Changes in carbohydrate content during wheat maturation—what is measured by near infrared spectroscopy?

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The role of bread, pasta and related products produced from milled wheat seeds is important to the human diet, so monitoring changes of starch content in developing grain is essential. Immature wheat grains are also used as a functional food, particularly as a source of water-soluble carbohydrates. The amount and variation in content of different carbohydrates changes considerably during maturation and these changes were non-destructively monitored in developing grain using near infrared (NIR) spectroscopy. Characteristic changes in three carbohydrate absorption bands [1585–1595 nm (Carbohydrate I), 2270–2280 nm (Carbohydrate II) and 2325–2335 nm (Carbohydrate III)] were identified and it was concluded that the different dynamics of carbohydrates (starch accumulation as well as synthesis/decomposition of water-soluble carbohydrates) could be followed sensitively by monitoring these three different regions of NIR spectra. Carbohydrate I represents the effect of starch accumulation during maturation based on the vibrations of intermolecular hydrogen bonded O–H groups in polysaccharides. Carbohydrate II is the manifestation of O–H stretching and C–C stretching vibrations existing unengaged in water-soluble carbohydrates while Carbohydrate III describes the changes in C–H stretching and deformation band of poly- and mono-oligosaccharides. NIR spectroscopic techniques are shown to be effective in monitoring plant physiological processes and the spectra have hidden information for predicting the stage of growth in wheat seed.

Keywords: seed development, maturation, carbohydrates, physiological process, near infrared spectroscopy

Introduction

Cereal grain structure, stage of development and tissue characteristics, as well as the morphogenesis of the cereal endosperm, all define aspects of quality that are relevant to current uses.[1] Structural and compositional differences of tissues, granules and milled fractions all influence the chemical, physical, rheological and functional properties of wheat materials.[2] During different stages of maturity, the properties of wheat can change significantly due to the accumulation or depletion of different carbohydrates, which produce significant characteristics during seed development.[3]

The first instrumental observations of carbohydrates from wheat can be traced back to the beginning of the 18th century. While visible light constitutes just a small part of the electromagnetic spectrum, the description of starch granules and their behaviour by Antony van Leewenhoek using the first light microscope was a huge step forward in science.[4] A few centuries later, near infrared (NIR) spectroscopy is commonly used for following the chemical, physical, technological or physiological processes of cereal materials that cause changes in composition, interactions, or indicate the structure of carbohydrates.[5]

Absorption bands relating to wheat and its components (for example, starch and pentosans) were first identified at the end of the 1970s by Law and Tkachuk.[6] Over the last 20 years, a number of workers have identified relationships between NIR spectra and properties of wheat and its components. The chemical hydrolysis of starch can be sensitively monitored in the combination region of the NIR spectrum through the monomers liberated during the process.[7] Physical processes such as flour milling or extrusion cooking can initiate intensive intra- and intermolecular changes in starch which have been followed with NIR methods.[8,9] Similarly, gelatinization and degradation of starch may be measured on the basis of spectral changes associated with different states of hydrogen bonding of O–H in starch.[10] NIR spectroscopic monitoring also allows an understanding of the dough formation that is an essential technological step in the production of bakery products.[11] More recently, starch–protein–water interactions in mixtures were investigated with dynamic NIR spectroscopy and a simultaneous gelatinization and strong

changes in ordering of water were suggested.[12] Physiological processes (for example, plant growth, maturation) are probably the most complicated and, in many details, unclear parts of the life cycle of cereal products strongly influence the chemical, physical and technological procedures mentioned above. The non-invasive, real-time nature of NIR techniques make it useful as a tool for better understanding of changes in plant materials during development.[13,14]

The aim of the present study was to investigate the maturation processes in wheat seeds that involve changes to carbohydrates. Current results focus on the spectroscopic observations that allow useful insight into the chemical changes that occurred in the maturing seed and may help to define the growth stage.

Experimental

Six different winter wheat varieties, with different harvest dates, were grown as field trials at the Agricultural Research Institute of the Hungarian Academy of Sciences (ARIHAS), Martonvásár, during 1997. The varieties investigated were GK Öthalom, Bánkúti 1201, Jubilejnaja 50, Mv 23, Fatima and Mv 15. Primary ears were collected twice or three times weekly for each variety. Sampling began 12 days after flowering (DAF) and the 16 sampling dates covered the entire maturation period of 41 days (12–53 DAF). Because seeds exhibit high biological variability, six independent ears were collected on each occasion for each variety.

The seeds were collected from ears directly after sampling and were investigated in intact form. The samples obtained on the first sampling date (12 DAF) differed from the others in that the immature kernels were so small that they had to be prepared together with their bracts (palea and lemma); otherwise, 40–60 seeds were taken from each ear and the average fresh weight of the samples (mg seed^{-1}) was measured.

Then the seeds from all six ears were physically combined to form one spectral sample. Five independent scans were recorded from each spectral sample using an NIRSystems Model 6500 monochromator system (Foss-NIRSystems, Silver Spring, MD, USA) fitted with a sample transport module and standard sample cups equipped with threaded back. Samples were scanned (32 scans co-added) from 1100 to 2498 nm in reflectance mode (R mode: PbS detector). Data were collected every 2 nm (700 data points per spectrum) and the raw spectra were transformed into second derivatives (D2OD) using a 10 nm segment and 0 nm gap size.[15] Spectral and reference data were processed using NIRSystems Spectral Analysis Software (NSAS), Ver. 3.30 and with PQS32 Evaluation Software, Ver. 1.37 (Metrika R&D Co., Budapest, Hungary).

A segment size of 10 nm was adopted because preliminary results using segment sizes of 4, 10 and 20 nm showed no significant alterations to carbohydrate peaks in D2OD spectra. Second derivative spectra have a negative peak that matches exactly the absorption maximum (positive peak) of an absorbance band in log 1/*R* and these negative peaks were used to monitor changes of carbohydrates in wheat seeds.

Moisture content of intact, fresh seeds was determined in triplicate using oven drying (105°C for four hours) immediately after collecting spectra to avoid moisture loss. Nitrogen content of whole, dried seeds was measured in triplicate by a combustion method using a LECO FP-528 Protein-Nitrogen Analyzer (LECO Corp., St Joseph, MI, USA). Because the chemical composition of wheat is characterized by a high content of starch, a significant protein content and a low almost constant lipid and ash content[16] (82%, 14%, 2%, 2% on dry weight basis respectively),[17] the measured moisture and protein content allowed an estimate of the carbohydrate concentration as % dry matter–% protein.[18] The remaining fresh samples and the dried materials were then frozen (–15°C) for further reference tests.

To identify the most characteristic (i.e. most highly resolved) carbohydrate peaks during maturation, main constituents [unmodified wheat starch (Sigma Chemical Co., St Louis, MO, USA) and wheat gluten (ICN Biomedicals, Irvine, CA, USA)] were also scanned in pure form using the same settings as for the wheat kernels. In addition, NIR spectra of distilled water and aqueous solutions of some mono- and disaccharides [fructose, glucose and sucrose (Reanal Finechemical Co., Budapest, Hungary)] were collected in transmittance mode (T mode: PbS detector) in a 1 mm cuvette at room temperature. For each of these water-soluble carbohydrates, a dilution series of five samples at concentration levels of 0, 40, 100, 200 and 400 g L^{-1} was prepared.

Results and discussion

The dynamics of water, net dry matter and calculated starch content in wheat seeds during maturation is shown in Figure 1. Before discussing the trends in these plots, we would like to explain why data expressed on a per grain basis as well as percent fresh weight is shown. In the 1970s, cereal chemists decided that different stages of kernel development are more clearly expressed by data based on mg seed^{-1} rather than % of dry weight because the dry matter of the developing grain changes continuously tending to hide significant metabolic events.[19–21] The reason for also showing data based on % of fresh weight is that concentration is determined by NIR spectroscopy through Beer–Lambert law[22] (i.e. there is a linear variation of band intensities with analyte concentration).

Three stages of grain development could be recognized in Figures 1(a) and 1(c): the first (between 15 and 23 DAF), in which a rapid increase both in moisture and dry weight content took place; the second (between 23 and 38 DAF), in which there was no significant net deposition of water into kernels, but a continuous, linear dry matter accumulation occurred; the third (between 38 and 53 DAF), in which the amount of dry matter remained constant but there was a

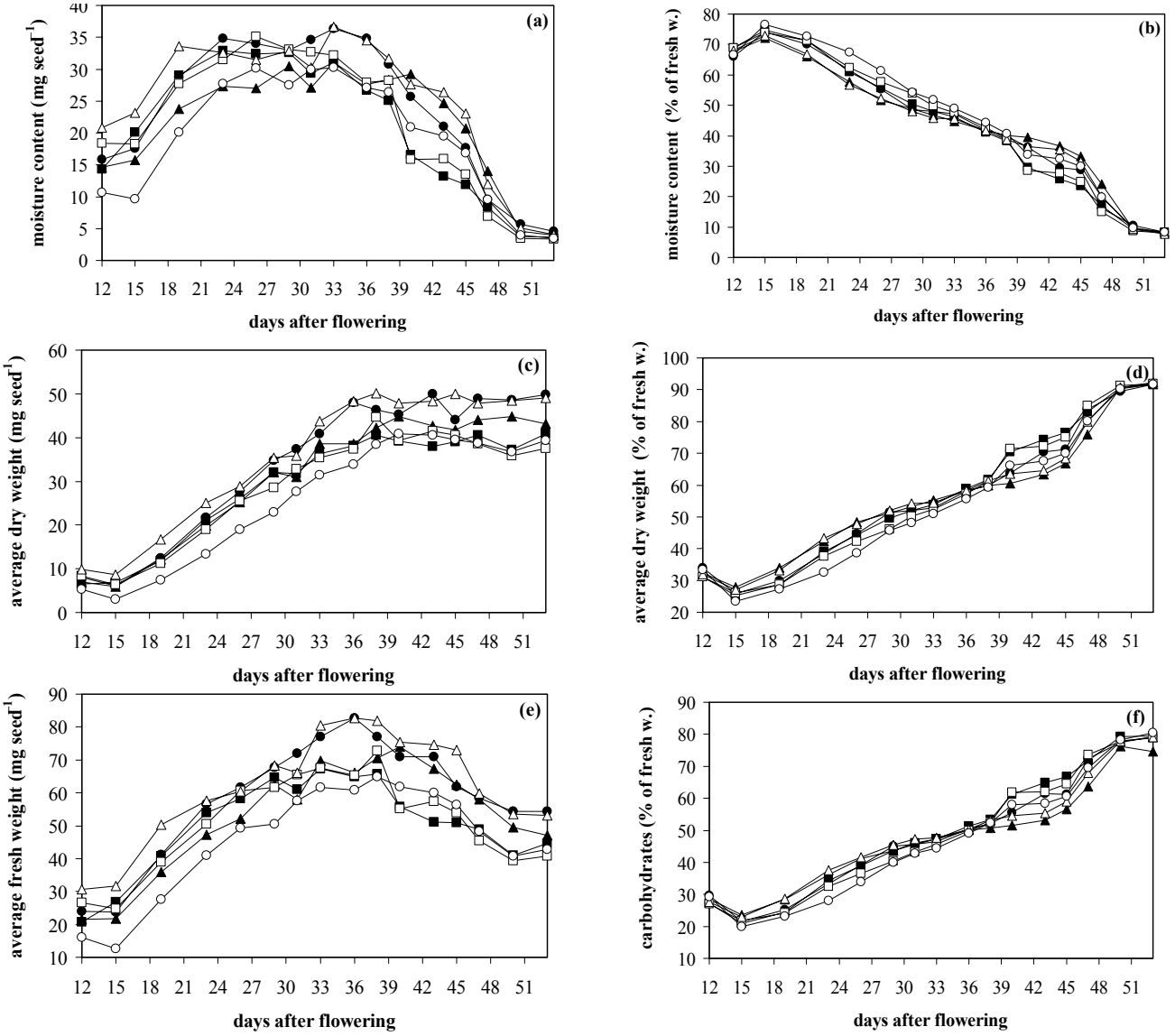

Figure 1. Changes in composition for seed samples of different wheat varieties during maturation; (a) moisture content (mg seed^{-1}), (b) moisture content (% of fresh weight), (c) average dry weight (mg seed^{-1}), (d) average dry weight (% of fresh weight), (e) average fresh weight (mg seed^{-1}), (f) carbohydrates (% of fresh weight). ■=GK Öthalom, ▲=Bánkúti 1201, ●=Jubilejnaja 50, □=Mv 23, △=Fatima, ○=Mv 15.

continuous decrease in moisture content.[3,23–27] These trends are summarized in the fresh weight curves [Figure 1(e)].[27]

Moisture content, as percent of fresh weight [Figure 1(b)], decreased in a linear fashion throughout the period of measurement in accordance with published data.[25] The plot of dry weight content based on percent fresh weight shown in Figure 1(d) illustrates the limitations of such a plot in terms of identifying aspects of plant physiology. Taking into consideration that the wheat seed is a solution, the concentration of dry matter depends not only on the absolute amount of the dry material (as dissolved material), but also on the quantity of moisture (as solvent). The different dynamics of these two components [see Figures 1(a) and 1(c)] results in an apparent linear increase in dry weight as percentage of fresh weight [Figure 1(d)]. In the first stage of grain development (15–23 DAF), dry weight expressed as concentration gradually increases, because the rate of dry matter accumulation is higher than the rate of water deposition. In the second phase (23–38 DAF), an increase in dry matter expressed as concentration is still present due to the amount of moisture remaining relatively constant while dry matter continues to deposited. In this third and final phase (38–53 DAF), an increase in concentration is caused by the drying process: i.e. the dry weight is relatively constant while there is a continual decrease in moisture content. The curves shown in Figure 1(f) relate to calculated values for carbohydrate concentration, as mentioned above in the Experimental section, so in order to extract the information regarding more specific to different carbohydrates (for example starch and water-soluble carbohydrates), NIR spectra were analysed.

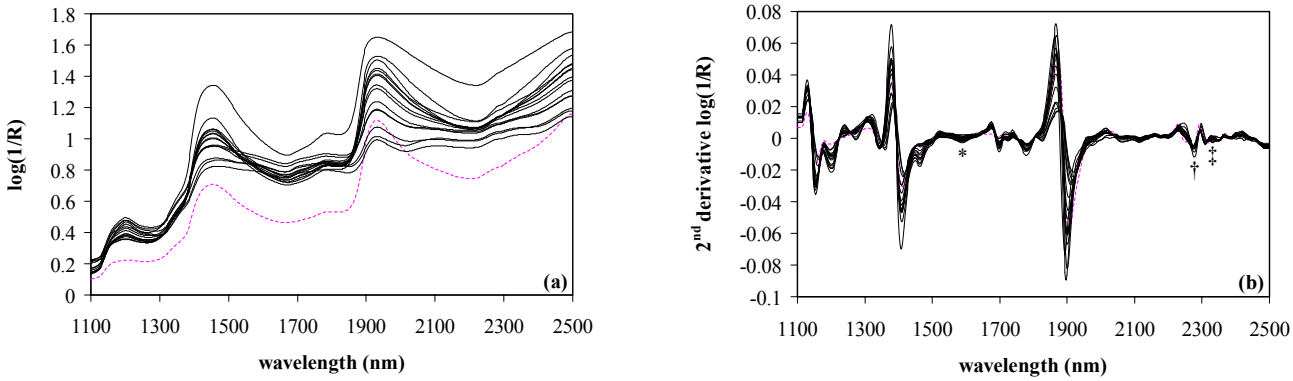

Figure 2. Average spectra of wheat seed samples (Jubilejnaja 50) during maturation; (a) raw spectra, (b) second derivative spectra. broken line = samples of 12 DAF (immature kernels with their bracts); * = Carbohydrate I, † = Carbohydrate II, ‡ = Carbohydrate III.

Reflectance spectra of maturing seed samples showed very high variability throughout the whole wavelength range [Figure 2(a)]. This variability remained, even after transformation (calculation of second derivatives) of raw spectra [Figure 2(b)]. Significant regions were observed in the spectra where the changes of concentrations of main constituents could be followed but, in this paper, only the status and changes of carbohydrate peaks were analysed in detail. The wavelength regions identified as relating to carbohydrates were those between 1195 and 1205 nm, 1585 and 1595 nm, 1695 and 1700 nm, 1775 and 1780 nm, 2110 and 2120 nm, 2195 and 2200 nm, 2270 and 2280 nm and 2325 and 2335 nm respectively. The cut-off wavelengths for the wavelength regions that encompass the carbohydrate bands were specified based on the local minimum values of D2OD spectra of maturing wheat.

Because of the complexity of NIR spectra, the most characteristic absorption bands of carbohydrates were selected using the spectra of main components of developing wheat [wheat starch, wheat gluten and water (see Figure 3)] and the spectra of the dilution series of selected mono- and oligosaccharides [fructose, glucose and sucrose (see Figure 4)] with the aim of eliminating the overlapping effects. Unfortunately, in wheat spectra it is difficult to interpret the variation in many D2OD peaks because the changes can be due either to starch, to gluten or even a starch–gluten interaction[11] and, in most cases, the characteristic bands of starch and gluten overlapped (for example the wavelength regions from 1195 to 1205 nm and from 1695 to 1700 nm due to C–H stretching, second and first overtone, respectively).[22] Similarly, during maturation, the starch peak from 1775 to 1780 nm is hidden by O–H bending and asymmetric stretching band of water.[28] In the case of wheat starch, a double peak could be seen at 2093 and 2110 nm, broadening the band centred at 2100 nm[29,30] due to the combination of O–H deformation and C–O stretching.[22] This very wide band is often used for determining the starch content of a product,[31] but amylose and amylopectin, the constituents of starch, also show differences in absorption at this point in the spectrum (Figure 5) and it is known that the amylose content of wheat varies from 17% to 29% with an average of 22–25%.[16] Wheat gluten proteins also have a characteristic band at 2110 nm,[5,13,32] presumably due to an amino group in side chains of amino acids (for example, wheat glutens have high amounts of glutamine and proline[33] as well as the lysine,[34] which is a limiting factor in nutrition) as a result of N–H symmetric stretching.[22] This interference from proteins in measurement of starch is found mainly at the onset of

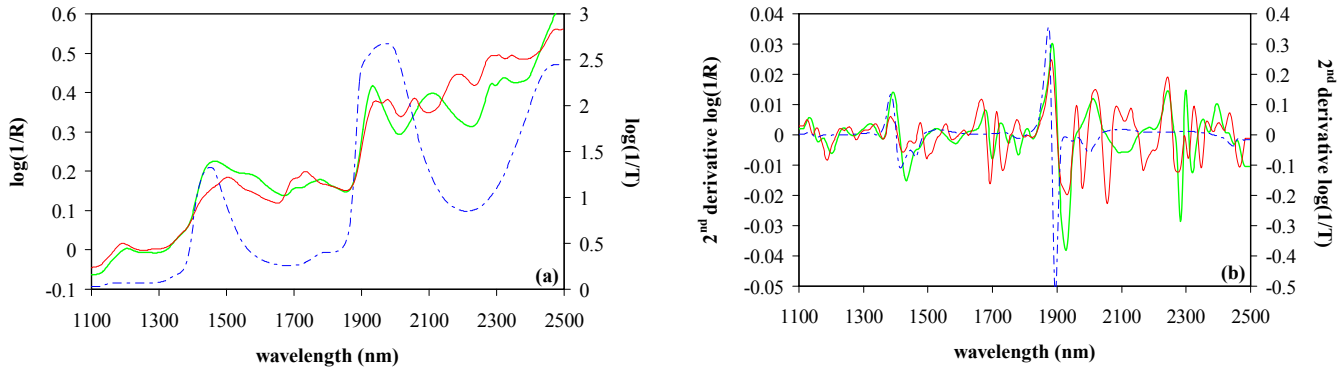

Figure 3. Average spectra of main components of developing wheat; (a) raw spectra, (b) second derivative spectra. thick line = wheat starch, thin line = wheat gluten, dotted line = water.

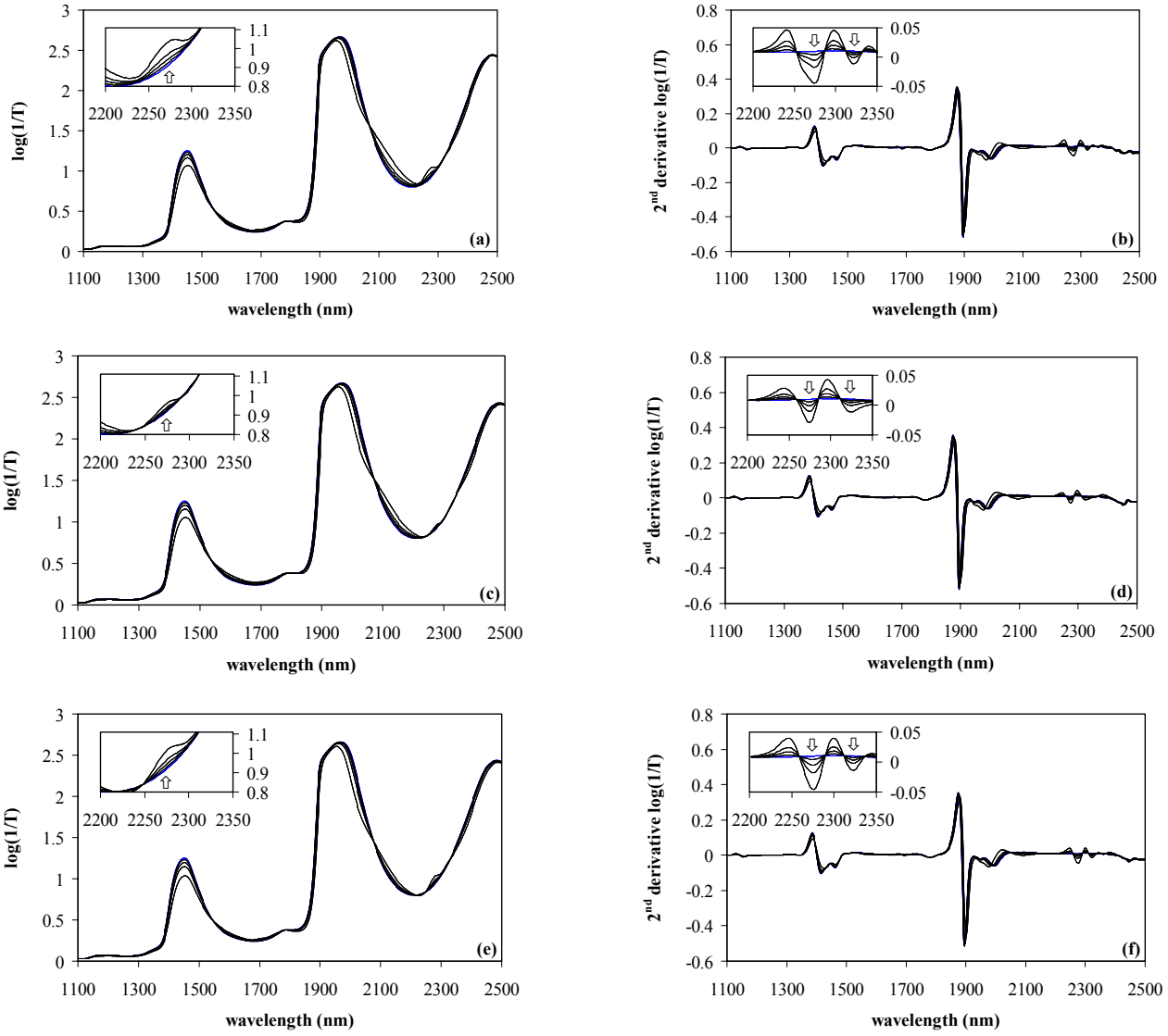

Figure 4. Average spectra of the monocomponent dilution series of some mono- and oligosaccharides; (a) fructose, raw spectra, (b) fructose, second derivative spectra, (c) glucose, raw spectra, (d) glucose, second derivative spectra, (e) sucrose, raw spectra, (f) sucrose, second derivative spectra. The concentration levels of 0, 40, 100, 200 and 400 g L^{-1} follow the arrow in enlarged pictures on the top-left corner.

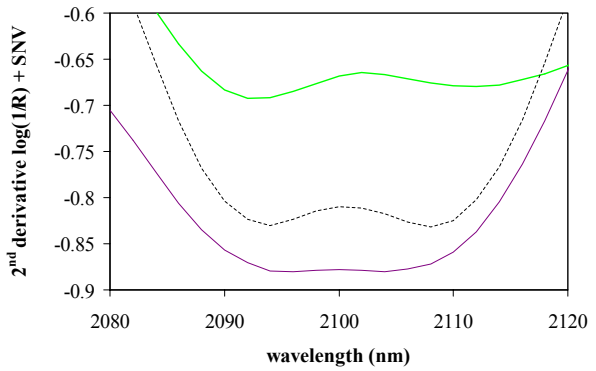

Figure 5. Second derivative spectra of starch and its constituents after standard normal variate (SNV) transformation. thick line = wheat starch, thin line = amylose from potatoes, broken line = amylopectin from potato starch.

protein synthesis where amino groups are present not only in side chains but also at the terminal ends of free amino acids. Starch, gluten, proteins and probably water may all be responsible for changes of absorption band between 2195 and 2200 nm in spectra of developing grain.[32,34]

Because of the problems listed above, three characteristic carbohydrate absorption bands were selected for study. These were the wavelength regions (a) between 1585 and 1595 nm (labelled Carbohydrate I), (b) from 2270 to 2280 nm (Carbohydrate II) and (c) from 2325 to 2335 nm (Carbohydrate III).

Over the last few years, plant physiologists have developed a model that describes the changes that occur to different sugars in wheat seeds during maturation. The amount of water-soluble carbohydrates (monosaccharides, sucrose and fructans) increases to a great extent immediately after the onset of anthesis showing a pronounced maximum at

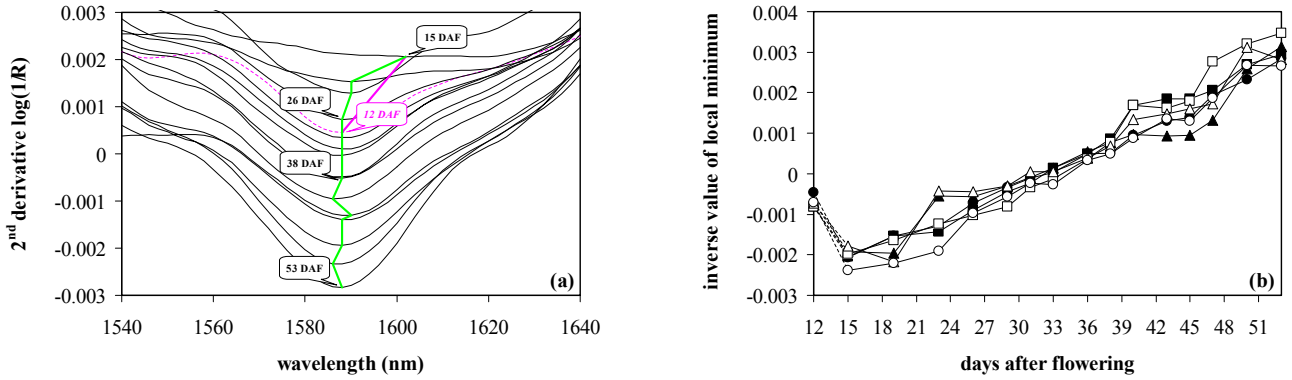

Figure 6. (a) Changes of Carbohydrate I peak in second derivative spectra of wheat seed samples (Jubilejnaja 50) in the 1585–1595 nm region during maturation. broken line = samples of 12 DAF (immature kernels with their bracts). (b) Changes of inverse value of local minimum of Carbohydrate I peak of wheat seed for six varieties during maturation. ■ = GK Öthalom, ▲ = Bánkúti 1201, ● = Jubilejnaja 50, □ = Mv 23, △ = Fatima, ○ = Mv 15.

about 12/14 DAF; thereafter, a progressive decrease occurs until 26/28 DAF then the sugar pool is maintained at a relatively constant level, which is necessary for continued starch synthesis.[3,19–21,24,27,35,36] The aim of this study is to determine whether the bands selected can be used to follow these changes in carbohydrate chemistry during the process of grain maturation.

Figure 6(a) shows second derivative values for the Carbohydrate I absorption band (between 1585 and 1595 nm) for the Jubilejnaja 50 wheat variety measured over the period 12 to 53 DAF. The line connecting the local minima for the Carbohydrate I peak showed a characteristic trend over time that related well to the trends seen in Figures 1(d) and 1(f). During the period 12 to 15 DAF, the D2OD values declined as did both average dry weight and carbohydrate content expressed as a percentage of fresh weight; thereafter, there was a period where there was a continuous increase in concentration in this narrow wavelength range matching increases in carbohydrate content and average dry weight. Figure 6(b) summarises, for all wheat varieties, the magnitude of the negative peak for carbohydrate band I during maturation. The correlation between the carbohydrate concentration [Figure 1(e)] and the inverse of D2OD values for the local minimum of the Carbohydrate I band [Figure 6(b)] was high (R^2 = 0.931–0.974 for six varieties). This was the highest correlation for any wavelength region between carbohydrate concentration and D2OD. The D2OD spectrum of wheat starch contains an absorption band at 1585 nm due to first overtones of the O–H stretching vibrations arising from hydrogen bonding.[8,31] This peak is known to characterise the intermolecular hydrogen-bonded hydroxyl groups in starch, which may be used to measure structural changes such as starch damage.[8,22] In our study, instead of starch damage, the opposite phenomenon (i.e. starch formation) can be detected during maturation. Starch synthesis proceeds rapidly in endosperm cells soon after fertilization and starch content increased almost linearly after anthesis.[35,36] A wheat endosperm has large A- and small B-type starch granules and these are formed at approximately 4 and 14 DAF respectively. After initiation, both types of granules persist and grow

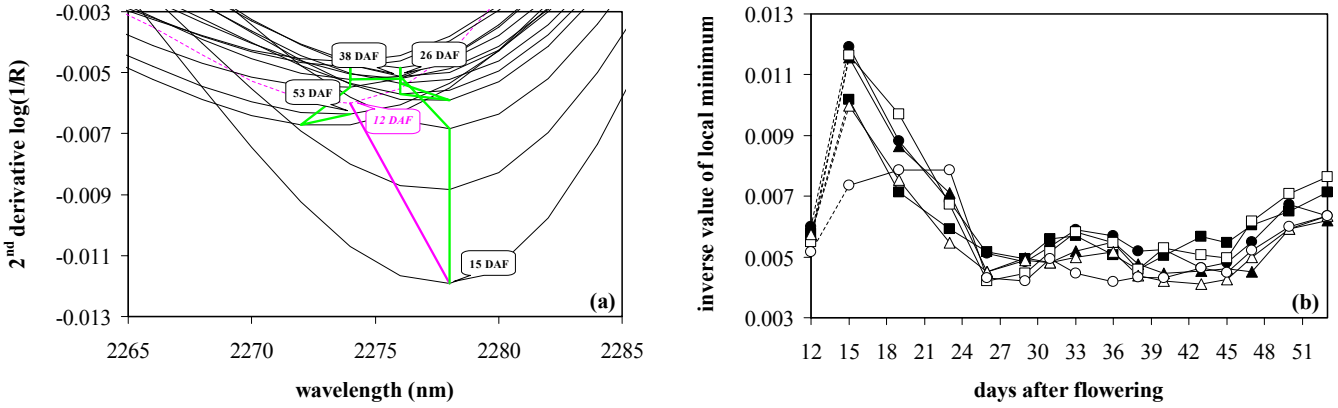

Figure 7. (a) Changes of Carbohydrate II peak in second derivative spectra of wheat seed samples (Jubilejnaja 50) in the 2270–2280 nm region during maturation. broken line = samples of 12 DAF (immature kernels with their bracts). (b) Changes of inverse value of local minimum of Carbohydrate II peak of wheat seed for six varieties during maturation. ■ = GK Öthalom, ▲ = Bánkúti 1201, ● = Jubilejnaja 50, □ = Mv 23, △ = Fatima, ○ = Mv 15.

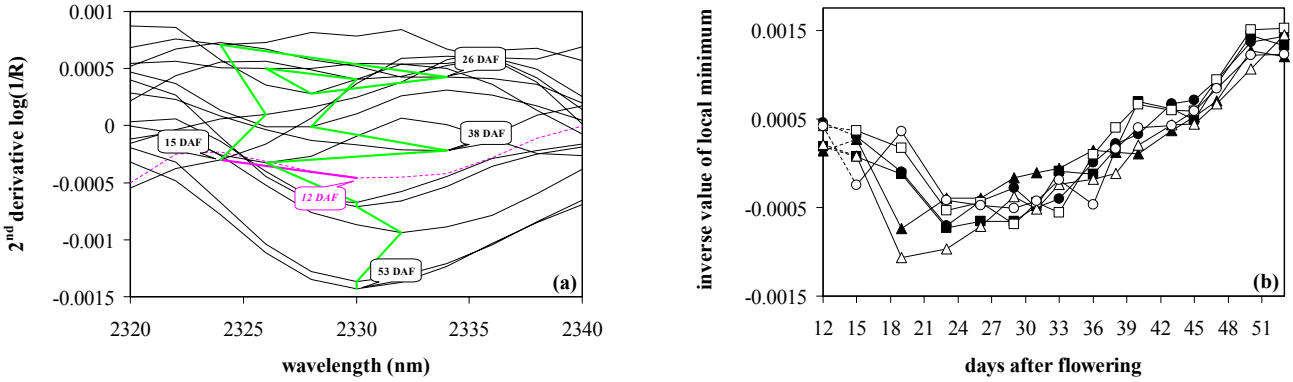

Figure 8. (a) Changes of Carbohydrate III peak in second derivative spectra of wheat seed samples (Jubilejnaja 50) in the 2325–2335 nm region during maturation. broken line = samples of 12 DAF (immature kernels with their bracts). (b) Changes of inverse value of local minimum of Carbohydrate III peak of wheat seed for six varieties during maturation. ■ = GK Öthalom, ▲ = Bánkúti 1201, ● = Jubilejnaja 50, □ = Mv 23, △ = Fatima, ○ = Mv 15.

until maturity.[37] At first, the high moisture environment in the wheat seed supports the formation of a weak association of starch molecules within the granules, which then become more closely associated as moisture content decreases during the ripening process. Water-binding capacity declines as the grain matures,[38] demonstrating a progressively closer association of the starch molecules in the granules, thus reducing the accessibility of free hydroxyl groups for hydrogen bonding to water and simultaneously augmenting the amount of intermolecular hydrogen-bonded hydroxyl groups in starch.

Changes in another characteristic carbohydrate band (Carbohydrate II, between 2270 and 2280 nm) during maturation are shown in Figure 7. Spectra for the Carbohydrate II band [Figure 7(a)] relating to the early phase of maturation (15–26 DAF) showed changes, not only in the magnitude of the D2OD local minimum, but also shifts in wavelength. This shift was greatest from 12 DAF, where the seeds were so small that they had to be prepared together with their bracts (palea and lemma), to 15 DAF, the spectrum of the first "true" seeds. The period that followed (16–26 DAF) is characterised by a reduction in water-soluble carbohydrate content and the inverse value of local minimum of Carbohydrate II band [Figure 7(b)], matched the trend during this period.

The differences between varieties were not significant; only the variety Mv 15 showed a retarded synthesis during early as well as the late phases of maturation. Water-soluble carbohydrates such as fructose, glucose and sucrose possess distinct absorption bands around 2275 nm[7] due to combinations of O–H stretching and C–C stretching vibrations[22] within this combination region of the NIR spectrum. This clear and consistent peak, which diminished gradually during ripening, was assumed to associate with non-starchy polysaccharides and soluble pentosans.[13] Sugars make a significant contribution to the dry weight of grains during the early stages of seed development.[20,35,36] Because the accumulation of water-soluble carbohydrates raises the osmotic pressure in the young seed cells, there is a consequent influx of water that swells the cells beyond their normal capacity.[39,40] Rising starch synthesis in endosperm cells then produces a fall in the sugar content (15–26 DAF) but the existence of significant amounts of water-soluble carbohydrates in wheat seeds even when starch accumulation has declined (26–38 DAF) suggests that the supply of sugar precursors does not limit the process of starch synthesis.[3,39,40]

Changes in the third carbohydrate peak (Carbohydrate III, between 2325 to 2335 nm) are shown in Figure 8. Here, a segment size of 4 nm was selected to enhance separation of absorption bands within the triple peak from 2300 to 2370 nm [Figure 8(a)]. This carbohydrate peak shares many of the characteristics observed at the Carbohydrate I and Carbohydrate II peaks. Changes in absorbance maximum [Figure 8(b)] after 24 DAF are similar to those seen for Carbohydrate I [Figure 6(b)] while, during the period 15–24 DAF, there are similarities to Carbohydrate II [Figure 7(b)]. The Carbohydrate III band probably relates to the bond vibration of the C–H stretch and C–H deformation combination.[6,7,22,31]

The results presented in this paper suggest that the three carbohydrate bands identified in the spectra of developing seeds can be used to monitor changes in chemical composition of samples and/or the interactions between components such as starch and soluble carbohydrates, and the changes in absorption generally follow accepted models of seed development. The responses seen in the Carbohydrate I peak can be related unambiguously to starch accumulation because this band relates to vibrations of intermolecular hydrogen-bonded O–H groups in polysaccharides, mainly in starch or its constituents (amylose and amylopectin). In the case of the Carbohydrate II peak, there was an opposite phenomenon: the combination of O–H stretching and C–C stretching vibrations reflected the absorption capacity of dissolved water-soluble carbohydrates (monosaccharides, sucrose and fructans) much better because of the relative independence of molecule groups in a dilute environment than the effect of intermolecularly-bonded, rigid polysaccharides produced during programmed cell death of endosperm cells. Probably the sensitivity of Carbohydrate III to both sugars and polysac-

charides (due to the combination of C–H stretching and C–H deformation vibrations) could be explained by the presence of C–H groups in carbohydrates, which presumably were less susceptible to the interactions in a continuously changing environment found in wheat seed during maturation.

In the introduction, we listed many applications of NIR to measure starch or changes to starch in different processes. In the human diet, starch forms a significant source of energy (in Europe and North America mainly as bread and pasta products produced from milled wheat seeds contributing, on average, approximately 30% of the UK diet by weight,[4]) so monitoring changes of starch content in developing grain could be important. There is, however, another potential application of NIR measurement of wheat seeds during maturation. Immature wheat grains have, among other water-soluble carbohydrates, high levels of fructose and its oligomers, as well as a more balanced amino acid composition than do mature seeds. These properties could suggest the utilisation of this material as a functional food[41] and NIR would allow optimisation of the harvest of immature plants by detecting levels of water-soluble carbohydrates and amino acids.[42]

Conclusions

The results presented in this paper confirm that there are many absorption bands in NIR spectra that can be sensitive indicators of the changes that occur to different carbohydrates (for example, starch and water-soluble carbohydrates) during maturation. Spectroscopic methods offer the opportunity, and potentially the ability, to detect fine details of physiological processes. The spectra have many hidden details that can help us to understand the biochemical background of processes in intact biological samples such as the maturing wheat seed.

Acknowledgements

The authors gratefully acknowledge Ian A. Cowe (Foss Analytical) for his invaluable help in revising the manuscript. Special thanks are due to László Láng (ARIHAS) for organising the plant trials. This work was supported by National Foundation of Science and Research Hungary project numbers: T 031902 and A 143; by National Research and Development Project Hungary project numbers: 4/035/2001.

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Received: 18 June 2004
Revised: 22 March 2005
Accepted: 3 May 2005
Web Publication: 23 May 2005