

# Using Visible and Near-Infrared Reflectance Spectroscopy and Differential Scanning Calorimetry to Study Starch, Protein, and Temperature Effects on Bread Staling

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ABSTRACT

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Starch, protein, and temperature effects on bread staling were investigated using visible and near-infrared spectroscopy (NIRS) and differential scanning calorimetry (DSC). Bread staling was mainly due to amylopectin retrogradation. NIRS measured amylopectin retrogradation accurately in different batches. Three important wavelengths, 970 nm, 1,155 nm, and 1,395 nm, were associated with amylopectin retrogradation. NIRS followed moisture and starch structure changes when amylopectin retrograded. The amylose-lipid complex changed little from one day after baking. The capability of NIRS to measure changes in the

retrograded amylose-lipid complex was limited. Two important wavelengths, 550 nm and 1,465 nm, were key for NIRS to successfully classify the starch-starch (SS) and starch-protein (SP) bread based on different colors and protein contents in SS and SP. Low temperature dramatically accelerated the amylopectin retrogradation process. Protein retarded bread staling, but not as much as temperature. The starch and protein interaction was less important than the starch retrogradation. Protein hindered the bread staling process mainly by diluting starch and retarding starch retrogradation.

Bread staling is a complex process that occurs during bread storage. It is a progressive deterioration of qualities such as taste, firmness, etc. The mechanism of bread staling is still not clear yet even though it has been studied for 150 years. Generally, the fact that starch retrogradation is responsible for bread staling is accepted. Starch retrogradation is mainly due to amylopectin retrogradation. Amylose contributes to bread staling primarily in the first 24 hr after baking (Kim and D'Appolonia 1977b; Zobel and Kulp 1996). Previous research on bread staling, however, has not always produced consistent results, and the underlying mechanism is not fully understood. For instance, whether protein is a major contributor to bread staling has generated lively debate (Erlander and Erlander 1969; Kim and D'Appolonia 1977a; Maleki et al 1980; Martin and Hosney 1991; Every et al 1998). Currently, no single technique has provided a complete picture of all events related to this process.

Suzuki et al (1986) used visible and near-infrared reflectance spectroscopy (NIRS) to study bread constituent content and reported that moisture and protein could be precisely determined, whereas sugar and lipid analyses were less accurate. Wilson et al (1991) applied NIRS to study the bread staling phenomenon. The staling rate measured by NIRS agreed with that obtained from differential scanning calorimetry (DSC) measurements. Osborne (1998) showed similar results when studying starch crystallinity in stored bread crumbs using NIRS. Xie et al (2003) compared the NIRS method with the texture analysis (TA) method when assessing wheat bread changes in storage. Their results indicated that the NIRS method was superior to the TA method for measuring bread staling during storage. NIRS followed the changes in bread physical state that caused the light scattering properties to change, and also followed specific functional molecular group changes such as hydrogen bond changes associated with the O-H bond in water and starch (Wilson et al 1991; Osborne 1996, 1998). Others have reported that NIRS successfully measured starch crystallinity, sucrose crystallinity, and starch damage by following hydrogen bonds in water

and starch (Osborne and Douglas 1981; Davies and Miller 1988; Millar et al 1996). Therefore, NIRS has the potential to provide fundamental evidence for determining the mechanism of bread staling.

DSC is also a useful tool for studying bread staling because it measures starch retrogradation accurately, and starch retrogradation largely accounts for bread staling (Fearn and Russell 1982; Wilson et al 1991; Osborne 1998). When a staled bread sample is heated in DSC, an endotherm is observed when crystallized starch melts again. Amylopectin endothermic peak temperature ranges are 50–80°C, depending on storage temperature, starch concentration, and storage time. Below 100°C, no endothermic peak is associated with the amylose fraction (Siljestrom et al 1988; Wilson et al 1991; Zobel and Kulp 1996; Klucinec and Thompson 1999). An endothermic peak at ≈100°C may correspond to the transition of the amylose-lipid complex (Eliasson 1994). However, the DSC method is a destructive method and can only provide the information about starch retrogradation, while the NIRS method follows both physical and chemical changes in bread staling without any damage to the integrity of the bread.

Much research has focused on studying bread staling or amylopectin crystallinity in bread using NIRS (Wilson et al 1991; Osborne 1996, 1998; Xie et al 2003). However, the research has provided only limited information about starch, protein, and temperature effects on bread staling. The primary objective of this study was to investigate the potential of NIRS as a fundamental tool to study bread staling with the help of DSC. A second objective was to investigate starch, protein, and temperature effects on bread staling using NIRS and DSC.

## MATERIALS AND METHODS

### Materials and Bread Preparation

Wheat starch, pregelatinized wheat starch (PREGEL N), and vital wheat gluten were obtained from Midwest Grain Products, (Atchison, KS). Wheat starch contained a maximum of 0.3% protein and 9–12% moisture. The PREGEL N contained a maximum of 0.7% protein and a maximum of 10% moisture. Vital wheat gluten had a minimum of 75% protein, 5–8% moisture, and 1–2% fat. Methyl cellulose (Dow Chemical Co., Midland, MI) contained 85–99% methyl cellulose and 1–10% moisture. All the specifications were provided by the manufacturers.

Two kinds of bread samples were prepared for this study. The formulas for the starch-starch (SS) bread with 0% protein and starch-protein (SP) bread with ≈15% protein are shown in Table I. The effective protein was ≈10–11% because vital wheat gluten is

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only  $\approx 75\%$  effective. In the recipe for SP, 20% vital wheat gluten was added to obtain 15% protein in the formula. The recipes and breadmaking procedure were modified based on those published by Every et al (1998) and Morgan et al (1997).

Bread dry mix was obtained by mixing wheat starch, PREGEL N, methyl cellulose, vital wheat gluten, instant active dry yeast, and calcium propionate using a paddle in a standard 12-qt mixing bowl with a mixer (A-200, Hobart Corp., Troy, OH) at speed one for 30 sec. Sugar was dissolved in one-third of the total water, added to the dry ingredients, and mixed at speed one for 30 sec. Corn oil and the rest of the water were added and mixed at speed one for 30 sec, then at speed two for 30 sec. Finally, the dough was mixed at speed three for another 6 min for SS and 1.5 min for SP. The dough had a batter-like consistency. A total of 14 loaves of SS or SP dough of 210 g each was made from each batch. The dough was proofed in a pup loaf size pan at 40°C and 70–80% rh for  $\approx 30$  min or until the dough height was 2 cm over the pan. Dough was baked at 218°C for 26 min. Bread was kept on the shelf at room temperature and humidity for  $\approx 1.5$  hr. Each loaf was cut into slices 12.5 mm thick, wrapped into a single plastic bag, and stored in the incubator under one of the two storage conditions described below.

### Experiment Design and Bread Tests

A 2x2 treatment combination was used with a randomized complete block design in this experiment. Treatments were two protein levels, 0 and 15%, and two storage conditions, 12.5  $\pm$  1°C, 55  $\pm$  3% rh and 31.5  $\pm$  1°C, 88  $\pm$  3% rh. Storage conditions were chosen in preliminary tests to keep moisture loss at <1% for both the SS and SP. Approximately 14 loaves of SS or SP were made from one batch on the same day for a block. Seven loaves of SS and seven loaves of SP were stored under one of the two incubator conditions. Five blocks (batches A–E) were used in this experiment. Overall,  $\approx 140$  loaves of bread were made.

Specific loaf volume (SV) was measured by the rapeseed displacement method. Moisture content was calculated as MC =

$[(W_b - W_d)/W_b] \times 100\%$ , where  $W_d$  is the total dry matter weight of flour and other ingredients, and  $W_b$  is the weight of bread loaf on the storage day (Wang and Sun 2002). Table II shows SV and MC data collected 1.5 hr after baking in all the five batches.

### Data Collection

A diode-array NIR spectrometer (Perten Instruments, Springfield, IL) was used to collect spectra. The NIRS specifications were reported by Xie et al (2003). One loaf of SS and one loaf of SP from each incubator were tested daily. Tests started  $\approx 24$  hr after baking. Measurements were taken on all the slices, except two or three heel slices from each end in a loaf. One NIRS measurement was taken from one slice. During scanning, the slice was scanned 15x in  $\approx 1$  sec and the average of 15 scans was saved as one measurement. NIRS measurements ( $\approx 6$ ) were taken from each loaf. The orientation of all the slices was kept constant during data collection. Only the central portion of each slice (diameter 12.7 cm) was scanned. The wavelength range of NIRS spectra was 400–1,700 nm. A reference standard (Spectralon) was used to collect the baseline. NIRS absorption data were recorded as  $\text{Log}(1/R)$ , where  $R$  is the relative reflectance.

After the NIRS test, two DSC tests were conducted on samples removed from a single loaf (DSC-Pyris1, Perkin-Elmer). Indium was used to calibrate the instrument. Samples were taken from the central portion of the central slice of each loaf using a cork borer (diameter 1.5 cm) immediately after NIRS testing. The core was compressed and a sample ( $\approx 45$  mg) was sliced from the middle of the compressed core. The sample was put into a preweighed large-volume aluminum DSC pan. The pan was hermetically sealed using a press. The pan and sample were then reweighed. DSC scans were conducted at a heating rate of 5–130°C at 10°C/min. An Al<sub>2</sub>O<sub>3</sub> pan represented the reference scan.

**TABLE I**  
Formulas for Starch-Starch (SS) and Starch-Protein (SP) Bread<sup>a</sup>

Ingredients	SS (%)	SP (%)
Wheat starch	89.31	69.31
Pregelatinized wheat starch	9.95	9.95
Gluten	0.00	20.00
Methyl cellulose	0.74	0.74
	100%	100%
Sugar	5.48	5.48
Instant active dry yeast	1.98	1.98
Corn oil	3.29	3.29
Water	105.95	105.95
Mold inhibitor(calcium propionate)	0.22	0.22

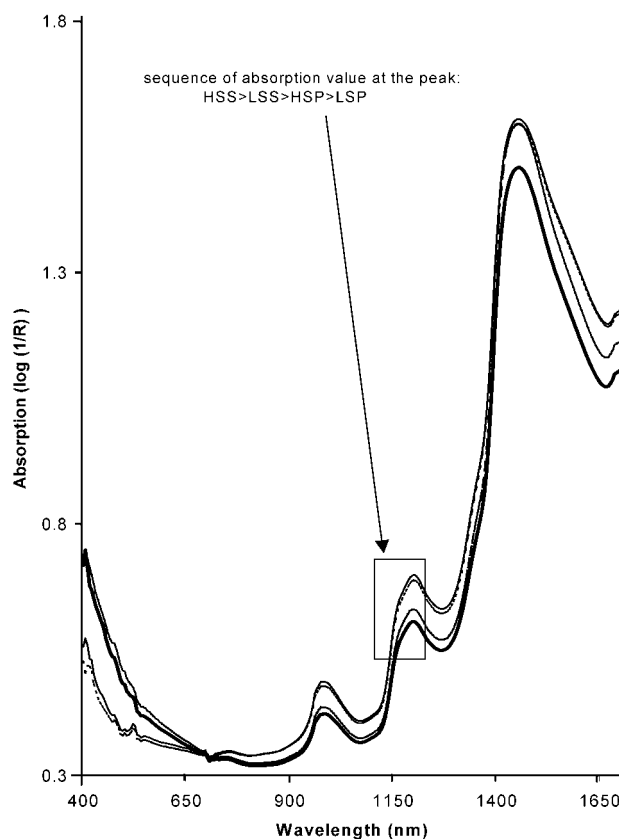
<sup>a</sup> All of the data based on flour as 100%.

**TABLE II**  
Bread Moisture Content (MC) and Specific Volume (SV) in Five Batches<sup>a</sup>

Batch	Formula <sup>b</sup>	MC	SV
A	SS	36.8	4.1
	SP	38.8	3.3
B	SS	36.9	3.6
	SP	36.8	4.0
C	SS	37.9	3.7
	SP	39.0	3.3
D	SS	39.0	3.3
	SP	37.1	3.6
E	SS	39.0	3.4
	SP	36.5	3.6

<sup>a</sup> All data averaged from four loaves in a batch.

<sup>b</sup> Starch-starch (SS) and starch-protein (SP).



**Fig. 1.** Raw spectra of the starch-starch bread stored at low temperature (LSS) and high temperature (HSS), and the starch-protein bread stored at low temperature (LSP) and high temperature (HSP) in batch A, day 1.

## Data Analysis

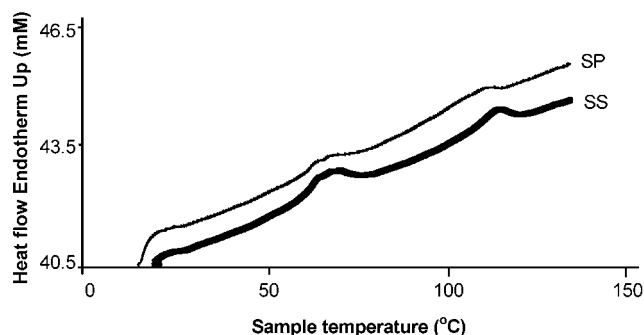
Grams/32 software (Galactic, Salem, NH) and a partial least squares (PLS) regression (Martens and Næs 1989) were used to analyze NIRS spectra data. The raw spectra of LSS (SS at low temperature), LSP (SP at low temperature), HSS (SS at high temperature), and HSP (SP at high temperature) in batch A on day 1 are shown in Fig. 1. All the raw spectra have a similar shape. Because of a high noise level at <550 nm, the wavelength range of 550–1,700 nm was used for data analysis. Beta coefficients of PLS were used to determine the important wavelength regions. The methods of developing calibration models were the same as described by Xie et al (2003).

The raw DSC thermograms of HSS and HSP in batch B on day 3 are shown in Fig. 2. Two endotherm peaks were observed in a thermogram. Endotherm onset ( $T_o$ ) and completion ( $T_m$ ) temperatures were determined according to the method published by Wilson et al (1991). These two points were taken as the points at which deviation occurred from the linear portions of the traces before and after the endotherm. Endotherm enthalpies of dry matter of bread were computed as J/g. The raw amylopectin retrogradation data were obtained by calculating the enthalpies of the first peaks at 50–80°C. The enthalpies of the second peak at 105–120°C were calculated as the raw amylose-lipid complex retrogradation data. The averaged raw amylopectin retrogradation value in one loaf was recorded as the DSC amylopectin retrogradation measurement of that loaf. The DSC amylose-lipid complex retrogradation measurements were obtained by following the same method.

The DSC amylopectin and amylose-lipid complex retrogradation measurements were correlated with NIRS spectra separately. After choosing the calibration batches, the others were used as validation batches. In each validation batch, the NIRS-predicted amylopectin retrogradation data for all slices of one loaf were averaged and recorded as the NIRS amylopectin retrogradation measurements for that loaf.

## RESULTS AND DISCUSSION

DSC easily tracked starch retrogradation in bread. Amylopectin melting peak temperatures were 50–60°C for bread stored at low temperature and 60–70°C for bread stored at high temperature. Figure 3 shows the amylopectin retrogradation development with time during bread storage in batch A. Other batches behaved similarly. The averaged correlation coefficient ( $R^2$ ) of LSS, LSP, HSS, and HSP in all of the batches was 0.94, 0.93, 0.93, and 0.85, respectively. Amylopectin retrogradation trends of LSS and LSP fit better in logarithmic regression lines than in linear regression lines, while those of HSS and HSP fit better in linear regression lines. The sequence of all regression lines of all treatments was consistent: LSS, LSP, HSS, and HSP from the top to the bottom

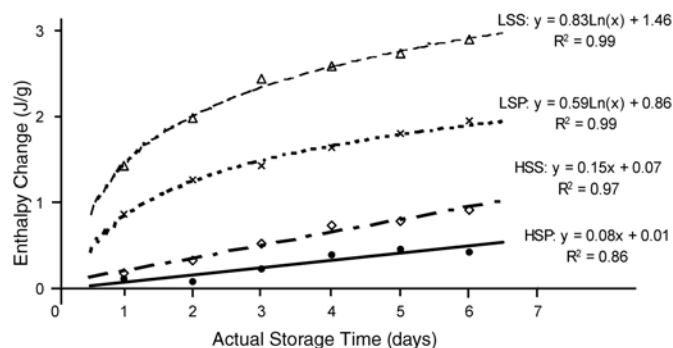


**Fig. 2.** Raw differential scanning calorimetry (DSC) thermogram of starch-starch (SS) and starch-protein (SP) bread in batch B stored at high temperature on day 3.

(Fig. 3). This indicates that low temperature accelerated the amylopectin retrogradation process, especially during the early three days of storage. Linear regression was also applied to LSS and LSP data to compare the amylopectin retrogradation rates of all the treatments. The slopes of all linear regression lines of all treatments decreased in the sequence LSS, LSP, HSS, and HSP for five batches. In each batch, the slope of LSS was always higher than that of HSS and the slope of LSP was higher than that of HSP. This indicates that amylopectin at low temperature retrograded more rapidly than that at high temperature. Starch crystallization has a negative temperature coefficient in the temperature range 4–60°C (Cornford et al 1964; Axford et al 1966; Slade and Levine 1987). High temperature retards bread staling by decreasing the starch retrogradation process.

It was also observed that the LSP regression line in each batch was always above the HSS line (Fig. 3). Furthermore, the slope of the LSP linear regression lines was higher than that of HSS. Even though LSP had 15% higher protein content than HSS, LSP staled faster than HSS. The results show that temperature dramatically affected the bread staling process. The effect of protein on bread staling was limited when compared with the temperature effect. At both temperatures, the regression line of SS was above that of SP (Fig. 3). This indicates that protein slowed down the bread staling process. The slope of the SS linear regression line was always higher than that of SP at both temperatures. In addition, the enthalpy ratios of SP and SS at day 4 of all the batches were <0.85. The reason to choose day 4 was that the staling was almost completed on day 4. If protein only diluted starch, the enthalpy ratio of SP and SS on day 4 would be close to 0.85 because the protein difference between them was 15%. The average ratio of all the batches was 0.49 at low temperature and 0.70 at high temperature. The DSC data used to calculate the ratio were adjusted from a dry bread basis to a dry starch basis because SS and SP had 45.7 and 38.8% of wheat starch, respectively. This shows that protein might retard bread staling not only by diluting starch as stated by Kim and D'Appolonia (1977a) and Every et al (1998), but also by interfering with amylopectin retrogradation. The lower ratio at low temperature also indicates that temperature affected the process of bread staling. Erlander and Erlander (1969) reported that protein inhibited starch retrogradation by forming a complex with starch. The amide group of glutamine protein interacts with a glucose unit by a hydrogen bond in either the amylose or the amylopectin chain. The interaction between protein and starch will be discussed later.

NIRS spectra correlated strongly with DSC-measured amylopectin retrogradation data in each batch (Table III). The range of  $R^2$  was 0.90–0.94 in the five batches. This indicates that NIRS was good at measuring amylopectin retrogradation in bread during storage. A calibration was developed by using samples in batches



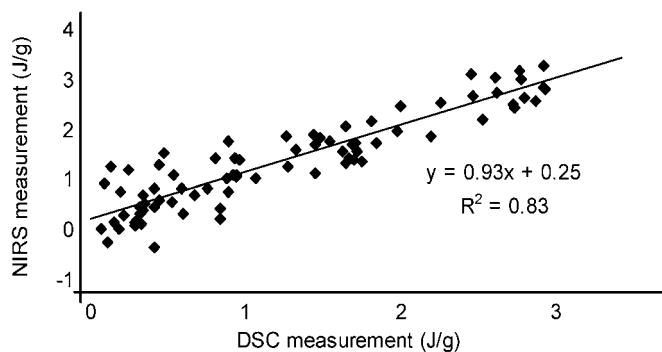
**Fig. 3.** Amylopectin retrogradation trend of starch-starch bread stored at low temperature (LSS) and high temperature (HSS), and the starch-protein bread stored at low temperature (LSP) and high temperature (HSP) from differential scanning calorimetry (DSC) in batch A.

D and E because they had the highest and lowest averaged  $R^2$ . The calibration was used to predict retrograded amylopectin in batches A–C. Figure 4 shows the correlation of retrograded amylopectin as measured by NIRS and by DSC. For the combined three batches, the overall  $R^2$  was 0.83 and standard error of estimation (SEE) was 0.37. The results in each validation batch are also summarized in Table III. The range of  $R^2$  was 0.90–0.96. The results show that NIRS could measure amylopectin retrogradation in other batches. The relative predictive determinate (RPD) is the ratio of standard deviation of reference data and standard error of estimation, which was used to evaluate the performance of the NIRS calibration (Williams 1987). An RPD = 1 means that the error of the calibration was at the same level as that of the reference method. The RPD values for each batch ranged from 3.7 to 5.0, indicating that NIRS can measure amylopectin retrogradation accurately.

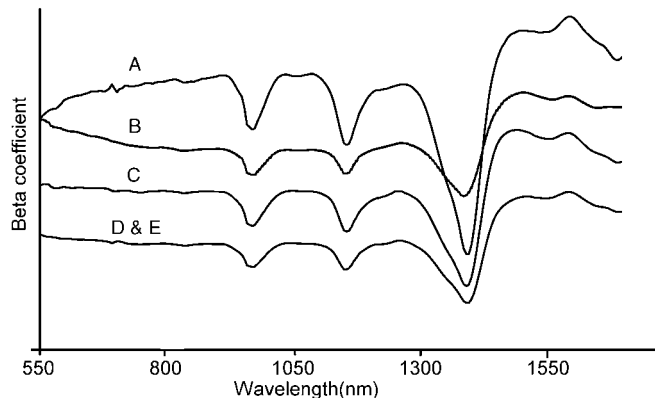
Figure 5 shows the beta coefficients obtained from batches A–C and calibration sample set batches D and E when three PLS factors were used. Three important wavelengths (970, 1,155, and 1,395 nm) are shown. The peak at 970 nm might correspond to the second overtone of O-H and might be due to moisture and starch structure changes. The peak at 1,155 nm might be due to the second overtone of C-H<sub>3</sub>. Finally, the peak at 1,395 nm might relate to the second overtone of C-H, C-H<sub>2</sub>, and C-H<sub>3</sub> (Murray and Williams 1987). Moisture works as a plasticizer in the starch-gluten network when starch retrogrades. Moisture molecules must migrate into the crystal region while starch chain segments are realigning. Amylopectin crystallization leads to the development of a crystalline structure with a  $\beta$ -type crystalline region. At saturation, the  $\beta$ -type crystalline region has 27% moisture (w/w) (Imberty and Perz 1988; Slade and Levine 1991; Zobel and Kulp 1996). This suggests that moisture migration contributed to the bread staling process. Amylopectin gelatinizes and loses its crystallinity during baking. During storage, amylopectin reforms into

double helical structures and reorganizes into the crystalline region. When bread is reheated, amylopectin crystallinity is disrupted again (Zobel and Kulp 1996). The peaks at 1,155 and 1,395 nm might correspond to starch structure changes. The important wavelength regions suggested in this study were similar to those given by Wilson et al (1991) and Osborne (1998), which were  $\approx$ 1,400 nm. They also indicated an important wavelength at 1,934 nm, which is a region where our instrument could not measure absorbance. Also, the wavelengths of 970 and 1,155 nm indicated in our study were not examined by Wilson et al (1991) and Osborne (1998).

NIRS-measured amylopectin retrogradation developments in validation batches A–C were studied. NIRS-predicted amylopectin retrogradation data for all the slices in a loaf were averaged and recorded as the NIRS measurement for the loaf. The results in validation batch B are shown in Fig. 6. The other two batches, A and C, behaved similarly. The sequence of regression lines of all treatments was the same as that obtained by DSC, except that HSS and HSP crossed around day 5 in batch A. The results indicate that NIRS could be used to study amylopectin retrogradation development in bread staling. It is reasonable to find this because previous results show that NIRS spectra had a high correlation to DSC-measured amylopectin retrogradation. The results confirm the conclusion drawn by Wilson et al (1991) and Osborne (1998) that NIRS could follow the process of bread staling. They found that the staling rate constant for DSC data was consistent with that calculated from NIRS data. The results also show that NIRS and DSC monitored different aspects during bread staling. In addition, the results show that NIRS could study the staling process of samples having extremely high or low protein contents and stored in various conditions. Previous research only investigated regular bread samples stored in room temperature and humidity. The  $R^2$  obtained by NIRS was lower than that obtained by DSC, perhaps



**Fig. 4.** Correlation of retrograded amylopectin measured by visible and near-infrared spectroscopy (NIRS) with that by differential scanning calorimetry (DSC) in validation batches A–C.



**Fig. 5.** Important wavelengths for amylopectin retrogradation from calibration batch D and E and validation batches A–C (beta coefficient spectra are offset for clarity).

**TABLE III**  
Results of Visible Near-Infrared Reflectance Spectroscopy (NIRS) Measured Retrograded Amylopectin

Batches	Cross-Validation			Validation <sup>a</sup>		
	Factors	$R^2$ <sup>b</sup>	SECV <sup>c</sup>	$R^2$	SEE <sup>d</sup>	RPD <sup>e</sup>
A	10	0.91	0.28	0.90	0.25	3.7
B	10	0.94	0.22	0.96	0.21	4.2
C	10	0.93	0.23	0.94	0.18	5.0
D	11	0.94	0.26	...	...	...
E	9	0.90	0.28	...	...	...
Average	...	0.92	0.25	0.91	0.21	4.3

<sup>a</sup> Calibration developed from batch D and E (9 factors) to measure staling of samples in batches A–C.

<sup>b</sup> Coefficient of determination is with retrograded amylopectin measured by differential scanning calorimetry (DSC).

<sup>c</sup> Standard error of cross-validation.

<sup>d</sup> Standard error of estimation.

<sup>e</sup> Relative predictive determinate.

because of the small sample size. In a loaf, six to seven slices were available for NIRS scanning. On each test day, only one loaf was tested for one treatment. Totally, six to seven measurements for each treatment on each day were available for NIRS data analysis. In future studies, 30 measurements per treatment on each test day would be recommended.

The amylose-lipid complex retrogradation was investigated using both DSC and NIRS in this study. Compared with amylopectin, the amylose-lipid complex had much less enthalpy and enthalpy change during storage. The amylose-lipid complex had an endothermic peak at  $114 \pm 5^\circ\text{C}$ , which was close to the value published by Eliasson (1994). Enthalpy values varied from 0.1 to 0.6 J/g for all treatments while that of amylopectin ranged from 0 to  $>3.0$  J/g. This implies that the amylose-lipid complex contributed less to bread staling than amylopectin. Figure 7 shows the amylose-lipid complex development in batch C. The regression lines for all treatments were almost flat. Similar results were obtained in the other batches. The results indicate that the amylose-lipid complex didn't change as much as the amylopectin did while in storage. This confirmed that amylose changed little one day after baking. Amylose contributes to bread staling primarily during the first day of storage but then changes little (Kim and D'Appolonia 1977b; Zobel and Kulp 1996). In all five batches, SS showed higher enthalpy values than SP, perhaps because less amylose was available in SP than in SS. The results agree with Kim and D'Appolonia (1977b). They noted that the effect of amylose on staling diminished as flour protein content increased because protein diluted starch.

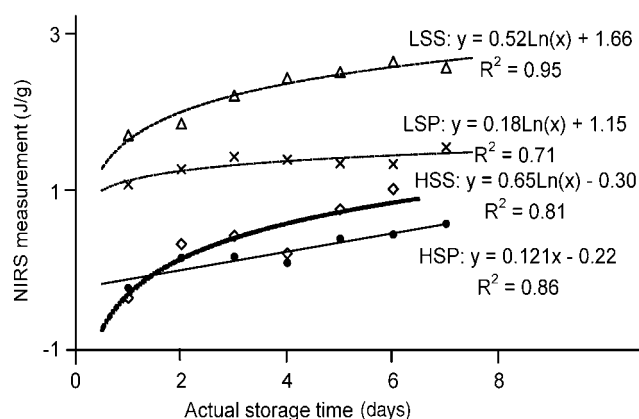
NIRS spectra correlated poorly with the amylose-lipid complex retrogradation data measured by DSC. In batch B, the  $R^2$  was 0.21 and 0.40 for SS and SP, respectively. The results show that NIRS had difficulty in measuring the retrograded amylose-lipid complex in both SS and SP. Because of the small enthalpy values of the retrograded amylose-lipid complex, DSC measurements could be affected by many factors such as lack of homogeneity in samples, uneven heating rates, and moisture distribution differences, etc. These factors also could cause difficulties for NIRS measurements. Even though NIRS did not accurately measure the amylose-lipid complex retrogradation, PLS results show that NIRS could differentiate between SS and SP successfully. The beta coefficients showed two important wavelengths (550 and 1,465 nm) that helped NIRS differentiate SS and SP. The peak at 550 nm showed that SS and SP had different colors. After baking, SS appeared whiter than SP because of the 15% protein in SP. The peak at 1,465 nm corresponded to the first overtone of N-H, which demonstrates that SS and SP had different protein contents.

NIRS has been used successfully in previous studies of bread staling, starch crystallinity, sucrose crystallinity, and starch damage by following hydrogen bond changes associated with O-H bonds in water and starch (Osborne and Douglas 1981; Davies and Miller 1988; Wilson et al 1991; Millar et al 1996; Osborne 1996). Gluten and starch can form a stable complex with chelation-type hydrogen bonds (Erlander and Erlander 1969). Hypothetically, this interaction could be detected by NIRS without any damage to the sample because it is a noninvasive method. Currently, no other analytical technique can investigate this interaction without sacrificing the integrity of the bread system. However, results from the current study show that this interaction was either too weak to be observed by NIRS or did not play an important role in bread staling.

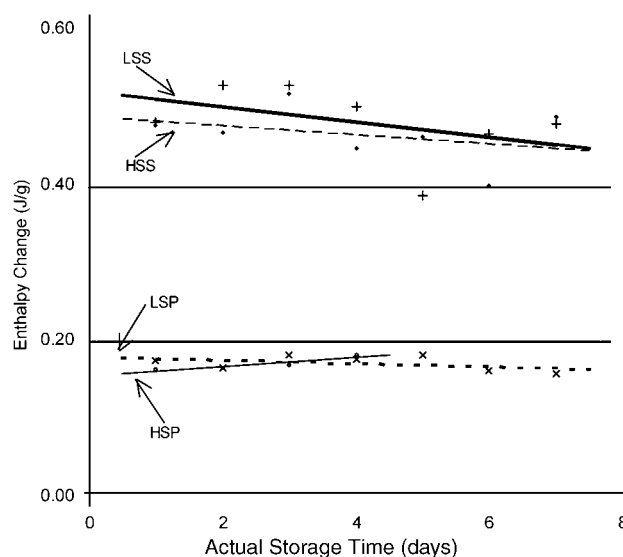
In brief, this demonstrates that the starch and protein interaction was not as important as starch retrogradation in bread staling. This confirms the conclusion drawn by Every et al (1998) that gluten-starch interaction was less important than starch retrogradation due to the lower concentrations of gluten. In addition, SS and SP had significantly different moisture contents and specific volume, which would affect the results because the NIRS absorption value was used directly in data analysis.

## CONCLUSIONS

NIRS could measure amylopectin retrogradation accurately in different batches. The averaged RPD value for NIRS measurements in validation batches was 4.3. NIRS and DSC obtained similar results when studying protein and temperature effects on amylopectin retrogradation development. However, NIRS had difficulty in measuring the changes of the amylose-lipid complex during storage. NIRS spectra not only provided information about changes in bread moisture and starch structure but also differences in bread color and protein content. Important wavelengths were 550, 970, 1,155, 1,395, and 1,465 nm. NIRS provides a useful approach to the study of the bread staling phenomenon. Bread staling is caused by changes in starch, protein, and moisture. Results confirmed previously reported findings that amylopectin retrogradation is likely the main factor in bread staling. The amylose-lipid complex contributes little to bread staling more than one day after baking. Temperature during storage significantly affects the amylopectin retrogradation process. Results also confirmed that protein retards bread staling mainly by diluting starch. The starch and protein interaction reduces the staling rate but is less important than starch retrogradation.



**Fig. 6.** Amylopectin retrogradation trend of starch-starch bread stored at low temperature (LSS) and high temperature (HSS), and the starch-protein bread stored at low temperature (LSP) and high temperature (HSP) as followed by visible and near-infrared spectroscopy (NIRS) in batch B.



**Fig. 7.** Amylose-lipid complex retrogradation trend of starch-starch bread stored at low temperature (LSS) and high temperature (HSS), and starch-protein bread stored at low temperature (LSP) and high temperature (HSP) measured by differential scanning calorimetry (DSC) in batch C.

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