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MONITORING OF WHEAT GERMINATION BY MEANS OF NIR METHOD

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Abstract

Germination is a complex manifestation of different physiological, morphological, chemical and enzymatic changes in seeds. There are various technical, technological and economical interests to predict the level of germination quickly and non destructively. Early stages of germination processes (0 – 72 hours) were investigated in wheat using NIR reflection and transmission methods in order to detect the changes of main constituents. The mechanism of water uptake as well as the hydration of proteins and carbohydrates were followed in whole seeds. The early stages of reserve mobilisation (proteolytic and amilolytic effects) were detected by means of NIR, rheological and electrophoretic methods. Most characteristic changes were observed in the forms and status of water during 24 hours of germination. The developed calibration methods allowed recognition of the germination processes very early (in four hours) and measurement of the degree of germination with acceptable accuracy.

Keywords: germination, near infrared spectroscopy.

Introduction

At the end of the period of growth and development all the reserves of a seed are accumulated and become air-dry. The dry seed lies at the turning point in seed life, between the phase of development and the phase of germination. By addition of water to a dry seed a new pattern of development starts where three phases can be recognised: imbibition, lag phase and germination. The early events are those leading up to visible germination, the protrusion of the root through the testa. If the temperature is appropriate and the supplies of oxygen adequate the seed enters the germination phase (ESASHI – LEOPOLD, 1968).

Air-dry seeds imbibe rapidly, the water content of the tissues reaching 30 – 40% in a day. By this time the seed gets swollen and heavier and is metabolising actively. As the seeds imbibe, solutes and gas enter the seeds as well as water and their volume increases. The swelling is not simultaneous and uniform throughout the tissues and inner layers. After

some minutes of imbibition the surface cells become ruptured so that their cytoplasm contents are free to leak out (DUNN et al., 1980, DUKE et al., 1981). The leakage of potassium is fast at the start of imbibition and many different substances leak out of seeds and embryos, including amino acids, organic acids, sugars, phenolics, phosphates, gibberellic acid and enzymes. The chemical milieu and the membrane system are reorganised and(or) repaired by the inflow of water (SIMON, 1978). Enzymes including those responsible for the metabolic events become active once more as soon as imbibition starts. The rate of a triphasic respiration rises rapidly, the substrates consumed in respiratory metabolism are sugars or oligosaccharides. The quantity of ATP present in seed tissues increases by as much as 10-fold during the first hour of germination. Protein synthesis starts up again: ribosomes, enzymes of glycolysis and new and specific germination proteins, amylases and proteases are synthesized (DASGUPTA – BEWLEY, 1982; SALGÓ et al., 1985). The protein synthesis is coded by the conserved mRNA during early imbibition but thereafter newly synthesized mRNA molecules play an important role. The synthesized new mRNA level increases rapidly in eight hours of imbibition. DNA synthesis follows the formation of proteins and RNA with some hours delay during imbibition (MURRAY, 1984). The aim of the present study was to recognize the early germination processes by near infrared technique.

Materials and Methods

400 g of seeds of ten different varieties of wheat were washed and soaked in tap water ($w/v = 1/2$) for four hours and then germinated according to SALGÓ et al. (1985). Samples were taken in defined times of germination. The moisture contents of whole seed samples were measured with the oven method (105°C, 3 hours) and simultaneously the samples were scanned with NIR System 6500 using a sample transport module. After drying the samples the seeds were ground with a Labmill QC114 and the whole meals were used for dough making. Wheat dough was made according to BLOKSMA et al. (1975). The doughs were investigated with Bohlin visco-oscillorheometer by means of the cone-plate method in the oscillation mode. Loss, storage and complex moduli of doughs were detected versus frequency of oscillation.

High molecular weight glutenin (HMWG) subunit structures of wheat samples were investigated with polyacrylamide gel-electrophoresis (PAGE) according to LAWRENCE et al. (1980). Near infrared spectra of samples were transformed and processed by a NSAS software package using PLS method.

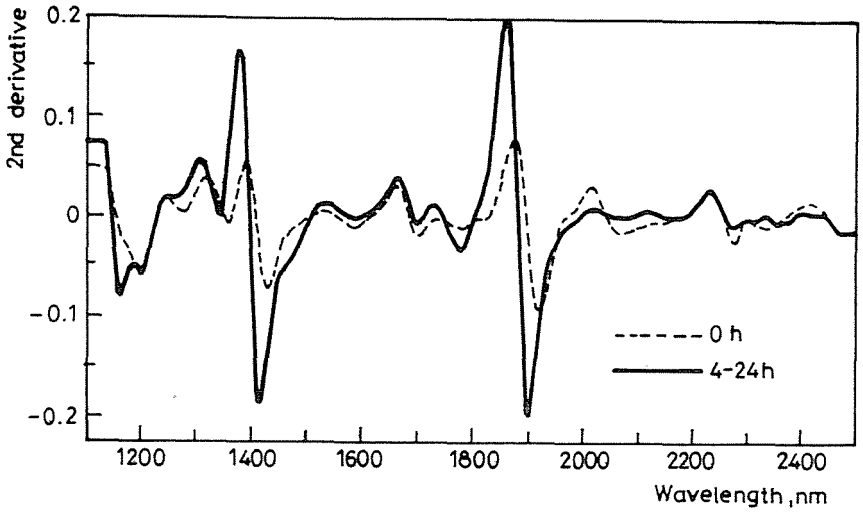

Fig. 1. Second derivative spectra of ungerminated and germinated wheat seed samples as function of the germination time

Results and Discussion

The early events of imbibition can be followed in second derivative spectra of seeds as function of the germination time in *Fig. 1.* Most characteristic changes were observed in two water absorption bands at 1430 and 1920 nm, respectively. The band in the 1400 nm region which has less interference from overtones arising due to contributing groups other than the O–H group is the characteristic range of different types of water ($S0$ = free water molecule, $S1$ = molecules with one OH engaged in hydrogen bond, $S2$ = with two OH engaged in hydrogen bond). Intensive shift in wavelength from 1431 to 1414 nm was observed due to the increased level of free water in four hours. The shift is not proportional to the germination time and it is influenced by the leaking solutes as well. Intensities of 2nd derivative spectra were decreased due to both effects (free water, solutes). The band in the 1920 nm region is associated mainly with $S1$ water species according to IWAMOTO et al. (1987). Definite but smaller change in free water was observed at 1918 nm compared to changes in the 1420 nm band. After four hours of germination a 16 nm shift in the band position was registered and the intensities changed slightly. The results indicated that the fine structure of water changes considerably during germination and the water uptake in the seed is quite complex. Characteristic changes of protein bands can be detected in the ranges 1150 – 1180 and 2260 – 2290 nm,

respectively. After a quick hydration of proteins in 4 hours, proportional but contradictory changes were observed in both spectral regions. Based on these observations it is logical to postulate that the metabolic events of germination became intensive after four hours of imbibition. The broad band of starch molecules (2280 nm) was overlapped by the protein band mentioned above, and thus the separation of changes in proteins or starch in this region is impossible. Time-dependent changes were observed in the starch and sugar peak in the range 2080 – 2130 nm. After hydration a slight increase was detected in the whole region indicating the change of saccharides as energy sources during early germination.

The hydrated protein complex of wheat seed was investigated either in dough form by means of rheological methods or in whole meal form with PAGE. The results indicated (data not shown) that four hours of germination caused irreversible changes in dough structure and the HMW glutenin subunit structure was damaged due to germination.

Calibration methods were developed for detection of moisture content and germination time in wheat seeds. The results are shown in *Figs 2* and *3*. The developed calibration method is based on the moisture content and allows detection of the degree of germination with 5.5% coefficient of variation.

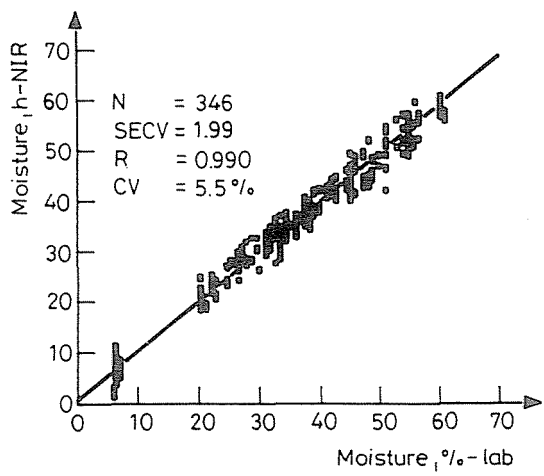

Fig. 2. Scatter plot of moisture content in dry and germinated wheat seed

As the sample population was big and consisted of the ten most common varieties used in Hungary it was stated that the g rmination can be predicted with acceptable accuracy using NIR. A significantly worse calibration equation was obtained when germination times were used as

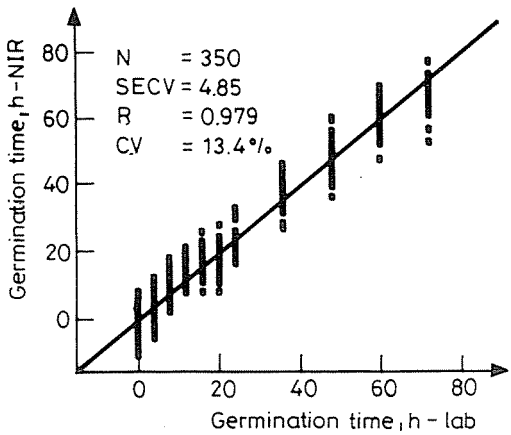

Fig. 3. Scatter plot of germination time in dry and germinated wheat seed

reference data. The results indicated that the 'biological variabilities' are much higher compared to the variability of single chemical constituents and consequently the accuracy of prediction decreased considerably. Similar results were obtained using transmittance technique but the precision of calibration was lower. Based on the developed calibration method the status of a germinated seed and the degree of germination are predictable and the method is applicable for the quality control or routine analysis in seed processing and storage.

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