

Dynamic Monitoring of Dough Mixing Using Near-Infrared Spectroscopy: Physical and Chemical Outcomes

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ABSTRACT

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The objective of the present study was to identify physical, chemical, and physicochemical mechanisms at the origin of the NIR spectra modifications recorded during dough mixing. An FT-NIR spectrometer over the 1000–2500 nm range with a fiber optic probe in contact with the dough during processing was used. The NIR spectra collections are analyzed as raw spectra and after second derivative treatment by using principal component analysis (PCA). The analysis of the three first principal components describe high cumulative variance (>95%). The PCA on the raw NIR spectra demonstrate the dominant contribution of physical mechanisms (granular state and surface aspect of the dough) and, to a lesser extent, of physicochemical mechanisms (water and protein modifi-

cations). The PCA of the second derivative spectra on the 1000–2325 nm wavelength range and on restricted wavelength ranges (1352–1485 nm, 1778–2052 nm, or 2109–2325 nm) allowed a physicochemical description of the NIR absorbance variations. The NIR absorbance variations mainly arise from the 1778–2052 nm range related to the O-H vibrations. NIR mixing times were determined from the PCA score plots based on raw and second derivative NIR spectra and were associated with changes in different dough physicochemical properties (glutenin depolymerization rate, extractable liquid phase, consistency, maximum strain, and stress at break).

Dough mixing is a very important stage in the breadmaking process. The extent of mixing has a critical impact on final bread quality (Sai Manohar and Haridas Rao 1992; Gras et al 2000; Olewnik et al 2004). The mixing process promotes different physical, chemical, and physicochemical modifications that contribute to the dough development. Blending action leads to an even distribution of the dough ingredients and ensures hydration and swelling of flour particles. Mechanical energy input induces conformational changes of the wheat protein components, promoting gluten network formation through breakage and formation of chemical links of both covalent (SS bonds) or noncovalent types (hydrophobic, hydrogen). Mixing produces an homogeneous gluten film regularly distributed around the starch granules. The dough must be mixed for a specific time (referred to as optimum dough development) to ensure optimal loaf volume and bread texture. Stopping mixing before the optimal point results in undermixed dough that gives bread of inferior volume and crumb quality. The optimal mixing requirement is a specific characteristic of each wheat flour. Beyond optimum dough development time, overmixing induces dough stickiness, decreases dough consistency (due to degradation by shearing effects), and negatively affects bread quality.

Most online monitoring systems for dough mixing are based on the indirect survey of changes in dough rheological properties using torque and consistency probes (Kilborn and Preston 1981; Bloksma and Bushuk 1988; Wilson and Newberry 1995; Olwenik et al 2004; Belton 2005). However, changes in dough properties during mixing primarily come from chemical modifications that can be assessed through spectroscopic investigations. Wesley et al (1997, 1998, 2002) patented a new approach based on a NIR spectroscopy technique to monitor dough development on mixing. The recent development of fast diode array spectrometers enables NIR spectra to be recorded in a matter of seconds and offers the possibility to monitor online the dough development using NIR spectroscopy and would lead to the industrial automation of the mixing process. Several studies in Europe (Millar et al 2000; Alava et al 2001; Aït Kaddour et al 2005), United States, and Australia (Wesley et al 1998; Psotka et al 1999; Huang 2001; Huang et al

2001; Olewnik et al 2004) have also investigated the ability of NIR spectroscopy to monitor dough mixing and extended applications over different mixing processes in laboratory or industrial conditions, wheat types, and dough formulations. Online NIR spectroscopy is the least disturbing method of exploring molecular changes. Because molecular absorbances in the NIR wavelength range are specific to the main dough components (water, protein, starch, and fat), NIR spectroscopy can detect the main chemical changes during dough development and generates chemical information to monitor dough mixing. The changes in NIR spectrum vs. mixing times were considered to construct the NIR mixing curve and to determine the optimum mixing time. The optimum dough development has been associated with a minimum (or maximum) value of NIR absorbance. Different NIR regions between 400 and 1700 nm have been identified as relevant to monitor dough mixing and were used to describe the underlying chemical changes (Wesley et al 1998; Alava et al 2001; Olewnik et al 2004).

The objective of the present study was to investigate the ability of the NIR spectroscopy method to monitor the French bread mixing process using FT-NIR spectrometry over the whole NIR wavelength range (1000–2500 nm) with a fiber optic probe positioned inside the mixer directly in contact with the dough. The NIR spectra collections are analyzed as raw spectra and after second derivative treatment by using the principal component analysis (PCA) method. The results are interpreted in terms of kinetics for the rheological, physicochemical, and chemical changes involved in dough development.

MATERIALS AND METHODS

Raw materials. Experiments were realized using nine different industrial common wheat flours (noted as flours 1–9) for bread, pastry, or biscuit applications, obtained from the Moulins Soufflet SA (Nogent/Seine, France). The flours were stored at 10°C until needed. Wheat flour moisture content, ash content, protein content, and alveograph properties were determined according to French standards NF V03-707, NF V03-720, NF V03-750, and NF ISO 5530.4, respectively. The characteristics of the selected flours are given in Table I. Tap water and standard bread yeast (SARL Auriac, S.I. Lesaffre, France) were incorporated in the bread formulation.

Dough mixer. A 6-kg Mahot mixer (VMI, labo 25 Montaigu, France) equipped with a rotating bowl and oblique shaft was used for dough mixing. Experiments were performed at a constant mixing rate (80 rpm), over long mixing times (20–30 min) to system-

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atically reach overmixing conditions. The standard formulation (3 kg of flour, 30 g of dry yeast, and water) was used for all tests. Moisture content of 69% (based on 12% moisture in flour) was used to prepare the dough. The ingredients were incorporated according to a specific order (flour, yeast, and water). The initial water, flour, and mixer temperatures were 17, 18, and 20°C ($\pm 1^\circ\text{C}$), respectively.

The mixer was equipped with a temperature consistency sensor (AAP 98, Captels Passage) (Fig. 1). The consistency sensor records the apparent force (in kg) exerted by the dough during mixing.

NIR spectrometry. NIR spectra during mixing were recorded using a Nicolet Antaris FT-NIR Analyser (Thermo Electron Corporation, France). The NIR instrument was connected to a fiber optic probe positioned inside the mixer directly in contact with the

dough (Fig. 1). The NIR spectrometer gathers spectral data over 1000–2500 nm at 2-nm intervals, recording 30 scans/20 sec that are averaged to give 1 spectrum, and represented as values of $\log(1/R)$ as a function of wavelength (nm). All the mixing experiments were performed at least three times. The NIR spectra were recorded during mixing and analyzed using MATLAB 7.0.4 software (The MathWorks, Inc). The second derivative treatment of the raw spectra was performed using the Stavitsky-Golay derivative. The smoothing and derivative parameters, filter width, polynomial order, and derivative order were 31, 2, and 2, respectively. The data matrix was mean-centred before principal component analysis (PCA) that was performed using the PLS-Toolbox v.3.5 (Eigenvector Research) for MATLAB. Triplicate results for each flour were analyzed by performing one PCA.

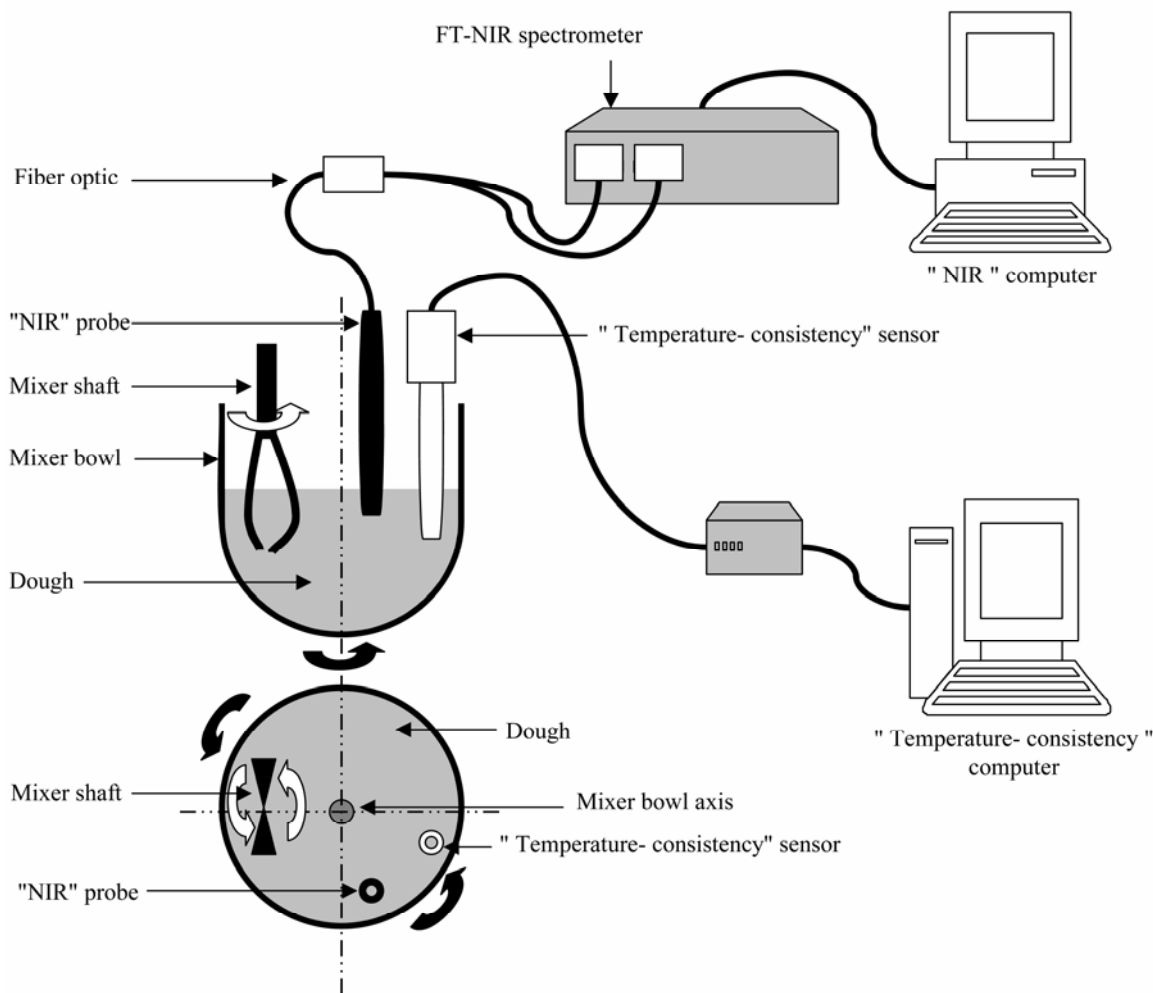


Fig. 1. Configuration used to record dough NIR spectra, dough temperature, and dough consistency during mixing.

TABLE I
Chemical and Physical Characteristics of Selected Flours

Flour	Ash (%mb)	Protein (% mb)	Moisture Content (%)	Alveograph Properties			
				<i>P</i> (mm)	<i>L</i> (mm)	<i>P/L</i>	<i>W</i> ($\text{J} \times 10^{-4}$)
Flour 1	0.50	9.40	13.3	34.0	133	0.44	230
Flour 2	0.40	12.4	13.8	83.0	190	0.44	383
Flour 3	0.49	11.3	13.1	91.6	114	0.80	275
Flour 4	0.42	9.50	14.7	79.9	104	0.77	230
Flour 5	0.44	8.60	13.5	87.0	71.0	1.22	222
Flour 6	0.53	9.20	14.3	80.0	84.5	0.95	240
Flour 7	0.45	9.30	14.5	76.7	95.5	0.80	268
Flour 8	0.52	8.90	14.9	84.3	80.0	1.05	249
Flour 9	0.35	10.7	13.3	87.4	73.5	1.19	275

^a Where *P* is the dough elasticity; *L* is the dough extensibility; *P/L* is the alveograph ratio; *W* is the deformation energy.

Dough rheological properties. Uniaxial extension tests were performed on dough samples using the TA-XT2 texture analyzer (Stable Micro System) equipped with a Kieffer extensibility rig (Dunnewind et al 2004). The dough samples were taken at different mixing times, shaped in a stencil form, and cut into pieces (5 cm long) with a trapezium-like cross-section. The dough samples were coated with vegetable oil to prevent drying and stored under a water vapor saturated atmosphere for a minimum of 30 min at 20°C to ensure the stress relaxation. Just before measuring, sample ends were clamped between the two plates of the Kieffer rig. Dough sample length between the two clamps is 14.6 mm.

Measurements were made at 20°C under extension at constant hook displacement rate (60 mm/min) until sample break. The force (N) and hook displacement (mm) were recorded as a function of time. Results are mean values of at least five measurements on at least three different dough preparations. The sample dough length (l_t) was calculated from the horizontal initial sample length (l_0) and the vertical measured hook displacement (dt) using Equation 1 as follows

$$l_t = \sqrt{d_t^2 + l_0^2} \quad (1)$$

The relative deformation (ϵ_t) of samples was calculated using Equation 2 as follows

$$R_t = \sqrt{\frac{R_0^2 l_0}{l_t}} \quad (2)$$

Assuming a constant cylindrically shaped dough sample and a constant dough sample volume between the two clamps through the test, the radius (R_t) and the cross-section of the dough sample decrease proportionally with the increase of its length (l_t) (Equation 3). Extension stress values (σ_t) were then calculated using Equation 4.

$$R_t = \sqrt{\frac{R_0^2 l_0}{l_t}} \quad (3)$$

$$\sigma_t = \frac{F_t}{\pi R_t^2} \quad (4)$$

Dough physicochemical characteristics. The dough extractable liquid phase content was measured by an ultra-centrifugation method. During mixing, dough samples (15 g) were taken and placed in a polycarbonate test tube (50 mL, 29 × 104 mm, Beckman) and immediately centrifuged at 100,000 × g during 1 hr at 20°C (centrifuge J-30 I, Avanti, Beckman). After centrifu-

gation, samples generally separate into four phases: liquid phase (upper phase), gel layer fraction, gluten fraction, and starch phase. The volume fraction of the upper liquid phase was estimated by weighing the liquid phase and was considered as the dough extractable liquid phase. The results are mean values of at least three measurements. The repeatability has been estimated from seven measurements on three different dough mixing after 6 min of mixing time. The coefficient of variation obtained ($\pm 4\%$) was applied to all the data points. Results are expressed in g of extractable liquid phase/100 g of dough.

Protein depolymerization rate. The effects of mixing on the content of SDS-soluble polymeric glutenins ($F_1 + F_2$) and SDS-insoluble polymeric glutenins (F_i) during dough mixing were evaluated by SE-HPLC (model Waters, LC Module 1 plus) using a Beckman column (TSG-G4000-SWXL) linked to a precolumn (TSK gel 3000-SWXL, Beckman) according to a specific method (Morel et al 2000). The dough samples were taken during kneading, immediately frozen by liquid nitrogen, stored at -26°C, lyophilized, and crushed during 1 min using a Danguomeau crusher (Fontenay-sous-Bois, ProLabo, France). The reproducibility has been estimated on three different dough mixing experiments. The coefficient of variation of the measurements was estimated at 4%.

RESULTS AND DISCUSSION

NIR spectra were recorded over the 1000–2500 nm wavelength range during dough mixing. However, the analyses of the NIR spectra have been restricted to the 1000–2325 nm range because the spectra between the 2325 and 2500 nm wavelength range were very noisy.

Direct description of the raw spectra. Typical NIR spectra that were recorded during dough mixing are presented in Fig. 2B. A broad band centred at 1460 nm and an intense feature centred at 1940 nm, probably due to water, are identified. Two other small absorption bands located at 1200 and 1783 nm were also observed. It was difficult to extract useful information directly from the raw NIR spectra because of low signal-to-noise ratio, large baseline changes from one spectrum to another, and broad absorption peaks. It is almost impossible to identify bands rising from dough components in the raw NIR spectra. Similar raw NIR spectra have been previously reported for dough mixing over restricted wavelength ranges, and some absorption peaks were identified at 985, 1205, and 1465 nm (Psothka 1999; Alava et al 2001; Olewnik et al 2004).

Direct description of spectra after second derivative. Typical NIR spectra after second derivative for wheat flour dough mixing are presented in Fig. 2. The use of the second derivative treatment

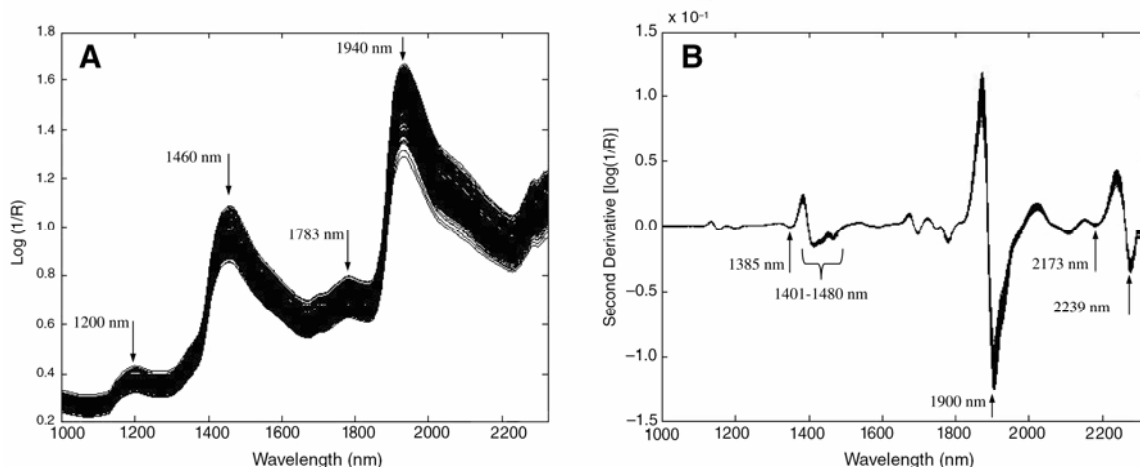


Fig. 2. Typical NIR raw spectra (A) and spectra after second derivative (B) recorded during dough mixing (standard formulation with flour 1). Arrows identify the position of the most important peaks.

removed the baseline shift between spectra caused by scattering effect. Overlapping absorbances are separated, and the peak resolution is high, making analysis easier. The direct description of the second derivative NIR absorption spectra demonstrated absorption features at specific wavelengths (1385, 1401–1480, 1900, 2173, and 2239 nm). The peaks can be associated with chemical changes of the dough components (Osborne and Fearn 1986), and more particularly with starch vibrations (1900 and 1385 nm), to protein vibrations (2173 and 2239 nm), and to C-H₂, CONH₂, H₂O, starch, and CONHR vibrations (1401–1480 nm). Alava et al (2001) also identified the OH first overtone for water molecules (1450 nm), wheat lipids (1410 nm), wheat gluten (1460 nm), and

wheat starch (1450 nm) from NIR investigation of dough mixing. Wesley et al (1998) found that the water first overtone (1450 nm) is overlain with absorbance due to the NH first overtone and the first overtone of the CH combination bands, and still remains relatively difficult to analyze.

PCA of the raw NIR spectra. PCA was used to analyze the NIR spectra recorded during dough mixing over the restricted wavelength range (1000–2325 nm). The PCA was applied to decompose and describe the dynamic spectral variations registered during dough kneading. The uneven blending of dough ingredients at the beginning of mixing was considered by eliminating the first four NIR spectra (0–2 min of mixing) identified as outliers after a first

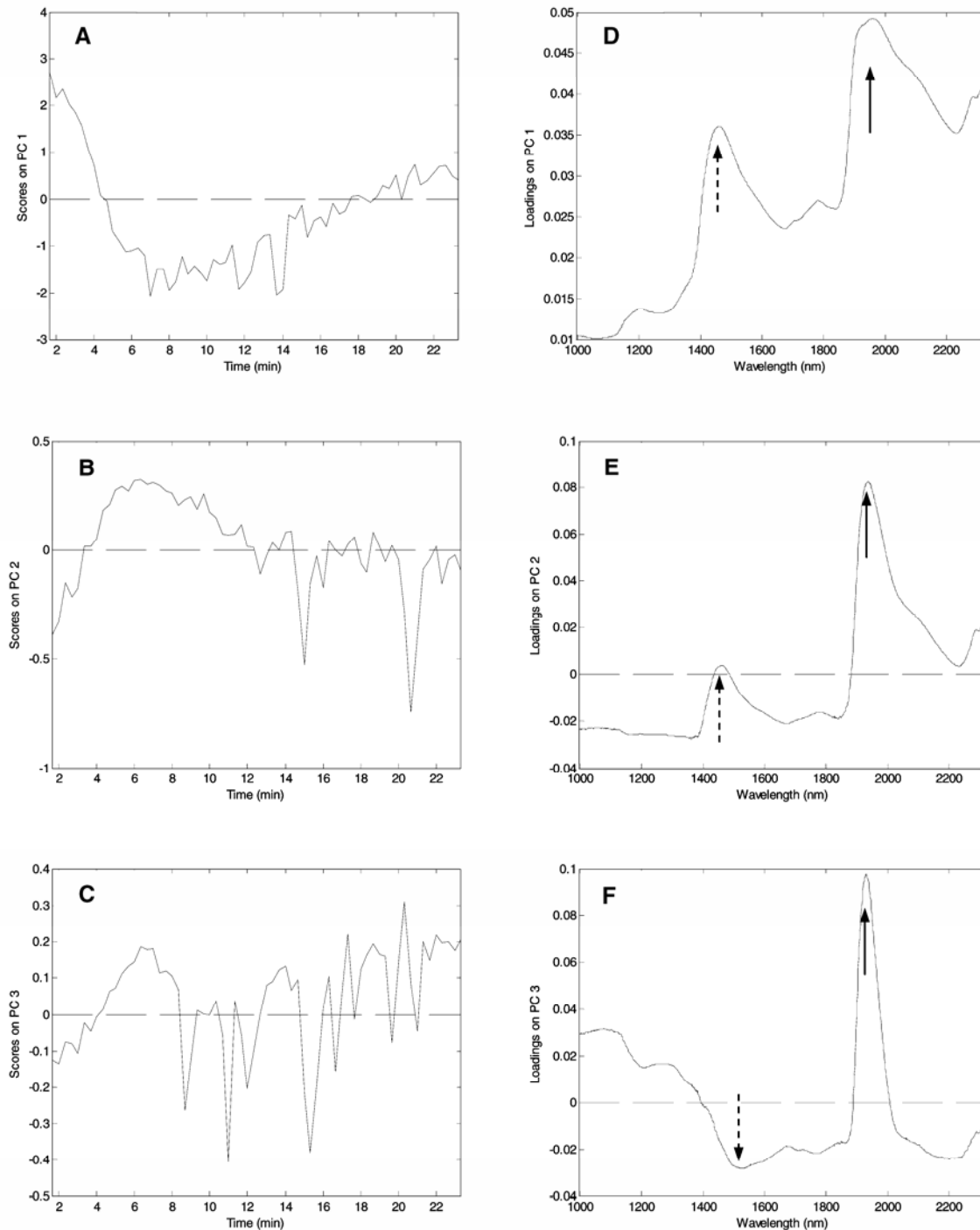


Fig. 3. Changes in NIR principal component scores PC₁, PC₂, and PC₃ (A, B, and C) as a function of mixing time and the respective spectral loadings (D, E, and F) calculated on raw spectra of 1000–2325 nm (standard dough formulation with flour 1). Arrows identify the position of the most important peaks (---1460 nm, —1940 nm).

investigation using PCA method. PCA was first conducted on raw NIR spectra. Typical NIR mixing curves obtained from flour 1, by plotting the changes in principal component scores (PC₁ to PC₃) as a function of mixing time are presented in Fig. 3 A, B, and C. The three first principal component scores describe high cumulative variance ($\geq 98\%$). PC₁ captures $>93\%$ of total variance. The change in the PC₁ with mixing time (Fig. 3A) shows an initial decrease until a minimum value at 7–9 min. For longer mixing times, a slight increase in the PC₁ is observed. The PC₂ captures 5.6% of total variance. The PC₂ trace (Fig. 3B) presents an initial increase to a maximum value at 6–7 min, then a decrease to reach a plateau value at 10–12 min until the mixing end. The PC₃ explains only 0.9% of variance (Fig. 3C). The PC₃ displays a rapid increase until 6–7 min of mixing, then a decrease from 7 to 8 min, and, finally, a noisy increase until the mixing end. The relatively high data variability in PC scores after 7 min of mixing could reflect the increase of the dough structural modifications (filamentous and sticky behavior) due to overmixing.

Mathematical modeling of the principal component changes during mixing was only conducted using PC₁ scores due to the noisy traces of the other PC scores. The PC₁ changes were modeled using Equation 5 (Alava et al 2001):

$$y = \frac{1}{2} \left[(k_1 + k_2) (t - t_0) + \frac{(k_2 - k_1)}{\alpha} \ln (e^{\alpha(t-t_0)} + e^{-\alpha(t-t_0)}) \right] + y_0 \quad (5)$$

The parameters k_1 , k_2 , t_0 , y_0 and α (Equation 5) were calculated to fit experimental data from a nonlinear least squares data fitting by the Gauss-Newton method using statistical software (Microsoft Excel 2000). The value t_0 was used to describe the turning point at which the rate of change of the gradient is a maximum and was called the NIR mixing time.

The proposed model fitted well the PC₁ values of the flour 1 with a determination coefficient of 0.84 and a standard deviation of 0.6. Calculation of the model parameters gave a value of 7 min (± 0.6 min) for the NIR mixing time.

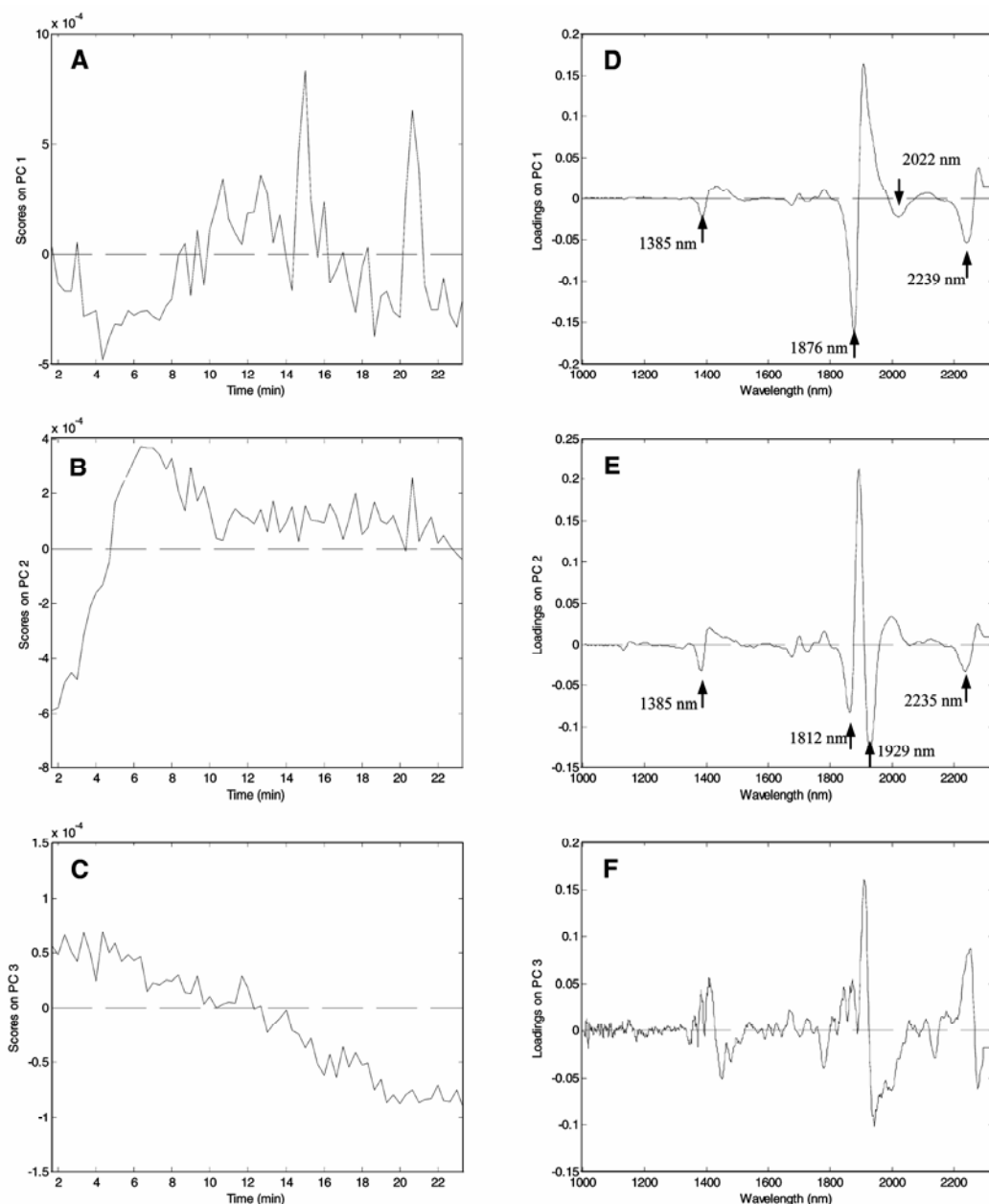


Fig. 4. Changes in NIR principal component scores PC₁, PC₂, and PC₃ (A, B, and C) as a function of mixing time and the respective spectral loadings (D, E, and F) calculated on second derivative spectra of 1000–2325 nm (standard dough formulation with flour 1). Arrows identify the position of the most important peaks.

The loading spectrum associated with the three PC scores are given in Fig. 3D, E, and F. The loading spectrum associated with the PC₁ (Fig. 3D) presents variations over the total spectrum range (1000–2325 nm). It displays a shape similar to the raw NIR spectra recorded during mixing (Fig. 2), with two broad peaks centred at 1460 and 1940 nm. The loading spectrum associated with the PC₁ would be mainly related to the physical evolution of the dough during mixing. It describes the specular and diffusive properties of dough and baseline shift evolution caused by the dough macroscopic structure modification during mixing. It bears most of the physical changes involved in dough from an heterogeneous blend of ingredients to a smooth and finally sticky and filamentous dough. The loading spectra associated with the PC₂ and PC₃ (Fig. 3E,F) present two peaks located at 1460 and 1940 nm. The predominant peak located at 1940 nm would be related to the combination of water absorption and protein vibrations bands identified at 1960 and 1980 nm (Law and Tkachuk 1977; Bertrand and Dufour 2000). The smaller band centred at 1460 nm is generally associated with the NH stretching first overtone of CONH₂ (1460 nm) and with the OH stretching first overtone of water (1450 nm) (Osborne and Fearn 1986). Loading spectra associated with the PC₂ and PC₃ probably reflect chemical and physicochemical information. The raw NIR spectra thus contain some chemical information related to protein vibrations and water absorption.

The present study demonstrated that PCA analysis of raw NIR spectra can be used to directly construct the NIR mixing curves, to identify the NIR mixing time, and to describe some physical mechanisms involved in the dough formation. Even when different authors have used raw NIR spectra to construct NIR mixing curves, no mechanism was associated with the spectral changes. Psotka et al (1999) developed simple NIR mixing curves using only one wavelength from raw NIR spectra (1380 nm). The NIR mixing time was determined at the turning point on the line configuration of the cumulative spectra data. Alava et al (2001) calculated peak area over a restricted wavelength range (1125–1180 nm) on the raw NIR spectra to construct the NIR mixing curves, while Olewnik et al (2004) used two wavelengths (1205 and 1455 nm).

Principal component analysis of the second derivative NIR spectra. Typical NIR mixing curves were obtained from flour 1 by plotting the changes in principal component scores (PC₁ to PC₃) from second derivative NIR spectra as a function of mixing time (Fig. 4A,B,C). The total variance explained by the three first

principal components is 96%. PC₁, PC₂, and PC₃ are associated with 74, 20, and 1% of variance, respectively. The PC₁ shows an initial decrease until reaching a more or less constant value after 5–6 min of mixing. The plot for the PC₂ shows an initial increase to 6–7 min, followed by a more or less regular decrease. PC₃ displays a regular linear decrease over all the mixing time. Because the PC₁ trace was relatively noisy, the calculation of kinetic parameters was conducted using the plots of PC₂ versus mixing time. The score evolution associated with PC₂ was modeled using Equation 5. The proposed model fitted well with the calculated values of PC₂, with $R^2 = 0.80$ and standard deviation = 0.3. The calculation of the model parameters gave a value of 6.5 min (± 0.3 min) for the NIR mixing time of flour 1. Alava et al (2001) also used the PCA of the second derivative NIR spectra recorded versus time to construct NIR mixing curves. They only considered the two first principal components to explain changes occurring during dough mixing.

As expected, the analysis of the second derivative spectra was more informative in evaluating the physicochemical and chemical changes during dough kneading. The loading spectrum associated with the PC₁ (Fig. 4D) presents a profile identical to the second derivative NIR spectra recorded during dough mixing (Fig. 2). The pseudo-sinusoidal shape of the loading spectrum associated with PC₁ probably describes an horizontal translation (Roger et al 2003) of the spectrum bands recorded during mixing (Fig. 1). We can observe that the peak centred at 1940 nm shifts to lower wavelength values (graph not shown). The wavelength shift occurs during the first 7 min of mixing and remains constant for longer mixing time (2.3 nm). The bands identified on the loading spectrum associated with PC₁ reflect starch (1385 nm) vibrations (Table II). This relates to changes in the starch and water vibrations. The 1375–1525 nm region of the NIR spectrum is associated with the OH first overtone and could describe the changes in H bonds between the wheat components and the water molecules. This region was previously reported by Alava et al (2001).

The bands identified on the loading spectrum associated with PC₂ reflect OH, H₂O and starch-water interaction (1929 nm) vibrations (Table II). The absorption feature at 1920–1960 nm on the loading spectrum associated with PC₂ (Fig. 4E) can be associated with changes in the absorption band centered at 1940 nm on the raw NIR spectra (Fig. 2).

The loading spectrum associated with PC₃ (Fig. 4F) could be interpreted by a temperature shift and related to the measured increase in the dough temperature during kneading (Fig. 5). The

TABLE II
Identification of Loading Peaks After PCA Over Different Spectral Ranges^a

Wavelength (nm)	Molecular Vibration/Structure	1000–2325 nm	1352–1485 nm	1778–2052 nm	2109–2325 nm
1373	C-H	–	LS ₂	–	–
1385	Starch	LS ₁ and LS ₂	LS ₁	–	–
1401–1480	C-H/CONH ₂ /H ₂ O/CONHR	–	LS ₁	–	–
1676	–	LS ₂	–	–	–
1812	–	LS ₂	–	LS ₂	–
1863	–	LS ₂	–	LS ₂	–
1876	–	LS ₁	–	LS ₁	–
1929	O-H/H ₂ O/starch-H ₂ O	LS ₂	–	LS ₂	–
2022	–	LS ₁	–	LS ₁	–
2120	–	LS ₁	–	–	LS ₁
2134	–	–	–	–	LS ₂
2173	Protein α -helix structure	LS ₁	–	–	LS ₁
2182	Protein β -sheet structure	–	–	–	LS ₃
2205	Protein β -sheet structure	–	–	–	LS ₂
2231	Amino acid	–	–	–	LS ₃
2235	–	LS ₂	–	–	–
2239	N-H/amino acids	LS ₁	–	–	–
2268	Nonordered protein structure	–	–	–	LS ₂
2276	OH/CH/starch/nonordered protein structure	–	–	–	LS ₁
2282	Protein α -helix structure/CH ₃	–	–	–	LS ₃

^a Where LS₁ is the loading spectrum associated with PC₁; LS₂ is the loading spectrum associated with PC₂; LS₃ is the loading spectrum associated with PC₃.

dough temperature increases almost linearly from 20.7 to 26°C after 21 min of mixing. The slight temperature increase is due to heat generated by viscous dissipation of the mechanical energy input. Complementary experiment based on temperature effect on flour 1 NIR spectra shows trace scores similar to those observed for PC₃ after PCA.

Principal component analysis on NIR restricted wavelength ranges. The PCA of NIR spectra after second derivative treatment clearly demonstrated the contribution of three spectral ranges (1352–1485 nm, 1778–2052 nm, and 2109–2325 nm) to describe dough development. To get a better determination of wavelengths at the origin of the score variations within each wavelength range and to identify additional peaks on loading spectra, it was decided to conduct the PCA method specifically on each spectral range. The contribution of the three spectral ranges to the NIR mixing curves was estimated by comparing the values of the determination coefficients calculated between the PCA scores obtained from the entire NIR range (1000–2325 nm) and those obtained from the three restricted NIR ranges (1352–1485 nm, 1778–2052 nm, and 2109–2325 nm) for each principal component (PC₁, PC₂, and PC₃). The PC₁ determined from the entire NIR range found its origin mainly in the 1778–2050 nm region ($R^2 = 0.99$) and in a lower portion of the 2109–2325 nm ($R^2 = 0.80$) or the 1352–1485 nm ($R^2 = 0.60$) regions. The PC₂ determined from the entire NIR range mainly found its origin in the 1778–2050 nm region ($R^2 = 0.99$) and in a lower portion of the 2109–2325 nm ($R^2 = 0.21$) and

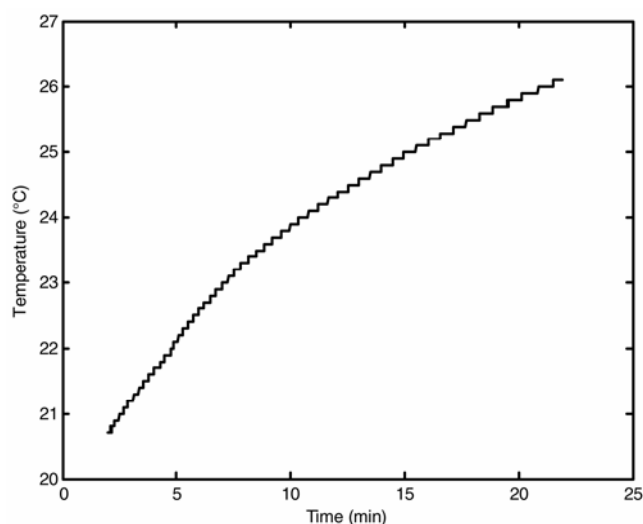


Fig. 5. Changes in temperature during wheat flour dough mixing (standard dough formulation with flour 1).

the 1352–1485 nm ($R^2 = 0.61$) regions. The PC₃ determined from the entire NIR range found its origin on the 1778–2050 nm region ($R^2 = 0.99$) and not in the 2109–2325 nm ($R^2 = 0$) or 1352–1485 nm ($R^2 = 0$) regions. It clearly appeared that the restricted area 1778–2052 nm was the most important region involved in the NIR mixing curves, as suggested by the loading spectra (Fig. 4D,E,F).

NIR mixing times calculated on the PC₂ scores for nine different flours were investigated from the entire spectral range and from the following selected ranges: 1352–1485 nm, 1778–2052 nm, and 2109–2325 nm. Results are presented in Table III. Similar mixing time values ($P \leq 0.05$) were reported for the 1000–2325 nm (5.8–18.2 min) and the 1778–2052 nm (5.7–20 min) ranges. On the other hand, significantly higher values ($P \leq 0.05$) of NIR mixing times were observed for the 1352–1485 nm (8.2–20 min) and the 2109–2325 nm (9.7–20 min) ranges. It clearly appeared that NIR mixing times calculated from the 1000–2325 nm spectral range are mostly associated with the 1778–2052 nm range as suggested by the loading spectra (Fig. 4D,E,F). The higher NIR mixing times values obtained for the 1352–1485 nm and 2109–2325 nm spectral ranges could characterize physicochemical mechanisms that are out of phase compared with those encountered in the 1778–2052 nm spectral range (Table II).

The complementary PCA on restricted spectral ranges were limited to the loadings associated with PC₁, PC₂, and PC₃ because the other loadings are too noisy to be analyzed. Table II presents the wavelengths peaks identified on the different loading spectra after PCA on the three restricted wavelength ranges. Some of the peaks identified on the loadings spectra were associated with molecular vibrations based on the literature data.

In comparison with the PCA on the entire NIR wavelength, the PCA on the 1352–1485 nm wavelength range identifies new peaks located at 1401–1480 nm in the loading spectrum associated with PC₁ and 1373 nm in the loading spectrum associated with PC₂. Those peaks can be associated with the CH vibration of the starch molecule (Table II) (Law and Tkachuck 1977; Osborne and Fearn 1986). The loading spectrum associated with PC₁ reflects the interaction of water molecules with the flour components, while the loading spectrum associated with PC₂ is specifically associated with the water-C-H interaction.

The PCA on the 1778–2052 nm wavelength range does not demonstrate a new peak in comparison with the PCA on the entire NIR wavelength. The PCA of 1778–2052 nm clearly shows that the spectral loadings and the scores associated with PC₁ are mainly related to the hydrogen bond vibrations.

In comparison with the PCA on the entire NIR wavelength, the PCA of the 2109–2325 nm wavelength range demonstrates similar loading spectra associated with the PC₁. New peaks were identified in the loading spectra associated with PC₂ and PC₃

TABLE III
NIR Mixing Time from Selected Flours Calculated Using the Second Derivative NIR Spectra Over Different Spectral Ranges^{a,b}

Flour	NIR Mixing Time			
	Entire Spectral Range		Restricted Spectral Range	
	1000–2325 nm	1352–1485 nm	1778–2052 nm	2109–2325 nm
Flour 1	5.8 (0.6)a	8.2 (0.3)b	5.7 (0.1)a	9.7 (1.4)c
Flour 2	8.3 (1.6)a	10.6 (0.0)b	8.2 (1.5)a	11.0 (0.1)c
Flour 3	7.9 (0.3)a	10.1 (0.0)b	8.0 (0.3)a	11.6 (0.2)c
Flour 4	8.0 (0.4)a	8.9 (0.0)a	7.8 (0.1)a	11.0 (0.6)c
Flour 5	13.0 (0.6)a	13.3 (0.4)ab	12.5 (0.2)a	14.2 (0.9)b
Flour 6	11.3 (0.6)a	12.8 (0.0)b	10.5 (0.5)a	13.7 (2.3)ab
Flour 7	11.2 (0.9)a	12.1 (0.1)ab	11.1 (0.9)a	12.9 (0.5)b
Flour 8	14.2 (0.9)a	15.0 (0.9)a	14.2 (0.7)a	16.8 (1.0)b
Flour 9	18.2 (0.0)a	20.0 (0.0)b	20.0 (0.0)b	20.0 (0.0)b

^a Values in parentheses are standard deviations.

^b Statistical analyses were performed separately to evaluate the difference between methods to calculate NIR mixing time by analysis of variance. Values followed by the same letter in the same row are not significantly different ($P < 0.05$).

(Table II). The peaks located at 2182, 2205, 2231, 2268, and 2282 nm (Table II) can be associated with the protein vibrations. These peaks mainly characterize modifications related to the protein structure. Seabourn et al (2003) indicated that FT-HATR middle-IR spectra of mixed doughs revealed changes in the four bands in the amide III region typically associated with secondary structure of proteins.

The calculated NIR mixing times values suggested the successive steps of an initial hydration of the starch (1352–1485 nm), a transfer of water as the protein hydrates, and then a transfer of water to the protein matrix as the dough develops.

After PCA of the NIR spectra collections of the other flours almost similar PC curves, loadings spectra, and physicochemical conclusions were obtained compared with the flour 1 results.

Physical and physicochemical description of wheat flour dough was conducted during dough kneading to check the physicochemical changes in dough properties gathered from the NIR spectroscopy results. Changes in dough rheological properties, in protein size distribution, and in the content of dough extractable liquid phase were measured during mixing.

Typical changes in the dough extractable liquid phase content during mixing of flour 1 are presented in Fig. 6. The extractable liquid phase content decreases significantly during the first 6 min

of mixing (from 15 to 13%). The extractable liquid phase then increases until the mixing end. Similar results were observed by Larsson and Eliasson (1996). They associated the decrease of the extractable liquid phase during the first stage of kneading to the hydration of the gluten network. This is consistent with the NIR results, and more particularly with the loading raw spectrum associated with PC₂ that was associated with the combination of the water absorption bands (O-H stretching) and to protein vibrations (1980 nm). The time at minimum content of the dough extractable liquid phase value is close to the NIR mixing time.

The effects of mixing on the content of SDS-soluble polymeric glutenin (F₁ + F₂) and SDS-insoluble polymeric glutenin (F_i) proteins during dough mixing are presented in Fig. 7. The insoluble glutenin polymeric content decreases steadily during the first 6 min of mixing and then leveled off at ≈2%. The decrease of the insoluble glutenin polymer fraction is concomitant with the increase of the soluble polymeric glutenin fraction. The changes are consistent with the NIR bands that were associated with the modification of the proteins during mixing (2173, 2182, 2205, 2231, 2268, and 2282 nm) and more particularly to α-helix, β-sheet, and to the nonordered proteins structures vibrations (Table II). The time at minimum content of the insoluble glutenin value is close to the NIR mixing time values.

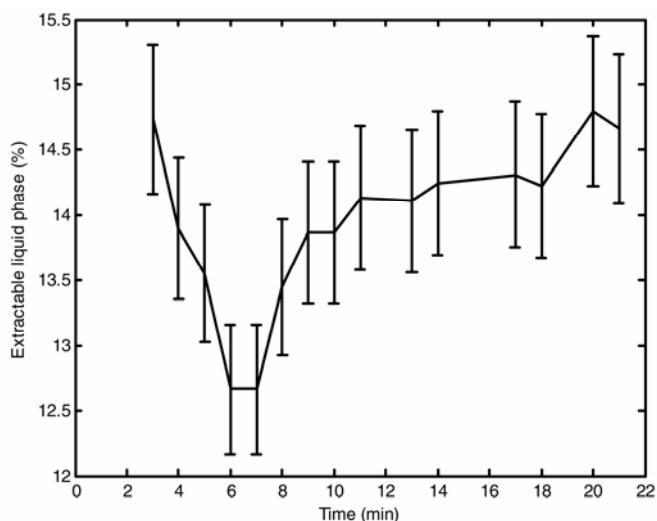


Fig. 6. Changes in dough extractable liquid phase content as a function of mixing (standard formulation with flour 1).

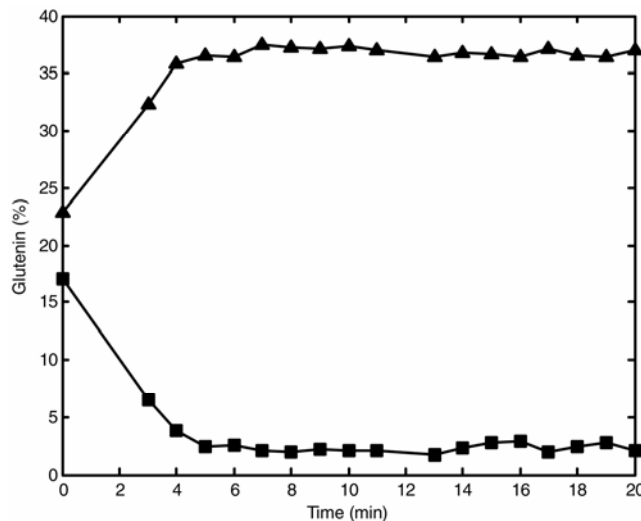


Fig. 7. Changes in dough SDS-insoluble polymeric glutenin proteins (F_i) (■) SDS-soluble polymeric glutenin (F₁ + F₂) (▲) as a function of mixing (standard dough formulation with flour 1).

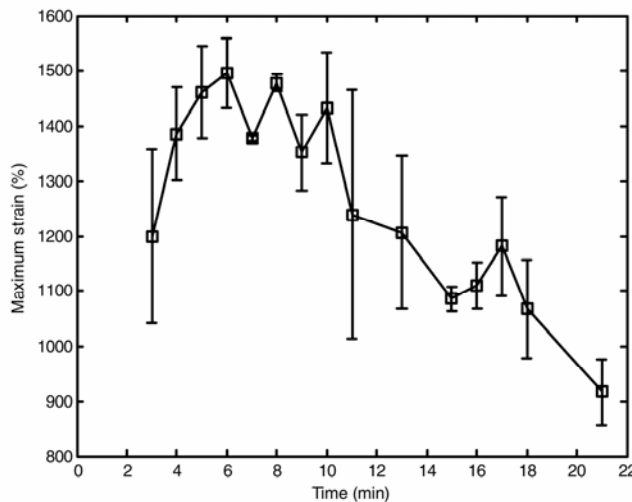
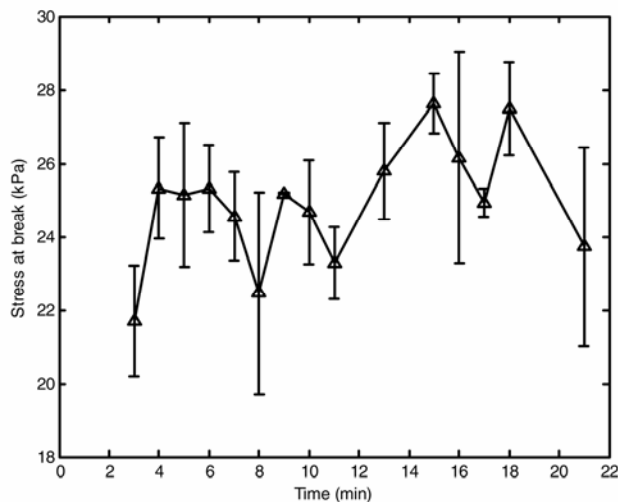


Fig. 8. Changes in dough extensional stress at break (Δ) and maximum strain (□) as a function of mixing time (standard dough formulation with flour 1).

TABLE IV
Time at Maximum Consistency and NIR Mixing Time Calculated for the Entire Spectral Range (1000–2325 nm)
Identified During Mixing for Selected Wheat Flours

Flour Dough	Time to Maximum Consistency	NIR Mixing Time from the 1000–2325 nm Range	
		Raw Spectra	Second Derivative Spectra
Flour 1	6.2 (0.4)ab	7.0 (0.7)c	5.8 (0.6)a
Flour 2	7.5 (0.2)a	10.5 (0.3)b	8.3 (1.6)ab
Flour 3	8.9 (0.1)c	9.4 (0.1)d	7.9 (0.3)b
Flour 4	9.5 (0.5)c	8.9 (0.6)c	8.0 (0.4)b
Flour 5	10.4a	13.6 (0.8)b	13.0 (0.6)b
Flour 6	10.8 (0.1)b	13.3 (0.5)c	11.3 (0.6)b
Flour 7	11.7 (0.6)b	12.0 (0.8)b	11.2 (0.9)b
Flour 8	12.6 (0.6)b	14.4 (1.1)c	14.2 (0.9)bc
Flour 9	15.5 (0.5)a	19.6 (1.1)c	18.2 (0.0)b

^a Values in brackets are standard deviations.

^b Statistical analyses were performed separately to evaluate the difference between methods used to calculate NIR mixing time by analysis of variance. Values followed by the same letter in the same row are not significantly different ($P < 0.05$).

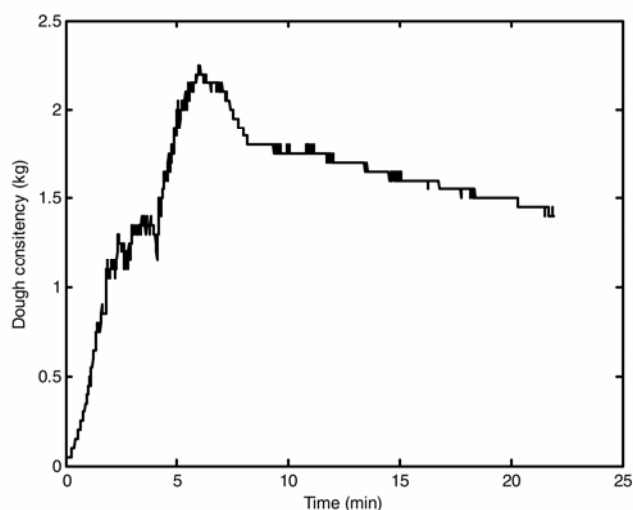


Fig. 9. Changes in dough consistency during wheat flour dough mixing (standard dough formulation with flour 1).

The changes in dough rheological properties (maximum stress and strain at break under extension) during mixing are presented in Fig. 8. The stress at the break curve presents a complex profile as a function of mixing time due to the relatively high data variability. The maximum values of strain (1,500%) and stress (25 kPa) at break under extension are reached after 6 min of mixing. For longer mixing time, strain and stress at break decrease. The results indicated the weakening of dough and strain (deformability) properties on overmixing (after 6 min of mixing). These results are in agreement with those of Gras et al (2000) and Zheng et al (2000). Changes in dough rheology could be related to the changes in NIR bands associated with protein vibrations. The dough strength characteristics are closely linked with the glutenin polymeric fraction.

NIR spectroscopy and dough quality. To evaluate the ability of the NIR spectroscopy to identify the optimum dough mixing time for the best end-product quality, we considered that maximum torque and dough consistency were relevant parameters (Kilborn and Preston 1981; Bloksma and Bushuk 1988; Wilson and Newberry 1995; Olwenik et al 2004; Belton 2005). Indeed, optimum dough mixing time at maximum torque value is usually related to the highest end-product quality by laboratory-scale mixers (Stear 1990). Optimum dough mixing time can also be evaluated using a consistency probe. Typical evolutions of dough consistency are presented in Fig. 9. As expected, the results show that mixing time had a profound effect on dough consistency. We observed an increase in dough consistency during the first 6 min, until reach-

ing maximum consistency. Later the measured force continuously decreases until mixing end.

The comparison of the NIR mixing times with times to maximum consistency for the nine different flours is presented in Table IV. Significantly different ($P \leq 0.05$) values were observed between the time to maximum consistency (6.2–15.5 min) and the NIR mixing times (7.0–19.6 min) from raw NIR spectra. The comparison of time values suggests that the NIR mixing times values could be higher than the times to maximum consistency. On the other hand, similar ($P \leq 0.05$) mixing time values were observed for the NIR mixing times from second derivative spectra and the times to maximum consistency, except for flours 3, 4, 5, and 9. The NIR mixing times obtained from second derivative spectra seem to be the closest to the time at maximum consistency. The NIR spectroscopy method could have the potential to identify the time associated with the optimum dough development and thus to the best end-product quality.

CONCLUSIONS

The results confirm the ability of the NIR spectroscopy to monitor dough mixing using chemometrics methods and the possibility to propose chemical interpretation of spectral change with mixing time. The PCA was applied on the raw NIR spectra and demonstrated that those spectra were principally associated with physical dough evolution (scattering and diffusive effects) and to a lesser extent to physicochemical mechanisms (water and protein modifications). The PCA investigated on the second derivative spectra in the entire wavelength range (1000–2325 nm) and on restricted spectral ranges (1352–1485 nm, 1778–2052 nm, and 2109–2325 nm) clearly show that variations of NIR absorbances are principally associated with the evolution of the hydrogen bond vibrations. The NIR monitoring of the dough mixing, using different industrial flours, shows the possibility of the method to discriminate flour behaviors during kneading. The NIR mixing time can be calculated from raw and second derivative spectra. The NIR mixing time values obtained after the second derivative treatment were associated with the time at maximum consistency, usually related to the best end-product quality.

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