adverse wet weather conditions.



(a) (b)

Figure 2. (a) PCA classification plot of PC 1 versus PC 5 and (b) classification image of PC 5.

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