Recent Developments in NIR Analysis of Grains and Grain Products

B. G. OSBORNE[1]
BRI Australia Ltd.
North Ryde, NSW, Australia

The Australian grains industry, as well as its Canadian, European, and U.S. counterparts, has been a world leader in the adoption of near infrared (NIR) spectroscopy, which has been used since 1975 to monitor growers' barley shipments in Queensland, Victoria, and South Australia and exported wheat shipments in New South Wales (1). Currently, NIR is the only quality testing technology available that is fast enough and cheap enough on a cost-per-test basis for widespread use in breeding programs, crop management, receival testing, and on-line process control. Consequently, NIR technology has been adopted by all of these sectors of the Australian grains industry.

Since 1990, Australian grain growers and the Commonwealth Government of Australia have coinvested through the Grains Research and Development Corporation (GRDC) in almost every significant grains research project in Australia. In 1996, the GRDC invested in a major new initiative: the Grain Industries Centre for NIR (2). The role of the NIR Centre is to provide strategic and applied research and support activities to realize nationally the full potential of NIR technology throughout the value-added chain and across all grain crops. The overall aims of the NIR Centre are

- Early generation, quality screening of breeding lines for key processing characteristics.
- On-farm monitoring of crops to target fertilizer applications, plan marketing strategies, and blend grain to meet specifications.
- Calibration of NIR instruments at receival silos to recognize a grain, assign it to a grade, and automatically report relevant quality characteristics.
- Rapid, accurate assays of animal nutritional factors.
- In processing, use data from on-line NIR testing to formulate raw materials and optimize the process to meet preset product targets.

This article reviews the key results of the first three years of research in relation to these aims. The NIR Centre program currently includes nine separate projects involving different combinations of 12 collaborators. The portfolio encompasses basic research, both strategic and applied, and projects focused on applications specific to individual sectors of the grains industry.

Basic Research

Wheat Protein Functionality. Almost all NIR applications for agricultural foods to date have been developed by exploiting the empirical relationships between spectral data and reference analytical data. This approach has proved highly successful in many cases but can lead to calibrations that are critically dependent on the characteristics of the samples used for the calibration. In such cases, calibration requires frequent adjustment to maintain accuracy, a situation that undermines the benefits of the NIR technique.

To achieve calibrations that are accurate and robust, some of the research in the NIR Centre is directed toward a more strategic approach to understanding the biochemical basis of calibrations. The first application to be addressed was the estimation of flour extensibility. The most important protein fractions that determine extensibility are glutenin and gliadin. Therefore, measurement of extensibility by NIR can be rationalized on the basis of unique spectral patterns due to these components. Figure 1 shows that glutenin and gliadin fractions isolated from wheat flour do have unique absorption patterns. Studies involving binary mixtures of the two components and ternary mixtures, including starch, have confirmed that gliadin and glutenin contents can be measured in mixtures by NIR. This finding formed the basis for a novel approach to ranking flour samples according to glutenin and gliadin contents that involved mathematical modeling of the flour spectra as linear combinations of the spectra of glutenin, gliadin, and starch (3). Although the model is not perfect, it does provide a basis for rationalizing NIR measurement of glutenin and gliadin in flour.

Wheat Starch Structure. Another area that has been studied strategically is the NIR spectroscopy of starch structure during processing of cereal foods. Starch plays a major role in the overall quality of many processed foods. It consists of two glucose polymers: amylose, which is linear, and amylopectin, which is highly branched. In the starch granule, amylose and amylopectin are intermingled, but when the linear segments of amylopectin align, they become ordered into crystallites. Crystallinity results from extensive hydrogen bonding, both intramolecularly and to water molecules, of amylopectin molecules. The addition of energy in the form of heat (as in baking), shear (as in roller milling), or heat and shear (as in extrusion cooking) causes progressive disruption of the hydrogen bonds and changes in the physical characteristics of starch granules. When starch is heated in water, the hydrogen bonds are progressively broken, causing the granule to swell and the amylose to gradually diffuse out of the granule, destroying the crystalline structure and resulting in the formation of a gel. This process, known as gelatinization, is central to many food-processing operations. If a starch gel or bread crumb is allowed to stand for a few hours or more, the reverse process (retrogradation) occurs, as crystallinity slowly redevelops. NIR is especially applicable to the study of chemical changes involving hydrogen bonding.

[1] Phone: +61 2 9888 9600; Fax: +61 2 9888 5821; E-mail: bosborne@bri.com.au

Publication no. W-2000-1220-02F.

© 2000 American Association of Cereal Chemists, Inc.

Accordingly, NIR has been used to study both gelatinization and retrogradation processes in wheat starch systems. It has been shown (4) that a series of bands at ≈1,410, 1,430, 1,460, and 1,510 nm show consistent changes over three experimental systems with changes in starch structure (Table I). For both wheat flour starch damage and extrusion cooking systems, the bands at 1,410 and 1,460 nm correlate positively while the bands at 1,430 and 1,510 nm correlate negatively with depolymerization phenomena. The assignments of these bands support the hypothesis that starch depolymerization may be measured on the basis of spectral changes associated with different states of hydrogen bonding of O-H in starch.

Ultra-Rapid Quality Testing. The success of NIR is largely attributable to its simplicity and speed as a routine analytical technique. A typical analysis of whole grain or flour can be completed in ≈1 min or at a rate of 60 samples per hour with most commonly available instruments. However, NIR Centre collaborators have shown that a new generation of diode array instruments offers unprecedented data acquisition speed with the potential for analysis of different grains (wheat, barley, oat, and pulse) and powdered samples (dried, ground plant tissue, and flour) at a rate of up to 400 samples per hour with an accuracy equivalent to the current best practices.

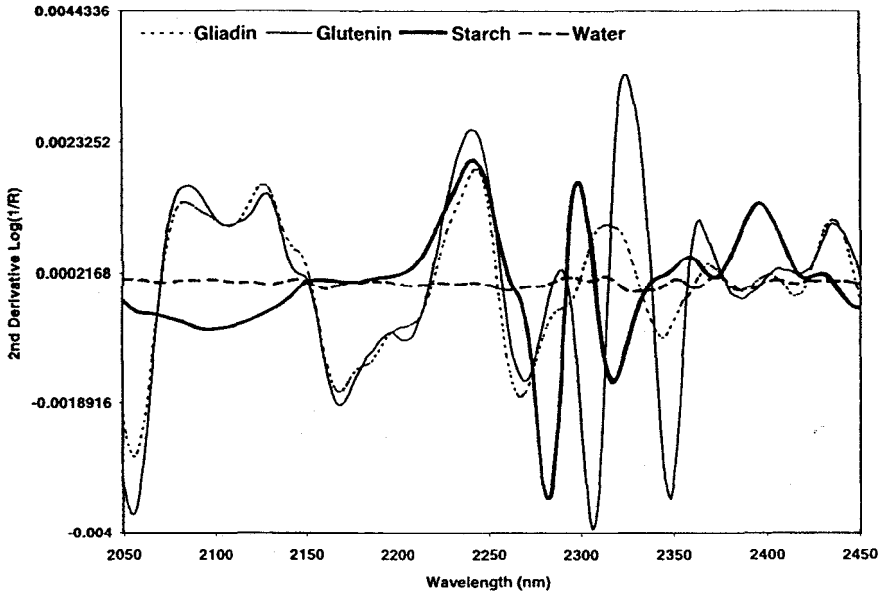

Fig. 1. Near infrared spectra of some major components of flour that influence dough quality.

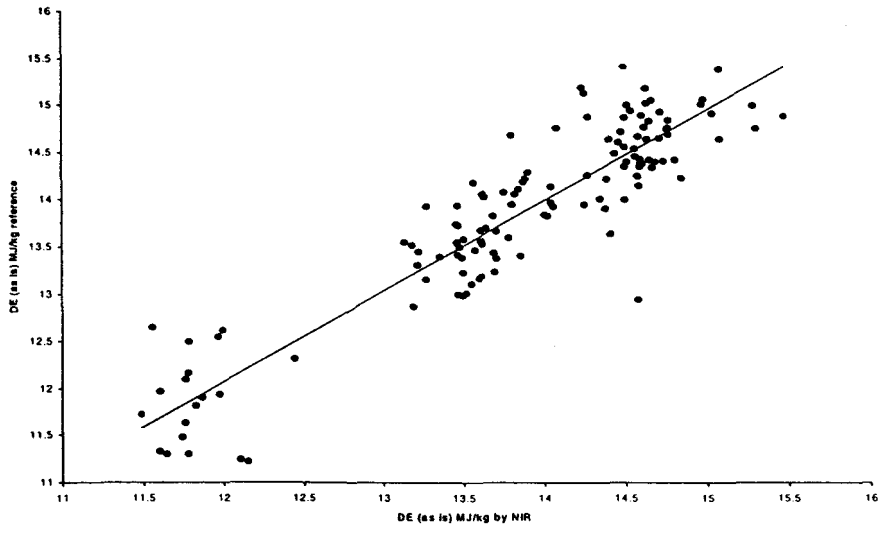

Fig. 2. Scatter plot of whole-grain digestible energy (DE) values after application of indicator variables.

Table I. Summary of Spectral Features and Correlations for Changes in Starch Structure in Three Experimental Systems

	Wavelength, nm (correlation)							
Application	**Minima**				**Maxima**			
Starch damage		1,432(–)			1,406(+)		1,468(+)	1,510(–)
Extrusion	1,410(–)		1,468(–)	1,526(–)		1,430(+)		
Retrogradation			1,460	1,516	1,416			

Plant Breeding

NIR is routinely used for quality testing of grain samples in most Australian wheat, barley, pulse, oilseed, and oat breeding programs. The quality-testing laboratories associated with these programs are equipped with a range of NIR instruments, including 11 NIRSystems 6500 or 5000 monochromators, two InfraAlyzer 500 monochromators, and five Perten DA-7000 diode array spectrometers. The traditional philosophy in quality assessment of plant breeding materials has been to use NIR technology as a more rapid, lower cost-per-test method to replace some traditional methods of testing protein, water, and hardness contents and water absorption in wheat and flour. However, the availability of whole-grain NIR research instruments provides breeding programs with the opportunity to develop NIR techniques to improve efficiency in quality screening of early generation lines, in which the numbers and quantities of samples preclude use of traditional methods.

One of the difficulties in developing calibrations for plant breeding material is that by the stage when samples are available in sufficient quantities for laboratory testing some selection has already taken place. Thus, the property ranges of interest have been narrowed. The aim of the two parallel projects on wheat and barley is to produce a wider range of materials than would otherwise be available to any single breeding program. The wider range of materials will be achieved by growing genetically diverse materials, including lines that would otherwise have been discarded based on quality, over a wide range of environments in the six collaborating breeding programs. The philosophy is to standardize the instruments then record NIR data for each sample set in the laboratory of origin. The data sets will later be merged into a national spectral library for each commodity, which will be available for use by each collaborator. Six of the collaborating quality-testing laboratories were equipped with NIRSystems 6500 monochromator instruments with a sample transport accessory to accommodate a whole-grain cell and ISI software. ISI software provides for creation of a standardization file from spectral data measured on different instruments, using a single sample in a sealed cell. In this exercise, the instruments were standardized so the spectra could be obtained when the same sample was measured on any of the instru-

ments, which are as similar as spectra obtained by repacking the same sample on one instrument (5).

After standardization of instruments, the next problem was to merge data sets that included reference analytical data measured in different laboratories. Indicator variables are "dummy" constituents that are used when the accuracy of a calibration may be influenced by one or more systematic factors in the data, such as reference data generated in different laboratories. The function of indicator variables is simply to activate or deactivate additional intercept terms in the NIR calibration equation. To provide a demonstration of the use of indicator variables and to test their effects in correcting for interlaboratory bias, a set of spectra of whole-wheat samples was selected, and changes were introduced into the reference Kjeldahl data to simulate possible interlaboratory biases in the protein contents. As expected, the introduction of the simulated interlaboratory biases decreased the r^2 and increased the standard error of cross-validation (SECV was calculated by sequentially removing subsets of samples from the calibration set, calibrating using the remaining samples, and predicting the samples removed [50 separate cross-validations were carried out]; SECV provided the best estimate of calibration accuracy obtainable from a single set of samples collected on the same occasion). However, application of indicator variables resulted in complete removal of the effects of the biases (Table II), and the biases calculated by subtraction of each pair of intercept terms were in close agreement with the known biases (Table III).

Indicator variables, therefore, enable calibrations to be developed with samples analyzed in different laboratories. They both compensate for the effect of interlaboratory biases on the accuracy of the calibration and allow the biases to be quantified without the need for a common set of samples to be analyzed in different laboratories.

Crop Production

Since 1990, NIR has been used in Australia to predict optimum fertilizer requirements of cereal crops by analyzing total nitrogen and carbohydrate in plant tissue samples. The tissue test for rapid determination of shoot nitrogen status in cereal crops was first developed for rice but has been extended to encompass wheat shoot nitrogen and fructans (6). The NIR-based tissue test was developed originally at Yanco Agricultural Institute in New South Wales, but testing services now operate throughout the Australian wheat belt to assess the nitrogen and energy status of cereal crops during the vegetative stage. The tissue-testing system is based on a plant sample, taken by the farmer at a specific growth stage, that is dried and analyzed by NIR. The nitrogen and fructan results are entered into a database that is used to determine the appropriate fertilizer recommendation. Recently, the test has been extended by development of calibrations for phosphorus and sulfur. The reliability of the recommendation does not depend solely on the accuracy of the NIR calibration for nitrogen. It also is important that the farmer takes the sample at the correct growth stage and that the database is valid for the crop assessed. Current research is focusing on using the NIR measurement itself to determine growth stage.

Receival Testing

Reliable segregation of grain into grades of appropriate quality is essential in meeting market requirements. Test methods must be simple and rapid to apply at the receival silo and must perform satisfactorily in the hands of relatively unskilled staff working under extreme conditions of temperature and sample throughput. NIR instruments have fulfilled these requirement, and there are currently more than 500 instruments across Australia that are used for load-by-load protein testing at receival. Whole-grain equipment is being progressively adopted for this application, although some ground-grain instruments are still in use. Calibration and maintenance of these instruments, which are distributed over a huge area, is the responsibility of the grain-handling company in each state. The NIR Centre offers support to these companies when required and carries out research on basic NIR calibration issues.

Animal Nutrition

Assessment of the nutritional quality of feed for livestock is important. The digestible energy (DE) value, together with true ileal digestible amino acids, is used to define the nutritive value of feed ingredients prior to incorporation into pig diets. To assess the potential of NIR to predict the DE content of cereals used in pig diets, 156 samples of wheat, barley, sorghum, triticale, and maize, with corresponding in vivo DE measurements, were obtained from Australia, Canada, France, and New Zealand. NIR spectra of both whole and milled grain were recorded and used to derive calibrations for DE. Indicator variables (discussed above) were used to identify and compensate for the inherent interlaboratory variability of the DE data (7). The SECV for DE in whole-grain cereals was 0.38 mJ/kg (Fig. 2), which is comparable to the precision of the reference in vivo methods. This is a particularly encouraging result that opens the way to more widespread use of NIR for nutritional assays.

Processing

Flour Milling. NIR has been used by flour millers to test both wheat and flour for many years, but accurate measurement of starch damage has proved elusive. Recent research with sets of Australian and New Zealand commercial flour samples augmented with samples produced on a pilot

mill (650 kg/hr) has resulted in a calibration with an r^2 of 0.940 and SECV of 0.41% (8). In this study, improving the accuracy of starch damage calibration depended on a more precise reference method (9) than the method used previously; use of a research monochromator instrument and its associated software (specifically, partial least squares regression); and careful control of sample temperature. In particular, the effect of variation in sample temperature on NIR starch damage is substantial. This means that sample temperature must either be controlled very closely or measured and a correction applied to the apparent NIR starch damage. The NIR calibration for starch damage was validated by using it to track changes that resulted from increases and decreases to reduction roll pressures during further pilot-milling trials. Random samples analyzed by the reference method (Fig. 3) confirmed that the NIR and reference starch damage data followed the same trends.

Table II. Calibration Statistics for Near Infrared Spectroscopy Analysis of Whole-Wheat Protein Content

Experiment	SECV[a] (%)	R^2
No biases	0.22	0.99
Simulated biases	0.42	0.95
Biases corrected using indicator variables	0.22	0.99

[a] Standard error of cross-validation.

Extrusion Processing. A wide range of products, such as breakfast cereals, snack foods, confectionery, ingredient supplies, and pet foods, are manufactured by extrusion cooking. The most significant changes in extrusion cooking involve the starch fraction. After crystalline regions of starch granules melt at high temperatures and pressures, application of powerful shearing forces in the extruder soften granular structures and cause disruption and dispersal. A continuous phase of starch polymers containing bubbles of steam in the extrudate allows an extensible foam-like structure to develop. The extent of transformation of raw material, referred to as the degree of cook, is crucial to final product quality. Degree of cook increases when there is an increase in the depolymerization of the starch molecules, which in turn results in an increase in the number of free hydroxyl bonds and a decrease in paste viscosity. The degree of cook of a powdered extrudate sample may be measured by means of a viscometer. However, viscometry can be carried out only on samples that already have been processed and, thus, is not suitable for feedback process control. A method for measuring degree of cook in real time would help enable the development of advanced control strategies. In this investigation, on-line NIR transmission spectroscopy with paired fiber-optic probes was used to monitor the physical state of starch polymers in the barrel melt as a means of measuring the degree of change that occurred under a given processing regime. An NIR measurement based on the band at 1,430 nm, identified as a result of the strategic research on wheat starch structure described above, was used to monitor changes that resulted from adjustments to different process variables (10).

Table III. Simulated Interlaboratory Biases in Protein Content (%)[a]

	Group 1	Group 2	Group 3
Group 1	0		
Group 2	0.25 (0.3)	0	
Group 3	–0.53 (–0.5)	–0.78 (–0.8)	0

[a] Calculated using indicator variables with added biases (in parentheses).

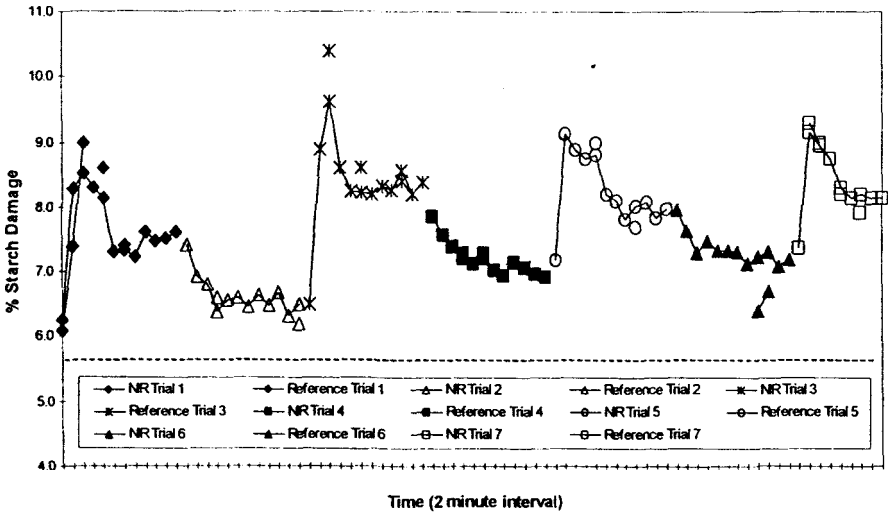

Fig. 3. Validation of near infrared spectroscopy starch damage measurement in a pilot mill.

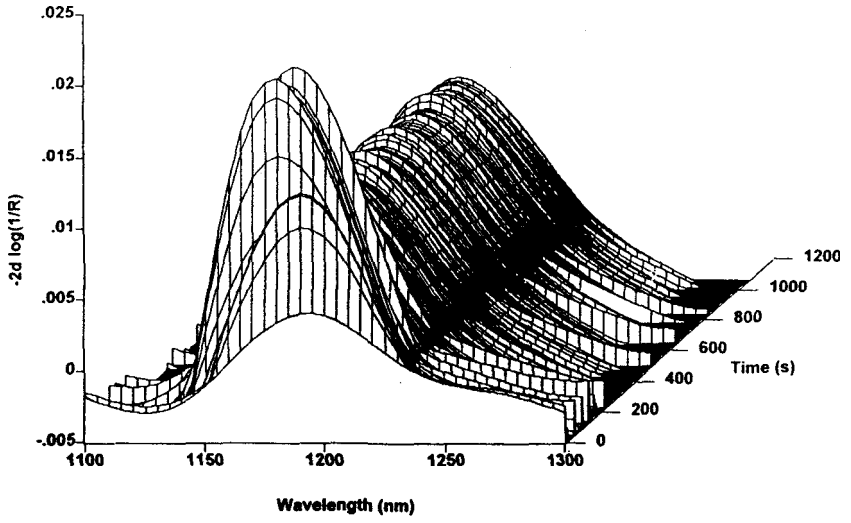

Fig. 4. Time-resolved near infrared spectra of dough during mixing.

Dough Mixing. Mixing dough to the point of optimum development is a crucial stage in production of bread products, but the time it takes for a flour to reach this point with a given formulation and mixer depends on its gluten strength. The farinograph is the standard laboratory instrument used to estimate the dough development time of a flour sample; however, a farinograph is not always an accurate predictor of bakery mixing time, and commercial bakers often use devices based on measurement of mixer power consumption or torque to assist them in determining the optimum mixing time of a dough. Recent research (11) has demonstrated that dynamic NIR spectroscopy with a diode array instrument capable of very fast data acquisition can provide an alternative, noninvasive method for estimating the mixing characteristics of a dough.

Figure 4 shows a series of spectra recorded at 2-sec intervals during dough mixing. The spectra have been transformed to a second derivative and inverted (i.e., the data were multiplied by –1) for clarity of presentation. The band at 1,160 nm decreases in intensity then increases again as the mixing progresses. This band is due to water, which

was confirmed by repeating the experiment with deuterium oxide in place of water in the formulation, resulting in the loss of this spectral information, because the heavier deuterium causes the band to shift to another part of the spectrum. A plot of absorbance at 1,160 nm versus time produces an NIR mixing curve that can be interpreted to provide an estimate of optimum mixing time. NIR mixing curves have been recorded with four flour and three mixer types. Dynamic NIR spectroscopy, therefore, has the potential to be used both in the laboratory to assess the mixing characteristics of different flour samples and in the bakery to control the mixing process.

Acknowledgments

I thank the Grains Research and Development Corporation for investing in this research through the Grain Industries Centre for NIR; I. Wesley (CSIRO Plant Industry) for Figures 1 and 4.

References

1. Miskelly, D. M., and Ronalds, J. A. Twenty-one years of NIR in Australia: A retrospective account with emphasis on cereals. NIR News 5(2):10, 1994.
2. Osborne, B. G. Australian grain industries centre for NIR. NIR News 8(1):10, 1997.
3. Wesley, I. J., Uthayakumaran, S., Andersen, R. S., Cornish, G. B., Bekes, F., Osborne, B. G., and Skerritt, J. H. A curve fitting approach to the measurement of functional proteins in wheat flour. J. Near Infrared Spectrosc. 7:229, 1999.
4. Osborne, B. G. Near infrared spectroscopic studies of starch and water in some processed cereal foods. J. Near Infrared Spectrosc. 4:195, 1996.
5. Osborne, B. G., Kotwal, Z., Wesley, I. J., Saunders, L., Dardenne, P., and Shenk, J. S. Standardisation of NIRSystems 5000/6500 instruments for the analysis of ground and whole wheat, J. Near Infrared Spectrosc. 7:167, 1999.
6. McGrath, V. B., Blakeney, A. B., and Batten, G. D. Fructan to nitrogen ratio as an indicator of nutrient stress in wheat crops, New Phytol. 136:145, 1997.
7. Van Barneveld, R. J., Nuttall, J. D., Flinn, P. C., and Osborne, B. G. Near infrared reflectance measurement of the digestible energy content of cereals for growing pigs. J. Near Infrared Spectrosc. 7:1, 1999.
8. Osborne, B. G. Improved NIR prediction of flour starch damage. Pages 434-438 in: *Proc. 48th Aust. Cereal Chem.* Conf. L. O'Brien, A. B. Blakeney, A. S. Ross, and C. W. Wrigley, eds. The Royal Australian Chemical Institute, Melbourne, 1999.
9. American Association of Cereal Chemists. *Approved Methods of the AACC, 9th ed.* Method 76-31, approved January 1995. The Association, St. Paul, MN, 1995.
10. Evans, A., Huang, S., Osborne, B. G., Kotwal, Z., and Wesley, I. J. Near infrared on-line measurement of degree of cook in extrusion processing of wheat flour. J. Near Infrared Spectrosc. 7:77, 1999.
11. Wesley, I. J., Larsen, N., Osborne, B. G. and Skerritt, J. H. Non-invasive monitoring of dough mixing by near infrared spectroscopy. J. Cereal Sci. 27:61, 1998.

B. G. Osborne

Dr Brian Osborne was born and educated in London, England, where he obtained his B.Sc. and Ph.D. degrees in Chemistry. He has 26 years of professional experience, including 21 years as a cereal chemist, and is a Fellow of The Royal Society of Chemistry, The Royal Australian Chemical Institute, and The Institute of Biology. Dr Osborne worked for 17 years at FMBRA, Chorleywood, in the Analytical and Milling Sections, culminating in his appointment as assistant director, cereals. In 1994, he emigrated to Australia to take up a newly created position as head, grain science & milling in BRI Australia Ltd. Dr Osborne's research interests have been in grain quality and processing, rapid quality testing of food (especially involving the application of near infrared [NIR] spectroscopy), residues and contaminants in food, breakdown pathways of additives during food processing, and food authenticity. He was awarded a D.Sc. degree for his contributions in food science in 1991 and the Tomas Hirschfeld Award for outstanding contributions to NIR spectroscopy by a scientist of international stature in 1993. In 1996, Dr Osborne became the National Coordinator for the Grain Industries Centre for NIR in Australia.