

# Rapid Determination of Dough Optimum Mixing Time for Early Generation Wheat Breeding Lines Using FT-HATR Infrared Spectroscopy

Bradford W. Seabourn,\* Feng Xie, and Okkyung K. Chung

## ABSTRACT

The traditional method in the United States for screening hard winter wheat (*Triticum aestivum* L.) breeding lines is based on the optimum mixing time (MT), an important rheological property of a wheat flour–water (dough) system typically obtained from the mixograph. This method is time consuming and requires some degree of subjective interpretation, especially with regard to mixing tolerance. The purpose of this study was to investigate the potential of Fourier transform horizontal attenuated total reflectance (FT-HATR) spectroscopy to objectively predict optimum MT in doughs from a short-duration mixing cycle (1 min). A total of 55 hard winter wheat flours with varying protein contents and MTs were scanned in the amide III region of the mid-infrared by FT-HATR immediately after being mixed 1 min with a mixograph. Regression analysis of the ratio of the band areas at  $1336\text{ cm}^{-1}$  ( $\alpha$ -helix) and  $1242\text{ cm}^{-1}$  ( $\beta$  sheet) versus optimum MT as determined by the mixograph showed a quadratic response with an  $R^2$  value of 0.81. Results from this study indicate that optimum MT could be predicted early in the mixing process based on changes in the secondary structure of the dough protein (gluten). This method could provide the basis for new technology to rapidly and accurately screen wheat samples in early generation breeding lines, thus saving considerable time and expense in the development of new cultivars.

B.W. Seabourn and O.K. Chung, USDA-ARS, Grain Marketing & Production Research Center, 1515 College Ave., Manhattan, KS 66502; F. Xie, Dep. of Grain Science and Industry, Kansas State Univ., 1515 College Ave., Manhattan, KS 66506. Received 10 Dec. 2007. \*Corresponding author (brad.seabourn@ars.usda.gov).

**Abbreviations:** ATR, attenuated total reflectance; FT-HATR, Fourier transform horizontal attenuated total reflectance; MT, mixing time; PC, protein content; SDBA, second derivative band area.

THE DEVELOPMENT OF rapid prediction methods for determination of wheat (*Triticum aestivum* L.) end-use functionality is a major focus of U.S. wheat-quality laboratories. The optimum mixograph mixing time (MT) in a wheat flour–water system (dough) is one of the important rheological properties that wheat breeders use to screen germplasm since MT is closely related to end-product quality, work input, and product processing time. The current standard method for wheat breeders to evaluate MT is to use a mixograph, farinograph, or similar device. For the end-use quality evaluation of hard winter wheats in the breeding laboratory, primarily the mixograph has been used. However, this method is subjective in its interpretation, as well as time consuming in its method. A rapid and objective method of flour evaluation based on the chemistry of the dough system and its response to work input, rather than physical response alone, is desirable.

It is generally recognized that when flour and water are optimally mixed, at least three things are accomplished: (i) a homogeneous mass of flour and water is formed, (ii) a three-dimensional protein network is developed with the unique capacity to hold gas, and (iii) air cells are incorporated into the dough. When water is added to flour, and the mixing process is started, flour

Published in Crop Sci. 48:1575–1578 (2008).

doi: 10.2135/cropsci2007.12.0669

© Crop Science Society of America

677 S. Segoe Rd., Madison, WI 53711 USA

All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Permission for printing and for reprinting the material contained herein has been obtained by the publisher.

protein (gluten) absorbs water and partially unfolds due to the shear and tensile forces imparted to the dough as a result of the mixing process (Baig and Hosene, 1977; Campos et al., 1997; Kilborn and Tipples, 1977; Larsen, 1964; Lee et al., 2001; Paredes-Lopez and Bushuk, 1983). It is believed that hydrophobic interactions and sulfhydryl-disulfide interchange reactions allow threadlike polymers to form in the presence of water (Shewry and Tatham, 1997). These polymers are then thought to interact with each other through hydrogen bonding, additional hydrophobic interactions and sulfhydryl-disulfide interchanges, and physical entanglements created during the mixing process to ultimately form a continuous sheetlike film of protein with the unique ability to retain fermentation gases (Bloksma, 1975; Mecham et al., 1963; Tsen, 1969). These entangled protein polymers conform to some as yet unknown extent with the traditional biochemical models of protein secondary structure, such as  $\alpha$ -helix,  $\beta$  sheet, and random coil.

Near-infrared spectroscopy has been used to model dough mixing time but has seen limited success (Delwiche et al., 1998; Delwiche and Weaver, 1994). In the present study, we investigated the potential of Fourier transform horizontal attenuated total reflectance (FT-HATR) infrared spectroscopy to objectively predict MT based on unique changes in the secondary structure of wheat gluten in flour as it is hydrated and mixed. In the mid-infrared region, wheat gluten protein has distinctive absorption bands that “fingerprint” its composition and biochemical structure and conformation (Susi, 1969). The structural repeat unit of proteins, the peptide group, gives nine characteristic mid-infrared bands, named amide A, B, I, II, and III to VII (Lin-Vien et al., 1991). The amide I ( $1700\text{--}1600\text{ cm}^{-1}$ ) and amide II ( $1600\text{--}1500\text{ cm}^{-1}$ ) bands are two major bands of the protein infrared spectrum and have traditionally been used to characterize protein secondary structure (Cooper and Knutson, 1995; Susi and Byler, 1983; Williams, 1986). The amide I region ( $1700\text{--}1600\text{ cm}^{-1}$ ) corresponds to the C=O stretch vibration and is directly related to the (poly) peptide backbone of the protein molecule. The amide II region ( $1600\text{--}1500\text{ cm}^{-1}$ ) represents C-N stretch strongly coupled with N-H bending and is sensitive to conformational changes in protein structure. The amide III region ( $1350\text{--}1200\text{ cm}^{-1}$ ) of the mid-infrared corresponds to N-H in-plane bending (40–60%) coupled with C-N stretching (18–40%) molecular vibrations and also includes C-H and N-H deformation vibrations. The most important advantage of using the signal in this region is that the –OH vibrations from water do not interfere with the protein spectrum as they do in the more traditionally used amide I ( $1700\text{--}1760\text{ cm}^{-1}$ ) and amide II ( $1600\text{--}1500\text{ cm}^{-1}$ ) regions. In addition, the absorption bands of secondary structures in the amide III region do not overlap as much as they do in the amide I, thus offering less ambiguity of band assignments (Singh et al., 1993).

Seabourn (2002) previously examined changes in the secondary structure of gluten proteins during dough mixing using FT-HATR. It was observed that long mixing doughs (MT >  $\sim 4.50$  min) had a higher ratio ( $1339\text{ cm}^{-1}/1242\text{ cm}^{-1}$ ) of second derivative band area (SDBA) at 1 min into the mixing cycle, followed by medium MT doughs (MT =  $\sim 3.00$  to 4.50 min), and with short MT doughs (MT <  $\sim 3.00$  min) having the lowest ratio. A band at  $1339\text{ cm}^{-1}$  was previously assigned to protein  $\alpha$ -helix secondary structure and  $1242\text{ cm}^{-1}$  to protein  $\beta$ -sheet secondary structure (Mathewson, 1985; Seabourn, 2002). The author suggested that infrared spectroscopic techniques that monitored changes in the secondary structure of flour proteins could potentially be used for rapidly screening wheat samples based on MT prediction. The objective of this study was to investigate the potential of using FT-HATR infrared spectroscopy as an objective measure to estimate optimum wheat flour MT in a flour–water dough system using a short-duration mixing cycle (1 min).

## MATERIALS AND METHODS

### Sample Preparation

Fifty-five straight grade flours derived from unblended pure experimental hard red winter wheat breeding lines were used in this study. Protein content (PC; 14% moisture basis) ranged from 8.7 to 14.2%, and dough optimum MT ranged from 1.63 to 7.38 min. Experienced operators at the Hard Winter Wheat Quality Laboratory (Manhattan, KS) determined (i) flour optimum MT and water absorption utilizing a mixograph according to AACC Method 54-40A; (ii) flour moisture by air oven according to AACC Method 44-15A; and (iii) protein content using a Foss NIR system according to AACC Method 39-11 (American Association of Cereal Chemists, 2000).

### Data Collection

An FT-HATR mid-infrared spectrometer (Nexus 870, ThermoElectron Corp., Madison, WI), equipped with a ZnSe sample cell (frequency range =  $20,000$  to  $650\text{ cm}^{-1}$ , index of refraction = 2.4), a mercury-cadmium-telluride (MCT/A) liquid nitrogen-cooled detector, and a KBr beam splitter, was used for recording spectra at room temperature ( $21.5 \pm 1^\circ\text{C}$ ) in the mid-infrared region ( $4000$  to  $700\text{ cm}^{-1}$ ). The spectrometer was purged with  $\text{CO}_2$ -free dry air for 24 h before recording spectra. A 128-scan co-added interferogram (mirror velocity: 1.8988; aperture: 69.00; sample gain: 1.0; Happ-Genzel apodization, Mertz phase correction) was collected for each dough sample at a resolution of  $2\text{ cm}^{-1}$  using Omnic software (v8.0.5, 2003, ThermoElectron Corp., Madison, WI). For each dough scan, the single beam spectrum of the dough was divided by the background (attenuated total reflectance [ATR] cell with no sample) single beam spectrum and then converted to an absorbance spectrum.

### Data Analysis

Omnic software and Grams/AI (v7.0, 2001, ThermoGalactic, Salem, NH) were used in data analysis. Before peak integration, the raw spectra were preprocessed according to the following procedure by Seabourn (2002): (i) baseline and ATR correction

(Griffiths and deHaseth, 1986), (ii) conversion to second derivative (5-pt and 2-degree polynomial), (iii) spectral smoothing (11-pt and 2-degree polynomial Savitsky–Golay), and (iv) spectral inversion. A self-developed C+ program was applied to integrate the peak areas by using the zero line as baseline. The SDBA was then regressed against optimum MT.

## RESULTS AND DISCUSSION

Figure 1 shows the raw spectrum of a dough sample (PC 12.3%, MT 3.5 min) collected by FT-HATR infrared spectroscopy. Two bands ( $1336\text{ cm}^{-1}$  and  $1242\text{ cm}^{-1}$ ) located in the amide III region can easily be observed. The raw spectrum was manipulated according to the method described above before integrating the individual band areas. The corresponding derivatized and inverted amide III region in Fig. 1 is shown in Fig. 2. Compared to the work of Seabourn (2002) and Mathewson (1985), the bands at  $1336\text{ cm}^{-1}$  and  $1242\text{ cm}^{-1}$  corresponded to  $\alpha$ -helix (Seabourn at  $1339\text{ cm}^{-1}$ ; Mathewson “no finding”) and  $\beta$ -sheet structure (Seabourn at  $1242\text{ cm}^{-1}$ ; Mathewson at  $1245\text{ cm}^{-1}$ ), respectively. The  $\beta$ -sheet band at  $1242\text{ cm}^{-1}$  centered within  $<1.0\text{ cm}^{-1}$  of the band location for all the samples, while the  $\alpha$ -helix band varied from  $1335.5\text{ cm}^{-1}$  to  $1342.2\text{ cm}^{-1}$ . Seabourn (2002) reported the same phenomenon in which this  $\alpha$ -helix band shifted slightly with samples of varying protein content and optimum MT.

The ratio of the SDBA at  $1336\text{ cm}^{-1}$  ( $\alpha$ -helix) and  $1242\text{ cm}^{-1}$  ( $\beta$  sheet) was calculated, and its relationship to optimum MT was studied. It can be seen from Fig. 3 that the ratio increased nonlinearly with MT. The data best fit a polynomial regression, and the  $R^2$  between the actual MT and the spectrophotometrically predicted MT was 0.81. The study’s results demonstrate that the ratio of the SDBAs at  $1336\text{ cm}^{-1}$  and  $1242\text{ cm}^{-1}$  was highly predictive of the optimum MT of the doughs.

Seabourn (2002) reported that the infrared absorption band associated with  $\alpha$ -helix decreased in area while the band associated with  $\beta$  sheet increased in area during the mixing process, and that these bands reached a plateau that was closely related to the optimum MT of the dough as determined by the mixogram midline. The results of this study show that  $\beta$ -sheet SDBAs for the longer mixing doughs were much smaller at 1 min in the mixing cycle in relation to the  $\alpha$ -helix band than for the shorter mixing doughs. In other words, the intimate relationship between the SDBA ratio and mixogram midline

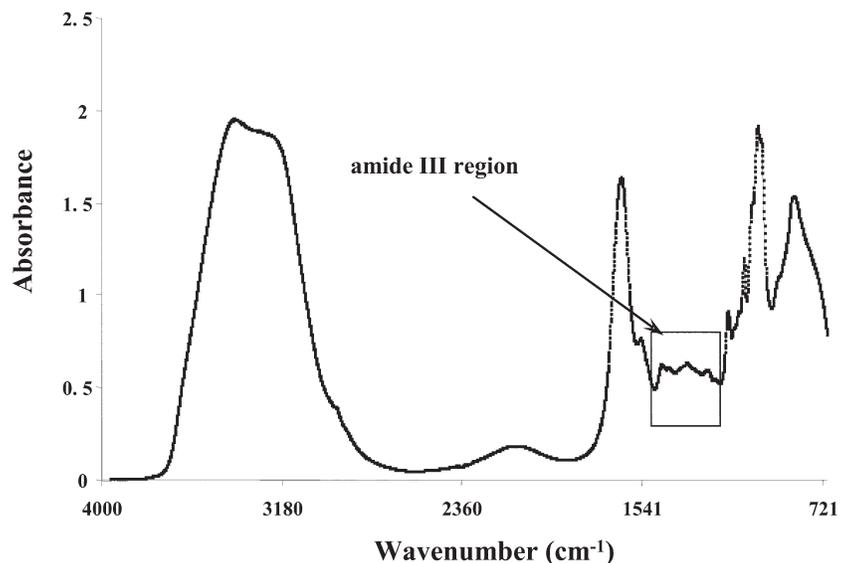


Figure 1. A raw spectrum of a dough sample collected by Fourier transform horizontal attenuated total reflectance infrared spectroscopy.

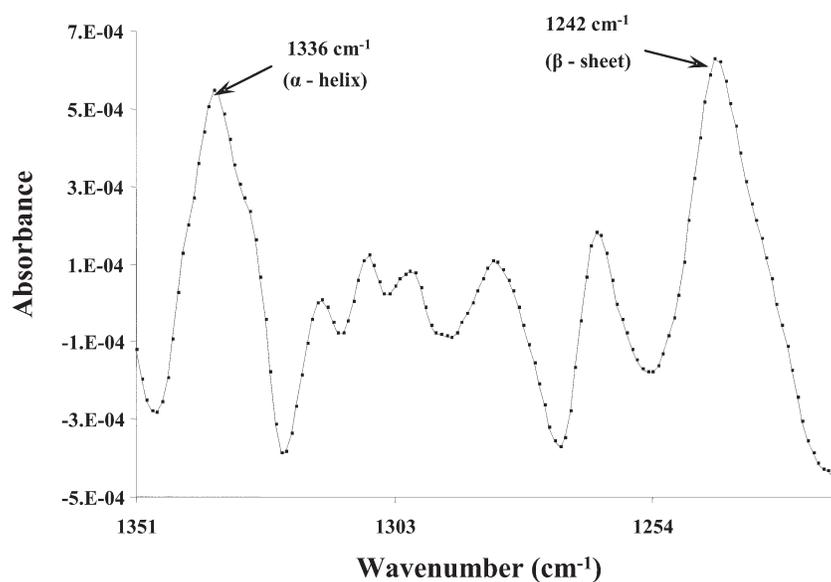


Figure 2. The corresponding inverted and smoothed second derivative spectrum (baseline and ATR corrected) of the amide III region shown in Fig. 1.

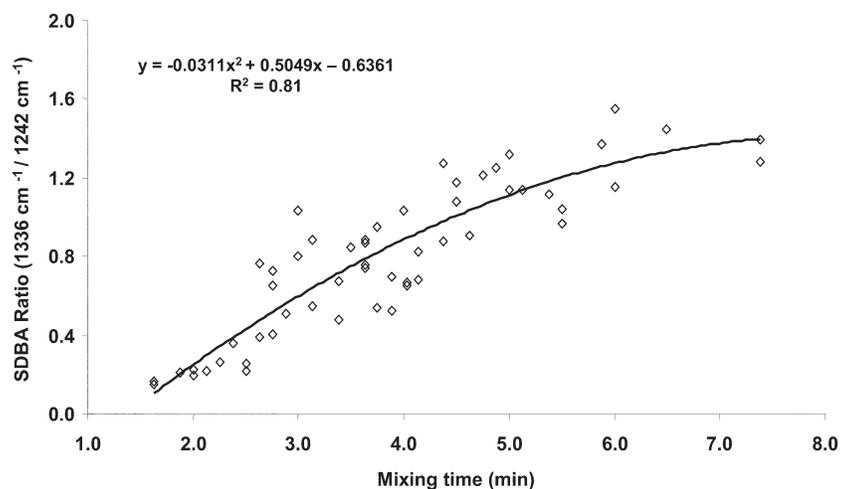


Figure 3. The relationship between dough optimum mixing time and second derivative band area (SDBA) ratio ( $1336\text{ cm}^{-1}/1242\text{ cm}^{-1}$ ) determined by regression analysis.

indicated flour samples with short MT tended to develop  $\beta$  sheet faster than those with long MT, and did so regardless of protein content. The results support the explanation given by Seabourn (2002) that flours with short MT are able to more rapidly hydrate and utilize hydrogen bonding and other noncovalent associations to develop the unique secondary structures such as  $\beta$  sheet because of their unique differences in gluten protein composition. The results observed in this study also confirm the observations of other researchers (Pezolet et al., 1992; Popineau et al., 1994; Wellner et al., 2005) that the  $\beta$ -sheet structure was the favorable conformation in doughs during mixing.

## CONCLUSIONS

The SDBA ratio of  $\alpha$ -helix vs.  $\beta$ -sheet ( $1336\text{ cm}^{-1}/1242\text{ cm}^{-1}$ ) measured at 1 min in the mixing cycle in a flour–water dough system was highly predictive of optimum MT. This FT-HATR technique demonstrates the ability to accurately predict optimum MT very early in the mixing process. Protein secondary structural characteristics are important factors in determining flour optimum MT since the protein network in a dough system is dependent on the formation of specific protein secondary structures such as  $\beta$ -sheet conformation. This technique could be the basis for new technology to rapidly and accurately screen wheat samples in early generation breeding lines, thus saving breeders considerable time and expense in the development of new cultivars.

## Acknowledgments

The authors gratefully acknowledge the USDA–ARS Hard Winter Wheat Quality Laboratory, Manhattan, KS, for providing essential funding and technical assistance. We also thank Dr. Liubo Chen, Department of Computer Sciences, Kansas State University, Manhattan, KS, for his assistance in developing the C+ program used to integrate the infrared peaks observed in this research.

## References

- American Association of Cereal Chemists. 2000. Approved Methods of the American Association of Cereal Chemists. 10th ed. AACCC, St. Paul, MN.
- Baig, M.M., and R.C. Hosney. 1977. Effects of mixer speed, dough temperature, and water absorption on flour–water mixograms. *Cereal Chem.* 54:605–615.
- Bloksma, A.H. 1975. Thiol and disulfide groups in dough rheology. *Cereal Chem.* 52:170r–183r.
- Campos, D.T., J.F. Steffe, and P.K.W. Ng. 1997. Rheological behavior of undeveloped and developed wheat dough. *Cereal Chem.* 74:489–494.
- Cooper, E.A., and K. Knutson. 1995. Fourier transform infrared spectroscopy investigations of protein structure. *Pharm. Biotechnol.* 7:101–143.
- Delwiche, S.R., and G. Weaver. 1994. Bread quality of wheat flour by near-infrared spectrophotometry: Feasibility of modeling. *J. Food Sci.* 59:410–415.
- Delwiche, S.R., R.A. Graybosh, and C.J. Peterson. 1998. Predicting protein composition, biochemical properties, and dough-handling properties of hard red winter wheat flour by near-infrared reflectance. *Cereal Chem.* 75:412–416.
- Griffiths, P.R., and J. deHaseth. 1986. Fourier transform infrared spectrometry. John Wiley & Sons, New York.
- Kilborn, R.H., and K.H. Tipples. 1977. Factors affecting mechanical dough development: I. Effect of mixing intensity and work input. *Cereal Chem.* 49:34–47.
- Larsen, R.A. 1964. Hydration as a factor in bread flour quality. *Cereal Chem.* 41:181–187.
- Lee, L., P.K.W. Ng, J.H. Whallon, and J.F. Steffe. 2001. Relationship between rheological properties and microstructural characteristics of nondeveloped, partially developed, and developed doughs. *Cereal Chem.* 78:447–452.
- Lin-Vien, D., N.B. Colthup, W.G. Fateley, and J.G. Grasselli. 1991. p. 484–490. *In* The handbook of infrared and Raman characteristic frequencies of organic molecules. Academic Press, New York.
- Mathewson, P.R. 1985. Fourier transform infrared spectroscopic analysis of protein secondary structure. p. 96–224. *In* Development of enzymological and Fourier transform infrared spectroscopic methods for analysis of proteolytic activity. Ph.D. diss. Kansas State Univ., Manhattan.
- Mecham, D.K., E.G. Cole, and H.A. Sokol. 1963. Modification of flour proteins by dough mixing: Effects of sulfhydryl-blocking and oxidizing agents. *Cereal Chem.* 40:1–9.
- Paredes-Lopez, O., and W. Bushuk. 1983. Development and “undevelopment” of wheat dough by mixing: Physicochemical studies. *Cereal Chem.* 60:19–23.
- Pezolet, M., S. Bonenfant, F. Dousseau, and Y. Popineau. 1992. Conformation of wheat gluten protein: Comparison between functional and solution states as determined by infrared spectroscopy. *FEBS Lett.* 299:247–250.
- Popineau, U., S. Bonenfant, M. Cornec, and M. Pezolet. 1994. A study by infrared spectroscopy of the confirmations of gluten proteins differing in their gliadin and glutenin compositions. *J. Cereal Sci.* 20:15–22.
- Seabourn, B.W. 2002. Determination of protein secondary structure in wheat flour–water systems during mixing using Fourier transform horizontal attenuated total reflectance infrared spectroscopy. Ph.D. diss. Kansas State Univ., Manhattan.
- Shewry, P.R., and A.S. Tatham. 1997. Disulphide bonds in wheat gluten proteins. *J. Cereal Sci.* 25:207–227.
- Singh, B.R., D.B. DeOliveira, F.N. Fu, and M.P. Fuller. 1993. Fourier transform infrared analysis of amide III bands of proteins for the secondary structure estimation. *Proc. SPIE* 1890:47–55.
- Susi, H. 1969. Infrared spectra of biological macromolecules and related systems. p. 575–663. *In* S. Timasheff and G. Fasman (ed.) Structure and stability of biological macromolecules. Marcel Dekker, New York.
- Susi, H., and D.M. Byler. 1983. Protein structure by Fourier transform infrared spectroscopy: Second derivative spectra. *Biochem. Biophys. Res. Commun.* 115:391–397.
- Tsen, C.C. 1969. Effect of oxidizing and reducing agents on changes of flour proteins during dough mixing. *Cereal Chem.* 46:435–442.
- Wellner, N., E.N.C. Mills, G. Brownsey, R. Wilson, N. Brown, J. Freeman, N. Halford, P. Shewry, and P. Belton. 2005. Changes in protein secondary structure during gluten deformation studied by dynamic Fourier transform infrared spectroscopy. *Biomacromolecules* 6:255–261.
- Williams, R.W. 1986. Protein secondary structure analysis using Raman amide I and amide III spectra. *Methods Enzymol.* 130:311–331.