

# Relationship Between NIR Spectra and RVA Parameters During Wheat Germination

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## ABSTRACT

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The process of germination in six different wheat cultivars was monitored using NIR spectroscopy and the Rapid Visco Analyser (RVA) method. Near-infrared spectra provided insight into both chemical and physical changes that occur in the seed, in particular mobilization processes involving carbohydrates. RVA curves also contain physical and chemical information and can be interpreted as physicochemical spectra. The process of germination was followed sensitively through the RVA curves and some rheological parameters (peak viscosity, trough, breakdown, final viscosity, and setback) were highly correlated ( $R = 0.95\text{--}0.98$ ) with

predicted values calculated from NIR spectra. Viscosity data calculated from RVA curves collected at 200–480 sec showed the most characteristic changes during the early heat treatment stage of the pasting procedure. Strong intercorrelations were found between viscosity data and NIR spectra from the beginning of the swelling and gelatinization processes in germinating seed. The NIR and RVA methods were sensitive tools for the rapid investigation of the germination process, which is important both from a physiological and technological point of view.

The processes that occur during the germination of seed play a central role in the propagation of cereal crops, influencing both the compositional and functional quality of cereals. This series of physiological changes can influence subsequent technological processes (e.g., malting) and can cause processing problems if preharvest sprouting occurs (Hareland 2003). During normal development, embryos complete germination several days before maturity (Golovina et al 2001). The viability of seed can also be influenced by different aging conditions and processes (temperature, humidity) (Galleschi et al 2002).

The germination process is hormonal- and enzyme-stimulated (Kuo et al 1996) and regulates mobilization of seed reserves. The biochemical, morphological, and hormonal changes during germination have been reviewed by Fincher (1989) and Simon (1984).

Hormones produced by the embryo activate cells in the aleurone layer to rapidly synthesize and secrete hydrolytic enzymes that catalyze the degradation of storage reserves in the cells of the starchy endosperm (Jones and Jacobsen 1991). The enzymes produced by the aleurone layer and scutellum are produced either by activation of preformed enzymes or by de novo synthesis.  $\alpha$ -Amylases,  $\alpha$ -glucosidases, debranching and cell-wall-hydrolyzing enzymes (different  $\beta$ -glucanases, xylanases) are all released from the aleurone layer and migrate to the endosperm where polysaccharide reserves are degraded. Once  $\beta$ -amylases are activated, glucose is released and absorbed by the scutellum (Bewley and Black 1985; Corder and Henry 1989; Beldman et al 1996).

Different types of endopeptidases (Bottari et al 1996; Fontanini and Jones 2001) and exopeptidases (Dominguez et al 2002) are synthesized or activated during germination. The bulk of proteolytic activity ( $\approx 90\%$ ) is due to cysteine proteinase (Capocchi et al 2000) and, to a lesser extent, to aspartic proteinase and carboxypeptidases. The gluten proteins (protein reserves) are mainly degraded by cysteine proteinases whose activity is regulated by their own concentration and by thioredoxins in vivo (Marx et al 2003). Regulation and control processes of wheat seed reserve mobilization have been reviewed by Bewley and Black (1985).

Pregermination (sprouting) of wheat can also occur during the preharvest period, causing reduced grade and inferior quality of seed, and resulting in large economic losses. The biochemical, compositional, and functional changes that occur in sprouted wheat

can be determined by enzymatic (Kruger and Marchylo 1972; Salgó and Feller 1987; Kruger and Hatcher 1993), immunological (Verity et al 1999), and physical-rheological (Finney 2001; Hareland 2003) test methods.

Nondestructive near-infrared (NIR) methods (Osborne 1996; Pawlinsky and Williams 1998; Alava et al 2001; Gergely and Salgó 2003) and Raman spectroscopic methods (Piot et al 2000, 2002; Ma and Phillips 2002) have been used to measure the compositional and structural (morphological) changes in wheat seed. The effects of sprouting on wheat quality can also be evaluated using the Rapid Visco Analyser (RVA) (Ross et al 1987; Walker et al 1988). In addition, amylase activity (Collado and Corke 1999), endoproteolytic enzyme activity (Bleukx et al 1999), particle size effect (Becker et al 2001), and fine structure of amylopectin (Han and Hamaker 2001) can be measured using RVA viscosity profiles.

Several workers have examined the relationship between NIR spectra and RVA values. Shashikumar et al (1993), working with wheat, showed that correlations between NIR predictions of sprout damage (using both falling number and RVA as reference values) were fairly good ( $R = 0.75\text{--}0.87$ ). On the other hand, Meadows and Barton (2002) and Delwiche et al (1996) reported that NIR spectra did not correlate with the traditional RVA parameters in rice. Wesley and Blakeney (2001) used the relationship between NIR predictions and RVA values in starch-protein-water mixtures to follow the gelatinization process in real time. The 1160-nm combination band of water is strongly associated with gelatinization and strongly interacts in the protein-starch-water system.

The aim of present study was to investigate the relationship (intercorrelation) between NIR and RVA characteristics of wheat seeds germinated under controlled conditions.

## MATERIALS AND METHODS

### Samples

Six bread wheat (*Triticum aestivum*) cultivars (Bánkúti 1201, Fatima 2, Jubilejnaja 50, Martonvásári 20, Martonvásári 23, and GK Ótthalom) grown in Hungary in 2002 were used in the study.

For each cultivar, 150 g of intact wheat grain was soaked in tap water for 2 hr at room temperature. The soaked samples were then germinated in the dark for 4, 8, 12, 24, 48, 72, and 96 hr at 20°C on filter paper soaked with 300 cm<sup>3</sup> of water. Superficial water present on the grains after sampling was removed with filter paper. Wheat samples after germination were measured by near-infrared spectroscopy immediately as wet samples. After NIR measurement, samples were oven-dried (120°C for 2 hr) to constant weight.

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Dried, sprouted grains were then ground using an experimental micromill developed in Hungary (FQC 2000, Metefém Co. Ltd., Budapest) and passed through a 0.5-mm sieve.

### NIR Analysis

Dry, ground wheat samples with particle size <0.5 mm were measured in duplicate using the repack method. An analytical near-infrared reflectance spectrometer (FOSS-NIRSystems model 6500 monochromator system, Silver Spring, MD) fitted with a sample transport module using standard sample cups was used for the reflectance measurements (reflectance mode: PbS detector). Spectral data were recorded from 1100 to 2498 nm at 2-nm intervals (700 data points per spectrum) and stored as the average of 32 scans for each sample.

Partial least squares (PLS) calibrations were developed using NIRSystems Spectral Analysis software (NSAS, v. 3.53) using RVA parameters and RVA data points (viscosity values at a constant time-temperature) as reference data. Calibrations based on the full NIR spectral range were developed for raw spectra and also for 2nd derivative spectra. The raw spectra were transformed into 2nd derivatives using a 10-nm segment and 0-nm gap size.

### RVA Measurement

Rheological properties of samples were measured using the Rapid Visco Analyser (Newport Scientific RVA-4SA) controlled by ThermoLine for Windows software (v. 2.2, Newport Scientific Pty Ltd., Warriewood, NSW, Australia). Standard 1 measurement profile (ICC Standard Method No. 162) was used. The viscosity was recorded in cP units (1 cP = 1 mPa sec<sup>-1</sup>).

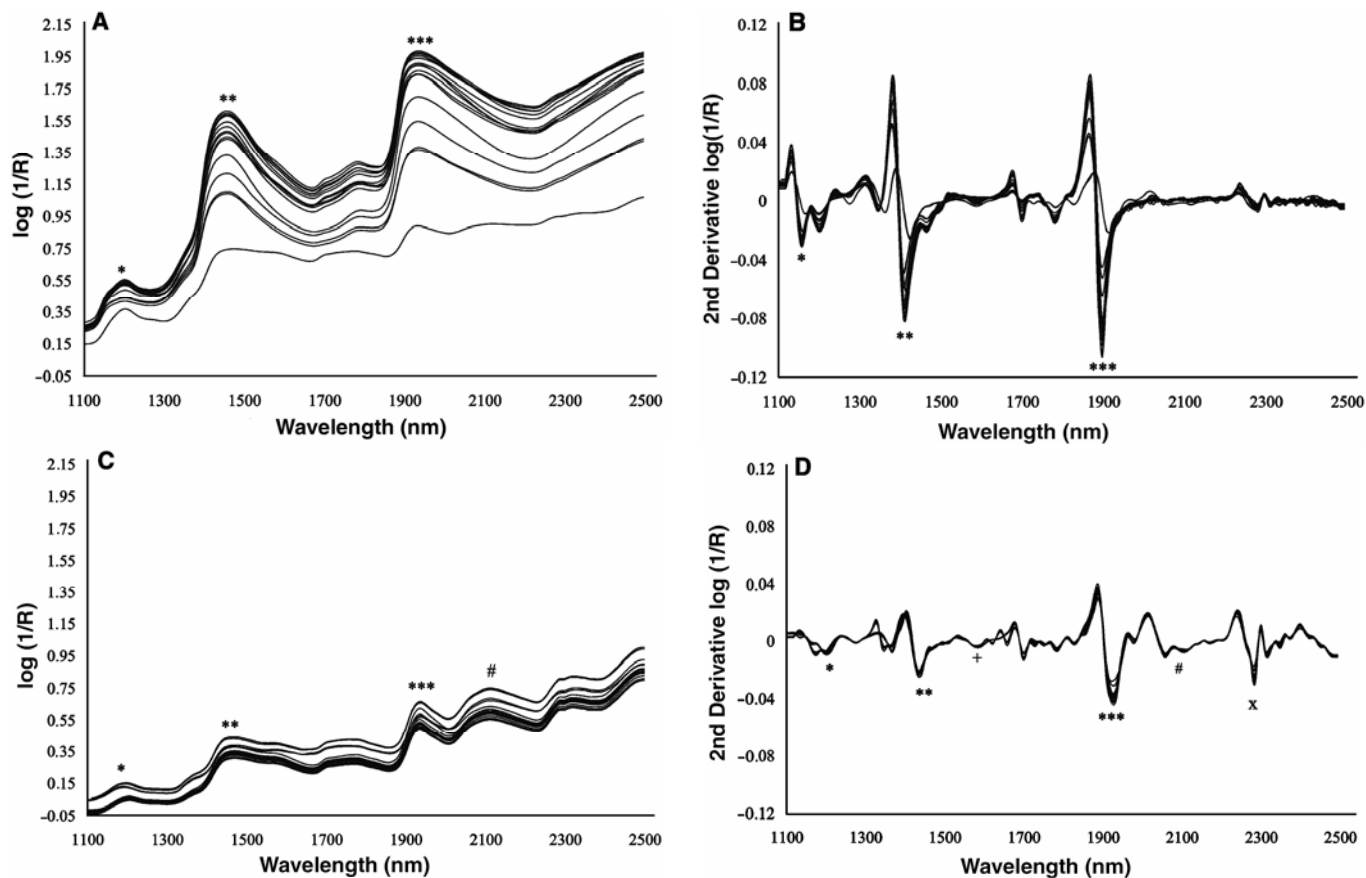
The sample (3.50 g of ground wheat with particle size <0.5 mm) was dispersed in distilled water (25.0 mL) and stirred in an

RVA canister at 960 rpm for 10 sec, then at 160 rpm for the remainder of the test. The standard temperature profile stages were 1) hold for 1.00 min at initial temperature (50°C); 2) heat to 95°C over 3.42 min; 3) hold at 95°C for 2.30 min; 4) cool to 50°C over 3.48 min; and 5) hold at 50°C for 2.00 min. The total test time was 13.00 min. The interval between viscosity and temperature readings was 4 sec (195 data points per RVA curve).

Values measured from the pasting profile were 1) peak viscosity (maximum paste viscosity achieved in stage 2, the heating stage of the profile); 2) trough (minimum paste viscosity achieved after holding at the maximum temperature, stage 3); 3) final viscosity (viscosity at the end of run); 4) pasting temperature (temperature at which starch granules begin to swell and gelatinize due to water uptake and defined as an increase of 25 cP over a period of 20 sec); 5) peak time (time at which peak viscosity was recorded); 6) breakdown (difference between peak viscosity and trough); and 7) setback (difference between final viscosity and trough). The raw RVA curves were transformed into 1st derivatives by taking differentials for each data point of the spectra using Microsoft Excel 2000 software.

## RESULTS AND DISCUSSION

The characteristic changes taking place in wheat seeds during germination could be observed in the NIR spectra (Fig. 1). Reflectance spectra of germinating seed samples (either wet or dry) showed a very high variation throughout the whole wavelength range (Fig. 1A,B). This variation remained high, even after transformation (calculation of 2nd derivatives) of raw spectra (Fig. 1C,D). All the relevant absorption regions or bands of macroconstituents (moisture, carbohydrates, proteins) showed characteristic



**Fig. 1.** NIR spectra of wheat samples (cultivar GK Ötthalom) during germination. **A**, raw NIR spectra, wet samples; **B**, 2nd derivative of NIR spectra, wet samples; **C**, raw NIR spectra, dry samples; **D**, 2nd derivative of NIR spectra, dry samples. \*, \*\*, \*\*\* Absorbance bands for moisture (1154–1166 nm, 1456–1472 nm, and 1932–1936 nm, respectively); +, absorbance bands for starch (1578–1580 nm); x, absorbance bands for mono- and oligosaccharides (2282 nm); #, absorbance bands for proteins (2056 nm, 2102–2108 nm).

changes. In Fig. 1A, the absorbance bands for moisture (1202 nm, 1456–1472 nm, and 1932–1936 nm) increase with water uptake over time. Similar changes can be seen in the 2nd derivatives (Fig. 1C). Dried samples (Fig. 1B,D) also showed changes in bands relating to carbohydrates and proteins (starch 1578–1582 nm, mono- and oligosaccharides 2282 nm, proteins 2056 and 2102–2108 nm).

NIR spectra of wet samples provide real-time analysis; they were measured immediately after sampling and are therefore suitable for in-field applications.

NIR spectra of dry samples were measured after the same sample preparation procedure as was used for RVA measurement. As expected, better correlations were observed between RVA parameters as reference data and NIR spectra of dry rather than wet samples. In addition, NIR spectra of dry samples can be evaluated more easily than those of wet samples because water peaks (Gergely and Salgó 2003) that mask the absorbance bands for proteins and polysaccharides are absent.

The changes taking place in wheat seeds during germination could be observed not only in NIR spectra but also in the RVA curves. Changes in RVA curves for wheat seed samples during germination are shown in Fig. 2. The characteristic shape of RVA curves for all cultivars investigated (there were differences only in the absolute viscosity values) were similar and therefore data is shown for only one cultivar (GK Ötthalom). The viscosity of the ground wheat-water suspension depends on temperature, amount of swelling, degree of gelatinization, and the degree of polymerization of storage compounds. Due to increased activity of amylase enzymes, the degree of starch polymerization in wheat seeds decreases continuously during germination (Fincher 1989). Thus, the pasting characteristic of ground, germinated wheat changes (the pasting curve flattens).

There were small differences in the shapes of RVA curves at the early stages of germination (0–6 hr) and more pronounced differences in the later phases of germination (10–98 hr). Degradation of starch in wheat seeds starts after a certain lag period. In ungerminated seeds, starch-degrading enzymes ( $\alpha$ -amylases,  $\beta$ -amylase, limit dextrinase, and  $\alpha$ -glucosidase) are present but their activity changes during germination. Starch granules are initially hydrolyzed by  $\alpha$ -amylase, which breaks the  $\alpha$ -1,4 glycosidic bonds between glucose molecules throughout the chains. Increasing  $\alpha$ -amylase activity during germination is a consequence of de novo synthesis of this enzyme (Bewley and Black 1985). Hydrolytic activity of  $\beta$ -amylase occurs only in the presence of  $\alpha$ -amylases (Maeda et al 1987). The activity of limit dextrinase shows a maximum after a prolonged period (Fincher 1989), while activity of  $\alpha$ -glucosidase increases rapidly during germination (MacGregor and Lenoir 1987). The differences between RVA curves for samples at different germination stages are due to these factors. It was difficult to read

the RVA parameters toward the end of germination (74–98 hr) because the characteristic form of the curves changed and became almost linear (Fig. 2).

Changes in RVA parameters during germination are shown in Fig. 3A–G. Pasting temperature is the temperature at which starch granules in the endosperm begin to swell and gelatinize due to water uptake, producing an increase in viscosity. Pasting temperature is influenced by the modification of starch. During germination, starch content of wheat seeds drops due to hydrolysis, producing a significant increase in pasting temperature (Fig. 3F). At the beginning of germination (0–6 hr), pasting temperature was  $\approx 65$ – $67^\circ\text{C}$  and then it rose to  $85^\circ\text{C}$ . At the end of germination (98 hr), gelatinization and pasting could not be observed, so it was impossible to read pasting temperature (data not shown). Peak time was relatively constant during germination (5.5–6.0 min) (Fig. 3G). However, at the later stages of germination, peak time seemed more variable, which was probably due to difficulties in reading the curves. The other RVA parameters show characteristic changes during germination that can be used as sensitive indicators in the detection of germination.

Peak viscosity, trough, final viscosity, and setback values (Fig. 3A,C,D,E) showed similar changes as a function of germination time with maximum values at 2–6 hr and with a marked tailing. The breakdown values (difference between peak viscosity and trough) decreased rapidly during the early phase of germination (0–6 hr) and it seems that early prediction of mobilization could be predicted using this parameter.

Correlation coefficients between predicted values from models based on full range NIR spectra and RVA parameters are summarized in Table I. Results indicated that the spectra of dry samples predict RVA parameters better than do the spectra of wet materials. Five RVA parameters (peak viscosity, trough, breakdown, final viscosity, and setback) showed high correlations with predictions from NIR data using either raw or 2nd derivative spectra. Peak time can also be predicted from NIR data, but the pasting temperature was not predictable with acceptable accuracy ( $R^2 = 0.31$ – $0.76$ ).

According to Shashikumar et al (1993), the RVA parameters obtained from the stirring number method (SN number, peak height, and final viscosity) can be predicted by NIR measurements with limited accuracy ( $R^2 = 0.56$ – $0.76$ ). RVA parameters using the RVA standard method seem more predictable by NIR during the germination process.

Meadows and Barton (2002) found that, in rice, traditional RVA parameters using the RVA standard method could be predicted by NIR fairly well, and that data points in RVA curves at a constant time-temperature correlated better with NIR spectra. The data points that showed maximum correlation ( $R$ ) were those in the gelatinization period of the RVA measurement (212–228 sec).

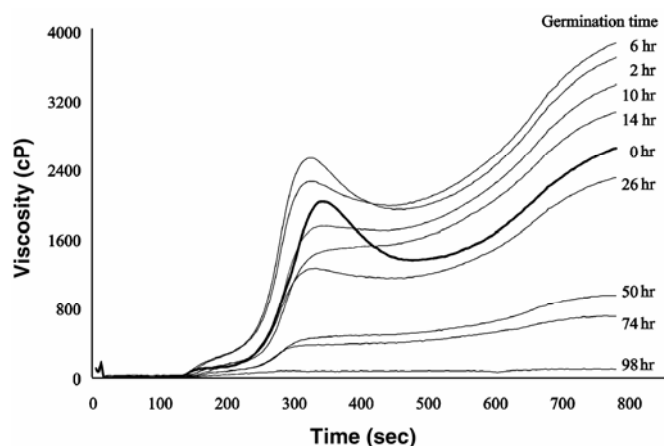


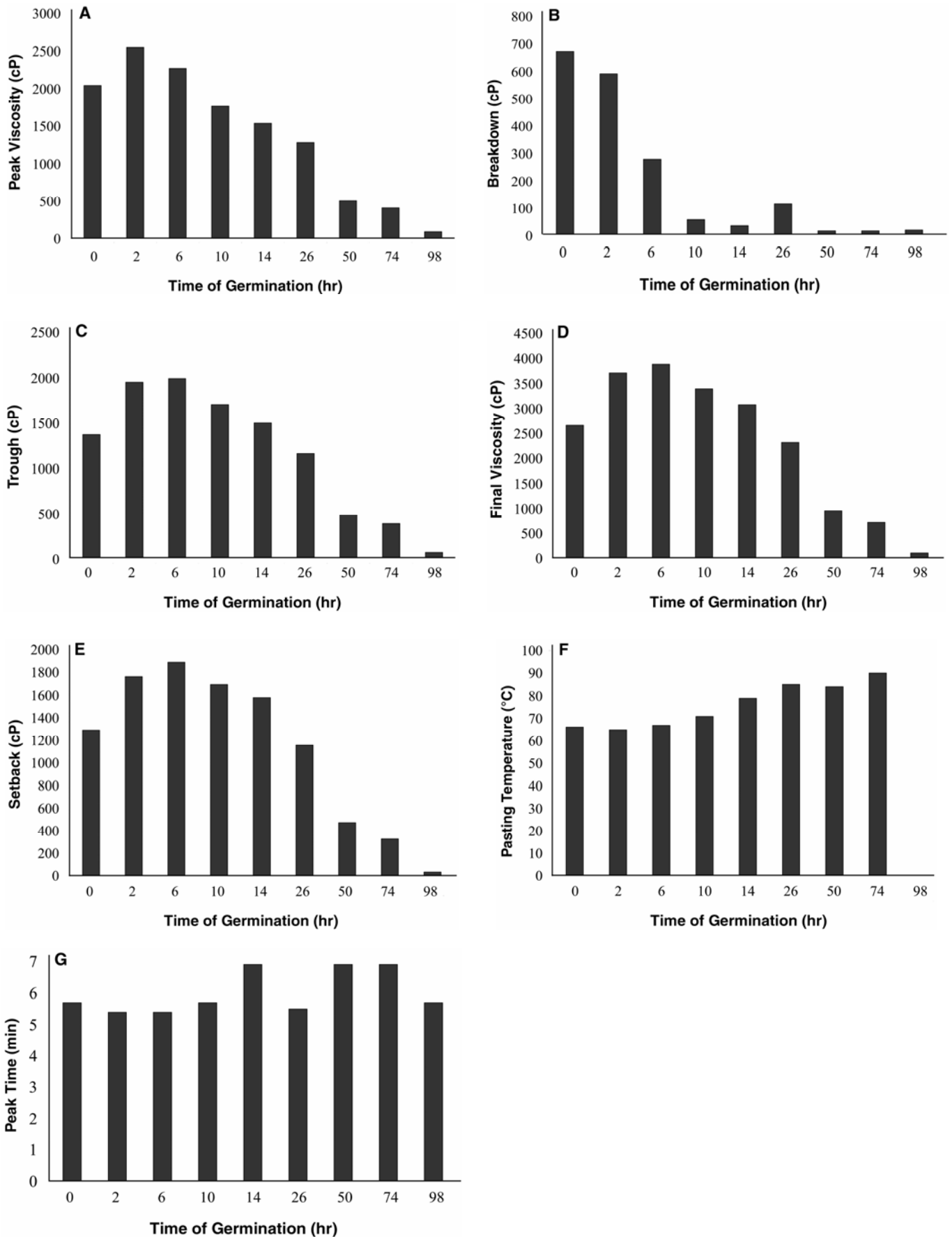
Fig. 2. RVA curves of ungerminated and germinated wheat samples (cultivar GK Ötthalom).

TABLE I  
Correlation Coefficients Between Predicted Values  
Based on Full Range NIR Spectra and RVA Parameters  
as Reference Data Calculated Using PLS

RVA Parameters <sup>a</sup>	NIR Spectra Parameters <sup>b</sup>			
	RW	DW	RD	DD
PV	0.93	0.91	0.97	0.97
TR	0.93	0.90	0.95	0.97
BD	0.91	0.86	0.98	0.97
FV	0.95	0.91	0.96	0.95
SB	0.96	0.93	0.97	0.96
PT	0.83	0.73	0.94	0.96
PAT	0.69	0.56	0.83	0.87

<sup>a</sup> PV, peak viscosity; TR, trough; BD, breakdown; FV, final viscosity; SB, setback; PT, peak time; PAT, pasting temperature.

<sup>b</sup> RW, raw NIR spectra, wet samples; DW, 2nd derivative NIR spectra, wet samples; RD, raw NIR spectra, dry samples; DD, 2nd derivative NIR spectra, dry samples.



**Fig. 3.** Change of RVA parameters during germination. **A**, peak viscosity; **B**, breakdown; **C**, trough; **D**, final viscosity; **E**, setback; **F**, pasting temperature; **G**, peak time.

Using wheat samples, we found good correlations between traditional RVA parameters and NIR spectra. However, based on the observation of Meadows and Barton (2002), we tried to improve intercorrelations by choosing new RVA parameters during the gelatinization period. For this evaluation, the RVA curves were transformed to 1st derivatives (Fig. 4). The most characteristic changes were observed at 200–480 sec, where the swelling and gelatinization processes were at their most intensive. The latter parts of the RVA curves (>550 sec) showed only slight variation. These results indicate that the temperature range causing gelatinization is broader in wheat when compared with rice, and all RVA data points showed high correlation coefficients ( $R^2 > 0.86$ ).

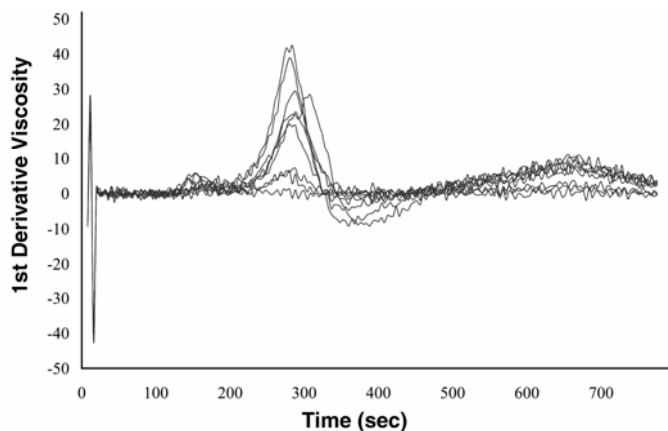
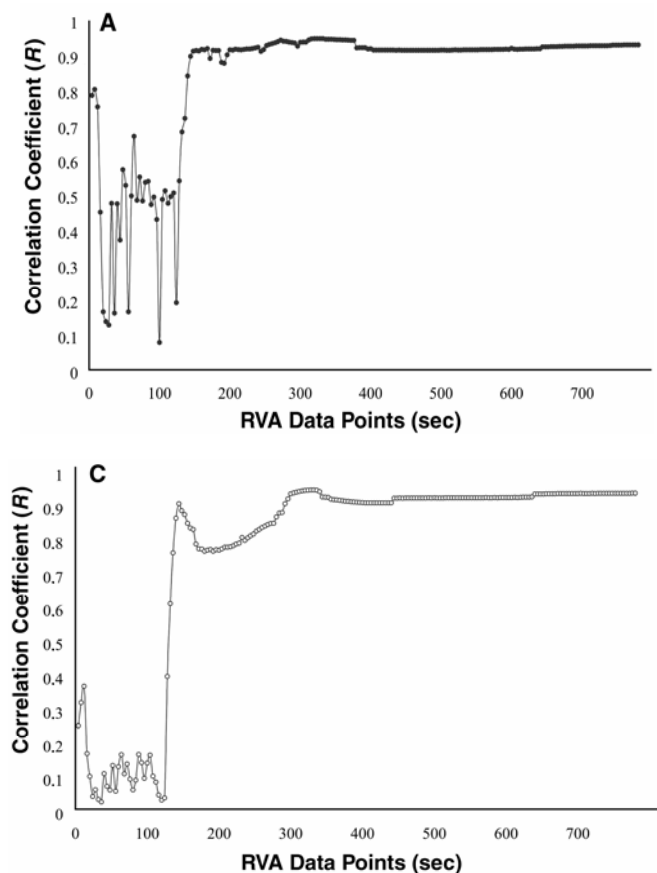


Fig. 4. 1st derivative of RVA curves of sound and germinated wheat samples (cultivar GK Óthalom).



Correlation coefficients between predicted values based on full range NIR spectra and each viscosity value from whole RVA curves were calculated for dry and wet samples using PLS as a calibration method. Figure 5A–D describes the linear correlation coefficients between NIR data and viscosity values over the whole RVA curve. Both raw and 2nd derivative spectra of dry samples (Fig. 5A,B) showed high correlation coefficients with viscosity values >150 sec where the gelatinization process started.

When correlations were calculated using spectra of wet samples (Fig. 5C,D), a characteristic decrease of  $R$  values was observed at 150–300 sec. Results indicated that the presence of water increased the variation of NIR spectra, and this variation remained high until the end of gelatinization ( $\approx 300$  sec) (Fig. 4), when the “free” water is no longer available.

## CONCLUSIONS

Seed germination processes can be sensitively followed using both the NIR and RVA methods. RVA parameters such as peak viscosity, trough, breakdown, final viscosity, and setback can be predicted from NIR spectra with acceptable accuracy and with good correlation coefficients ( $R = 0.95$ – $0.97$ ). High correlations exist between NIR spectra and viscosity values from the onset of swelling and gelatinization of starch. Results indicate good correlations between chemical and functional properties, thereby confirming that NIR spectra provide both chemical and physical information during germination.

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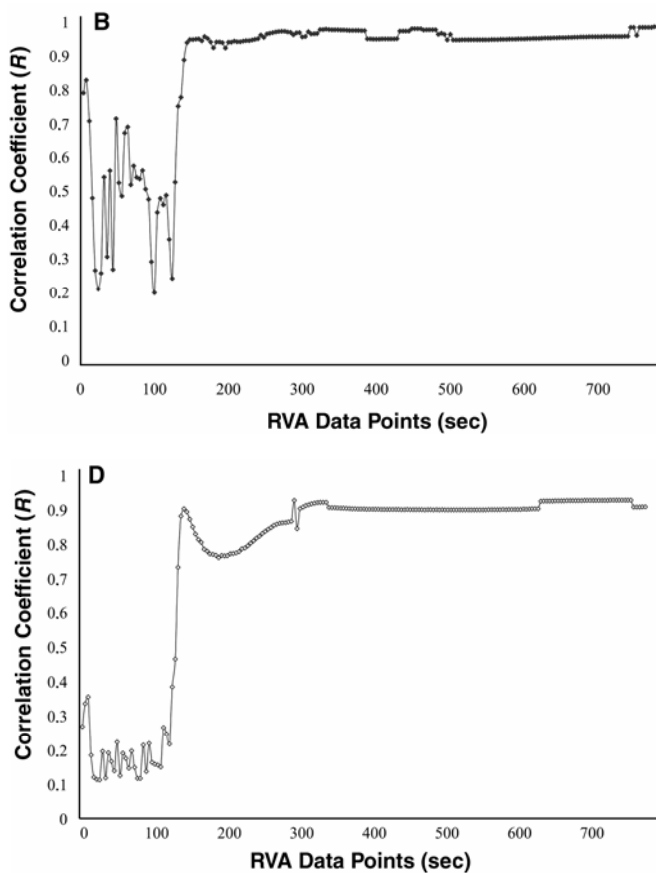


Fig. 5. Correlation coefficients between predicted values based on full range NIR spectra of wheat samples and viscosity values measured by RVA calculated using PLS. **A**, Raw NIR spectra, dry samples. **B**, 2nd derivative of NIR spectra, dry samples. **C**, Raw NIR spectra, wet samples. **D**, 2nd derivative of NIR spectra, wet samples.

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