The structural evolution of water and gluten in refined and whole grain breads: A study of soft and hard wheat breads from postmixing to final product

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Abstract

Background and objectives: Recent work investigating gluten secondary structure in dough systems has provided tantalizing insights as to how small perturbations in gluten structure may impact bread quality. Specifically, shifts in gluten structure from β-turns to β-sheets in doughs with bran may be partially responsible for quality defects in whole grain bread. This study aimed to further that knowledge by monitoring gluten secondary structure in soft and hard wheat refined and whole grain bread at different stages during processing. Commercial hard red spring (HW) and soft red winter wheat (SW) flours were used to make breads with whole grain doughs created by substituting bran for 15% of the refined flour. ATR-FTIR spectra were collected from samples and analyzed for gluten secondary structural content.

Findings: Freshly mixed doughs demonstrated the greatest β-sheet content (53%–57%) compared to any other dough stage. Refined HW doughs demonstrated continual relaxation of β-sheets to β-turns through dough processing (−13% to panning), whereas relaxed SW doughs (−6% to 10%) partially reverted to sheets during punching and panning (1%–3%). The data also confirmed earlier findings that the presence of bran triggers the formation of additional β-sheets (+4% to 5%) in both HW and SW doughs. Doughs with bran exhibited less structural relaxation (−3% to 6% β-sheets) during processing. Interestingly, HW doughs exhibited only 1%–2% more β-turns and only 1%–4% fewer β-sheets than SW doughs. Analysis of the bread during baking revealed that SW generates a larger sheets:turns ratio compared to HW in refined bread, 21.3 versus 3.6, respectively, that grows to 129 versus 5.3 on addition of bran.

Conclusions: Gluten structural relaxation takes place during proofing in optimally hydrated doughs and largely follows water relaxation trends. The ratio of sheets:turns increases rapidly as water is lost during baking. The extent of this build-up in the ratio is dependent on the wheat source for the flour (hard vs. soft wheat), presence/absence of bran, and the water loss (i.e., denaturation).

Significance and novelty: These results show that the ability of gluten to resist significant loss of bulk water during baking is related to better bread making performance. Additionally, the ability of gluten to resist substantial secondary structural
1 | INTRODUCTION

Water plays a crucial role in the formation of protein structural features because of continual rearrangement to achieve the lowest possible free energy state. In foods where water is relatively limited, both the overall water content and the water distribution are important drivers of protein structure up to a critical threshold where all proteins are optimally hydrated.

Beyond the role of water, the addition of mechanical energy can alter secondary structure of proteins in food systems. The most obvious example of this is the time relaxation phenomenon in wheat flour dough. As proposed by Belton (1999), mixing leads to the stretching of relatively flexible loops, or β-turns, eventually converting them to inflexible trains, or β-sheets. This is hypothesized to be the reason for the build-up in torque to a maximum at optimal dough development time. Results from Wellner et al. (2005) and Jazaeri et al. (2015) support this hypothesis in dynamic deformation and mixing studies, respectively. Mejia, Mauer, and Hamaker (2007) were additionally able to document the relaxation of secondary structures in a modified corn zein dough over a 7-min rest period, thus demonstrating support for secondary structural rearrangement during relaxation. However, the same authors were unable to definitively show relaxation of gluten protein secondary structure in the same 7-min time frame due to the longer relaxation time requirement of gluten.

The exploration of the role of protein secondary structure, specifically gluten secondary structure, in cereal-based systems is still in its infancy. One reason is that existing techniques were not suitable for the detection of secondary structural elements in gluten: X-ray diffraction requires the presence of a crystalline or semi-crystalline sample whereas circular dichroism (CD) spectroscopy requires the proteins be dispersed in a dilute solution. The gluten proteins glutenin and gliadin do not contain enough high order structure to generate crystalline samples of sufficient quality for X-ray diffraction, and they are also noted for their limited solubility in water. Additionally, neither technique is suitable for in situ measurement of protein structure in food systems making it difficult to draw conclusions about gluten secondary structure in dough or other applied systems. As a result, Fourier transform infrared (FTIR) spectroscopy has proven to be the best option for studying food systems such as dough because of the ability to nondestructively measure native systems with little to no sample preparation.

The second reason is that it is difficult to give absolute secondary structural distributions for wheat gluten due to its high degree of polymorphism. It can generally be stated that the major structures in flour and dough include β-sheets/β-strands, β-turns, aperiodic structures, and α-helices in order of decreasing amount based on existing reports (Bock, Connelly, & Damodaran, 2013; Bock & Damodaran, 2013; Cao, Falk, & Bock, 2017). However, Cao et al. (2017) benchmarked the secondary structure of more than 30 individual wheat varieties in flour, dough, batter, and bread samples and noted a significant amount of variation in the ranges for different structures depending on the particular variety of wheat, moisture content, and degree of processing (i.e., mixing and baking).

While most existing studies have focused on the secondary structural features of refined systems, there have been a few studies that have evaluated secondary structure in whole grain systems. Bock and Damodaran (2013) and Bock et al. (2013) found the presence of bran altered water state and structure in model gluten and bread dough systems, consequently leading to an increase in β-sheet structures at the expense of β-turns and/or aperiodic structures. This was hypothesized to be partially responsible for the detrimental impact of bran in whole wheat bread. The limitation of these and other studies is that they were conducted using static dough systems that failed to capture the full spectrum of processing.

Bock et al. (2015) tracked gluten structural features through a simplified pasta making process in both refined and whole grain macaroni and reported conversely that the presence of bran decreased β-sheet content with a concurrent increase in β-turns. The authors noted this difference and proposed that bran has an overt influence on gluten secondary structure but that it appears to be different depending on the process and product matrix.

In order to test this hypothesis, this study was designed to monitor gluten secondary structure through a simplified bread making process so as to facilitate a comparison between pasta and bread product systems. Refined and whole grain versions of bread were monitored from flour to final product, similar to that for pasta in Bock et al. (2015). Additionally, hard and soft wheat versions were compared to assess whether gluten proteins between wheats with different end uses adopt different secondary structural distributions when subjected to the same processing, thereby influencing quality of the resulting product. The results provide further insight into the role of...
2 | MATERIALS AND METHODS

2.1 | Flour and bran samples

Commercial flour samples were provided by P&H Milling (Acton, ON, Canada). They included a Canadian western hard red spring blend (12.1% protein content, 14% m.b.) and a Canadian eastern soft red winter blend (7.2% protein content, 14% m.b.), with corresponding bran samples included.

Model whole grain flours were created by substituting 15% (w/w) of the flour with bran and mixing to homogeneity in a Hobart mixer (A200T; Hobart Corp., Troy, OH, USA) according to the protocol described by Issarny, Cao, Falk, Seetharaman, and Bock (2017).

2.2 | Bread making and sample collection

All doughs and breads were prepared according to the AACCI approved method 10-10.03 using a 90-min fermentation and 33-min proof (AACCI International, 2011b). Optimal water absorption and dough development time were determined with a Farinograph-E (C.W. Brabender Instruments, Inc., South Hackensack, NJ) using AACCI approved method 54-21.02 (AACCI International, 2011a). Any necessary refinements for the water absorption and development time for the full formula doughs were made following the guidelines established by Finney (1984).

Dough samples were collected at each stage of the process for gluten secondary structure evaluation. These points included immediately postmixing; postproof #1 (52 min); postpunch #1; postproof #2 (25 min); postpunch #2; panning (13 min); and immediately prebaking (33 min). Samples were collected by cutting the dough with scissors to minimize structural alteration from dough handling and immediately assessed for secondary structure via FTIR spectroscopy.

In order to determine critical points at which to evaluate gluten secondary structure during baking, the internal bread temperature during baking was tracked using a modified Scorpion® humidity data logger (Reading Thermal Systems, Reading, PA) with temperature continuously recorded at one data point per second. A hole was drilled into the bread pan to allow a thermocouple probe to be inserted horizontally into the center of the dough mass parallel to the longest portion of the pan. Two replicate profiles were recorded to determine the average temperature profile depicted in Figure 1 with the selected evaluation points noted.

Bread samples were collected at 5, 8, 16, and 24 min of baking. The sample at 5 min was the point at which the internal temperature began to rise; 8 min was the inflection point of the internal temperature increase; 16 min was the point at which the temperature profile reached its asymptote; and 24 min was the full baking time of the bread. The samples were first allowed to cool for 1 hr before a sample was cut from the center of the bread for FTIR secondary structure assessment.

A set of two doughs was baked each time with the procedure completed in triplicate for a total of six loaves of bread for each treatment.

2.3 | FTIR spectroscopy

Fourier transform infrared spectra of flour, dough, and bread samples were collected on a Bruker Tensor 37 (Bruker Optics, Milton, ON, Canada) equipped with a potassium bromide (KBr) beam splitter and a deuterated tri-glycine sulfate (DTGS) detector. All samples were placed on a zinc selenide (ZnSe) horizontal multireflectance crystal and then clamped in place to ensure sample contact with the crystal and constant sample thickness. A total of 32 scans were collected at a resolution of 4 cm⁻¹ over a spectral range of 600–4,000 cm⁻¹ at ambient temperature. A total of six spectra were collected for each sample with a standard deviation of <5% using OPUS v. 7.0 software.

2.4 | Secondary structure analysis

Secondary structural analysis was conducted according to the method of Dong, Huang, and Caughey (1990) as modified by Bock and Damodaran (2013). Spectra were vector normalized to correct for differences in IR penetration depth before off-set correction to establish a zero baseline. Vector-normalized H₂O–D₂O reference spectra of the same water content were digitally subtracted along with a vector-normalized bran spectrum when appropriate. The difference spectra were subsequently analyzed in the 3,000–3,800 cm⁻¹ region to detect shifts in the state and/or structure of water due to processing and bran addition (Bock et al., 2013; Bock & Damodaran, 2013; Jain, Varshney, & Maitra, 1989; Sutander, Ahn, & Franses, 1994; Zelent & Vanderkooi, 2009).
The difference spectra were further second-derivatized using a five-point Savitzky–Golay derivative function and analyzed in the 1,600–1,700 cm⁻¹ amide I region to monitor changes in secondary structure. Secondary structure estimates were calculated from the second-derivative spectra as in Bock and Damodaran (2013) and other protein studies (Dong, Caughey, Caughey, Bhat, & Coe, 1992; Dong et al., 1990; Kalnin & Venyaminov, 1990; Kong & Yu, 2007; Susi & Byler, 1983). The contributions from different secondary structures (aperiodic, α-helices, β-sheets, and β-turns) are considered to be additive, and each structural feature will elicit a different absorption frequency in the amide I range even though the molar absorptivity of the C=O group of the peptide backbones is the same. Although amino acid side chains contribute to the IR absorption in the amide I region (Kalnin & Venyaminov, 1990; Kong & Yu, 2007; Susi & Byler, 1983), the contributions from different secondary structures are considered to be a minor constant factor in the secondary structure estimates given the overall similarity of samples (Bock & Damodaran, 2013).

The IR frequencies for each structural feature are listed in Table 1 along with values from other cereal studies. The IR frequencies of Bock and Damodaran (2013) were selected for use in this study given the similarity in model dough systems. The secondary structures were calculated by dividing the relative areas associated with the peaks at the denoted frequencies by the total area associated with all secondary structures as in Dong et al. (1990).

<table>
<thead>
<tr>
<th>Frequencies (cm⁻¹)⁴</th>
<th>Secondary structure⁴</th>
<th>Frequencies (cm⁻¹)⁵</th>
<th>Secondary structure⁵</th>
<th>Frequencies (cm⁻¹)⁶</th>
<th>Secondary structure⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,624</td>
<td>β-sheet</td>
<td>1,613–1,614</td>
<td>β-sheet, extended chain, glutamine side chains</td>
<td>1,618–1,622</td>
<td>β-sheet</td>
</tr>
<tr>
<td>1,627</td>
<td>β-sheet</td>
<td>1,629–1,632</td>
<td>β-sheet</td>
<td>1,627–1,633</td>
<td>β-sheet, extended chain</td>
</tr>
<tr>
<td>1,632</td>
<td>β-sheet, extended chain</td>
<td>1,649–1,650</td>
<td>Unordered (random), α-helix</td>
<td>1,641</td>
<td>β-sheet</td>
</tr>
<tr>
<td>1,638</td>
<td>β-sheet</td>
<td>1,665–1,670</td>
<td>β-turns</td>
<td>1,647–1,651</td>
<td>Unordered (random)</td>
</tr>
<tr>
<td>1,642</td>
<td>β-sheet</td>
<td>1,681–1,684</td>
<td>β-sheet</td>
<td>1,654–1,656</td>
<td>α-helix</td>
</tr>
<tr>
<td>1,650</td>
<td>Unordered (random)</td>
<td>1,698–1,699</td>
<td>β-turn</td>
<td>1,662</td>
<td>β-turn</td>
</tr>
<tr>
<td>1,665</td>
<td>α-helix</td>
<td></td>
<td></td>
<td>1,670</td>
<td>β-turn</td>
</tr>
<tr>
<td>1,666</td>
<td>β-turn</td>
<td></td>
<td></td>
<td>1,678–1,679</td>
<td>β-turn</td>
</tr>
<tr>
<td>1,672</td>
<td>β-turn</td>
<td></td>
<td></td>
<td>1,683</td>
<td>β-turn</td>
</tr>
<tr>
<td>1,680</td>
<td>β-turn</td>
<td></td>
<td></td>
<td>1,693</td>
<td>β-turn</td>
</tr>
<tr>
<td>1,688</td>
<td>β-turn</td>
<td></td>
<td></td>
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</tbody>
</table>

⁴Adapted from Dong et al. (1990). ⁵Adapted from Georget and Belton (2006). ⁶Bock and Damodaran (2013).

The difference spectra were further second-derivatized using a five-point Savitzky–Golay derivative function and analyzed in the 1,600–1,700 cm⁻¹ amide I region to monitor changes in secondary structure. Secondary structure estimates were calculated from the second-derivative spectra as in Bock and Damodaran (2013) and other protein studies (Dong, Caughey, Caughey, Bhat, & Coe, 1992; Dong et al., 1990; Kalnin & Venyaminov, 1990; Kong & Yu, 2007; Susi & Byler, 1983). The contributions from different secondary structures (aperiodic, α-helices, β-sheets, and β-turns) are considered to be additive, and each structural feature will elicit a different absorption frequency in the amide I range even though the molar absorptivity of the C=O group of the peptide backbones is the same. Although amino acid side chains contribute to the IR absorption in the amide I region (Kalnin & Venyaminov, 1990; Kong & Yu, 2007; Susi & Byler, 1983), the contributions from amino acid side chains were considered to be a minor constant factor in the secondary structure estimates given the overall similarity of samples (Bock & Damodaran, 2013).

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### 3 | RESULTS AND DISCUSSION

#### 3.1 | State and structure of water in doughs

The OH stretch bands (3,000–3,800 cm⁻¹) from the difference spectra for all doughs are presented in Figure 2. The difference spectra showed a trough at 3,410 cm⁻¹ and positive bands at 3,165 and ~3,580 cm⁻¹. The area of the trough represents the fraction of water that has been redistributed to the bands at 3,165 and ~3,580 cm⁻¹. The two sharper peaks between 2,800 and 3,000 cm⁻¹ are associated with lipids and were not assessed in this study.

Representative spectra for refined hard wheat doughs are shown in Figure 2a. The band at 3,165 cm⁻¹ represents a subpopulation of structured water molecules with a greater degree of hydrogen bonding (Cotugno, Iarobina, Mensitieri, Musto, & Ragosta, 2001; Jain et al., 1989; Liu, Wu, Ding, Chen, & Li, 2002), in this case to dough polymers (Bock et al., 2013). The band at 3,165 cm⁻¹ did not display a peak shift throughout the postmixing dough fermentation process, indicating that the energetic state of the water remained constant throughout the process. However, the absorbance intensity and area generally decreased the longer the time-lapse postmixing. The trend was not entirely consistent due to the processing being conducted by hand with greater associated variance in intensity compared to a more mechanized process. Nonetheless, the trend does show a significant decrease in band absorbance intensity and area as the fermentation period progresses.

The band at ~3,580 cm⁻¹ represents a subpopulation of water with relatively less hydrogen bonding, consisting generally of monomeric water molecules (Jain et al., 1989). It is also a higher energy subpopulation than the subpopulation at 3,165 cm⁻¹. The absorbance peak continually shifted for the ~3,580 cm⁻¹ band as the time postmixing increased, starting initially at 3,566 cm⁻¹ immediately postmixing and gradually shifting to 3,590 cm⁻¹ prebaking. Energetically speaking, this is shift toward higher energies. As with the band at 3,165 cm⁻¹, the absorbance intensity and band area decreased as time progressed postmixing.

Put together the changes in both subpopulations of water indicate a relaxation phenomenon where water gradually
partitions between lower and higher energy states as the fermentation process proceeds. Water begins in a more highly structured state immediately postmixing as evidenced by the greater absorbance intensity and band area at 3,165 cm$^{-1}$ combined with the initial peak frequency of 3,566 cm$^{-1}$. As time progresses, the structured water subpopulation at 3,165 cm$^{-1}$ decreases (loss of absorbance intensity and band area) correspondent with an increase in the area of the trough at 3,410 cm$^{-1}$ and an energetic shift of the subpopulation at 3,590 cm$^{-1}$.

A similar phenomenon is observed with refined soft wheat dough as shown in Figure 2b. However, the trend is not as consistent nor as large as that for refined hard wheat dough. The structured subpopulation centered at 3,190 cm$^{-1}$ does not show the same gradual decrease in absorbance intensity and band area. Rather, it is more abrupt in nature with samples forming three distinct groupings. The less structured subpopulation displays a similar absorbance frequency shift from 3,560 to 3,579 cm$^{-1}$, but does not demonstrate the same degree of absorbance intensity and band area decrease.

Although it is evident that a similar water partitioning is occurring in refined soft wheat doughs as fermentation progresses, it appears to be less significant and more abrupt when compared to refined hard wheat dough. The tighter wave number range of the overall OH stretch band also indicates that water is present in a smaller range of energetic states compared to that for refined hard wheat dough.

The OH stretch spectra for whole wheat hard wheat dough (Figure 2c) display a trend more similar to that for refined soft wheat dough. The structured water subpopulation presents at 3,159 cm$^{-1}$ while the less structured subpopulation presents at 3,583–3,600 cm$^{-1}$. The water subpopulations here show a more extreme partitioning in the presence of bran than observed for the original refined hard wheat dough. This is due to the greater water structuring ability of bran compared to gluten as described by Bock and Damodaran (2013) and Bock et al. (2013).

When compared to whole wheat hard wheat doughs, the OH stretch band for whole wheat soft wheat doughs (Figure 2d) again demonstrates a narrower distribution of water populations (3,161 cm$^{-1}$ and 3,575–3,579 cm$^{-1}$). It is still wider than that for refined soft wheat dough due to the presence of bran and its tendency to redistribute water within the system. However, it again indicates a lesser degree of relaxation during the fermentation process.

### 3.2 State and structure of water in breads during baking

The OH stretch bands from the difference spectra for refined hard wheat flour breads during baking are presented in Figure
The original band at 3,165 cm\(^{-1}\) displayed a gradual shift to higher wave numbers (3,178 cm\(^{-1}\)) over the course of baking, indicating that the energetic state of the structured water subpopulation increased throughout the process. The energetic shift was concurrent with an increase in the absorbance intensity and area, although the shift occurred sharply between 8 and 16 min of baking.

Additionally, the original band at ~3,580 cm\(^{-1}\) generally exhibited gradual peak shifts to lower energy states (3,551 cm\(^{-1}\)) along with increases in absorbance intensity and area over the duration of baking. The sole exception to the trend occurred at 8 min where a higher energy state and lower absorbance intensity and area were observed.

The observed changes in the original band at 3,165 cm\(^{-1}\) are consistent with a loss of water structuring as proteins go through the denaturation transition. This was most clearly observed as the sharp shift between 8 and 16 min as internal bread temperature initially reached ~60°C at 8 min and ~96°C at 16 min. That temperature range is consistent with protein denaturation in a moisture limited system such as bread dough (Georget & Belton, 2006). The increase in absorbance intensity and area indicate that an increasing proportion of water is structured as bread bakes, likely through increased interactions with partially gelatinized starch polymers. Despite this increase in structured water, it appears that the starch polymers do not structure water as tightly as gluten proteins as evidenced by the gradual shift to higher energy states.

The trends for the original band at ~3,580 cm\(^{-1}\) are consistent with loss of sample moisture. Higher energy water subpopulations revert to energy states closer to that of bulk water as the total population of water slowly decreases. The increase in proportion of less structured water is indicative of the weaker water structuring capabilities of the partially gelatinized starch polymers (Liu et al., 2002).

**FIGURE 3** Representative difference spectra of dough at various points during the baking stage of bread making: (a) refined hard wheat doughs; (b) refined soft wheat doughs, (c) whole wheat hard wheat doughs; and (d) whole wheat soft wheat doughs [Color figure can be viewed at wileyonlinelibrary.com]
The interesting phenomenon observed in the original band at ~3,580 cm⁻¹ at 8 min of baking may be related to the protein and starch transitions that begin at ~60°C and their associated influence on water structuring. As protein denaturation begins, it is conceivable that there would be a broadening of the OH stretch band as water is slowly being released by proteins. Starch polymers would begin restructuring this newly liberated water around the same time, but the observed changes in OH stretch difference curves indicate that the kinetics are not identical between the two transitions. While this is an initial explanation that is consistent with the results, it will require further study to fully confirm and explain the phenomenon.

Put together, the results for refined hard wheat flour dough show that a major transition occurs in water state and structure around 8 min of baking. All water becomes more similar to bulk water, with the more structured subpopulation showing slightly weaker structuring to the polymers in the system and the less structured subpopulation exhibiting lower energy states. This is consistent with the loss of water that occurs during baking.

Water structural trends observed for refined soft wheat flour breads during baking (Figure 3b) are roughly similar to those for refined hard wheat flour breads. The most significant difference is the significant decrease in the trough region (or bulk water) at ~3,410 cm⁻¹ as baking proceeds. This is partially reflective of the lower initial water absorption requirements of refined soft wheat flour doughs, about 3% lower in this particular study. As a result, the water subpopulations merge into a bimodal curve where the more structured water subpopulation dominates and the less structured water subpopulation appears as a shoulder.

The gradual loss of the original band at ~3,580 cm⁻¹ indicates that refined soft wheat flour breads are not as capable of retaining sufficient bulk water as refined hard wheat flour breads, and that the less structured water subpopulation must transition to a more structured state to compensate. Indeed, the initial 3% moisture content difference between refined hard and soft wheat flour doughs widens to 6% between the final breads under identical conditions (Table 2).

The addition of bran to whole wheat hard wheat breads induces more nuanced changes in the OH stretch difference spectra over the duration of baking (Figure 3c). Surprisingly, the major shift occurs more toward 16 min instead of 8 min, possibly due to the tendency of bran to maintain water structuring throughout baking. Not only does bran outcompete gluten for available water (Bock & Damodaran, 2013), it would be less likely to release that water during baking. This would potentially serve to delay protein denaturation and starch gelatinization. It also would create conditions for fewer transitions among water subpopulations.

The changes in the OH stretch difference spectra of whole wheat soft wheat breads (Figure 3d) are also more tempered in the presence of bran compared to its refined counterpart. Again, the major shift occurs later in the baking. However, the shift occurs at 24 min (full bake) for whole wheat soft wheat breads compared to 16 min for whole wheat hard wheat bread. If this is indeed related to a delay in protein denaturation in the presence of bran as postulated for whole wheat hard wheat breads, it is consistent with the OH stretch observations for refined soft wheat breads that indicate an inability to maintain bulk water which would be exacerbated in the presence of bran.

### Table 2  Bread and dough moisture contents for hard and soft refined and whole wheat doughs and breads

<table>
<thead>
<tr>
<th>Flour</th>
<th>Sample</th>
<th>Moisture content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refined hard wheat flour</td>
<td>Dough</td>
<td>38.4 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>32.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>8 min</td>
<td>30.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>16 min</td>
<td>26.8 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Full bake</td>
<td>25.4 ± 0.3</td>
</tr>
<tr>
<td>Refined soft wheat flour</td>
<td>Dough</td>
<td>35.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>28.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>8 min</td>
<td>23.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>16 min</td>
<td>21.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Full bake</td>
<td>19.9 ± 4.8</td>
</tr>
<tr>
<td>Whole wheat hard wheat flour</td>
<td>Dough</td>
<td>42.5 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>37.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>8 min</td>
<td>32.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>16 min</td>
<td>31.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Full bake</td>
<td>29.9 ± 0.3</td>
</tr>
<tr>
<td>Whole wheat soft wheat flour</td>
<td>Dough</td>
<td>39.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>31.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>8 min</td>
<td>25.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>16 min</td>
<td>23.7 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Full bake</td>
<td>24.8 ± 1.0</td>
</tr>
</tbody>
</table>

*Note. n = 6.*

### 3.3 Secondary structures in doughs

The evolution of secondary structures for hard wheat doughs during proofing is shown in Figure 4a. The overall trend for hard wheat dough is indicative of structural relaxation, similar to observations of Mejia et al. (2007). Beta-sheet structures initially comprise ~56% of all secondary structures immediately postmixing, but gradually decrease to ~45% by the time the dough is ready to enter the oven. Concomitantly, β-turns and aperiodic/α-helical structures increase by +5% and +6%, respectively, over the same time period. This is despite mechanical energy input at the punching and panning stages. It appears based on this evidence that the mechanical
energy was not of sufficient duration or intensity to significantly impede structural relaxation. However, it should be noted that structural relaxation may be more punctuated in a commercial process where energy input may be more intensive due to automation.

Soft wheat dough also exhibited structural relaxation during proofing, but the effect was more muted than that observed for refined hard wheat doughs (Figure 4b). Whereas relaxation for hard wheat doughs was more curvilinear with greater relaxation taking place in the earlier stages of proofing before stabilizing, structural relaxation in soft wheat dough was more linear over the proofing period. The total decrease in β-sheet structures was on the order of −9% with a concurrent increase in β-turns and aperiodic/α-helical structures of +7% and +2%, respectively.

Introducing bran to hard wheat dough resulted in significant inhibition of the structural relaxation process (Figure 4c). There was a slight decrease in overall β-sheet structures (−2%) compared to refined hard wheat dough, but what was most compelling was that structural relaxation during proofing only proceeded to reduce β-sheets by −2%. Beta-turns and aperiodic/α-helical structures increased by +1.5% and +0.5%, respectively, in the same time period. These results show that bran has a significant inhibitory effect on structural relaxation in hard wheat whole wheat bread doughs. This can be explained through altered water state and structure inducing structural limitation on gluten proteins (Bock et al., 2013; Bock & Damodaran, 2013). It should be noted, however, that physical interference may also exist for gluten proteins in the presence of bran and therefore cannot be discounted as a potentially contributing factor (Gan, Ellis, Vaughan, & Galliard, 1989).

Soft wheat doughs also exhibited more limited structural relaxation during proofing in the presence of bran (Figure 4d). Adding bran resulted in a slight increase in β-sheet structures (+4%) compared to the refined soft wheat counterpart, but relaxation only proceeded to decrease β-sheets by ~3% in soft wheat whole wheat doughs prior to baking. Just as for hard wheat whole wheat doughs, most of the decrease in β-sheet
structures was compensated for by an increase in β-turns (+2.5%) as opposed to aperiodic/α-helical structures (+0.5%).

3.4 Secondary structures during bread baking

Secondary structures were also tracked during the baking process to provide observations on the structural transformations taking place in the oven. Hard wheat doughs exhibited major structural changes within the first 5 min of bake time (Figure 5a). The distribution of secondary structures was 44.6% β-sheets, 34.4% aperiodic/α-helical structures, and 21% β-turns. This distribution was altered to nearly equal proportions of 34.2%, 35.0%, and 30.8%, respectively, by the 5-min mark of baking. It is an intriguing finding considering that the internal temperature of the bread dough has not yet begun to significantly increase (Figure 1). It suggests that structural transformation begins well before denaturation temperatures are achieved. As baking progresses beyond 5 min, β-sheets increase (+25%) at the expense of all other structures through the 16-min mark, just after observed spectral water alterations at 8 min of baking indicative of temperature induced water loss (Figure 3a). There is a small amount of structural reorganization between 16 and 24 min of baking (likely due to redistribution of the remaining water as seen in Figure 3a), mainly between β-sheets (−3%) and β-turns (+4%), but it is clear from the data that the most significant structural transformations take place in the first 16 min of baking.

Soft wheat doughs followed a similar structural progression through the baking process (Figure 5b), but, as with structural relaxation during proofing, it was muted compared to the observations for refined hard wheat dough. The structural transformations that took place during the first 5 min of baking were not as extensive. Beta-sheets and β-turns declined to 43% (−1.3%) and 19.1% (−5.0%), respectively, while aperiodic/α-helical structures increased to 37.9% (+6.3%). Structural transformations in soft wheat doughs continued through the end of baking. Unlike hard wheat doughs, soft wheat doughs continued to exhibit a build-up of β-sheets to 66.8% of total secondary structures through the full baking time. Beta-turns were nearly lost, decreasing to only 3.1% of total secondary structures, while aperiodic/α-helical structures exhibited a smaller decrease to 30.1%. The final secondary structural distribution is significantly different between hard and soft wheat breads, with the latter more heavily favoring β-sheet and aperiodic/α-helical structures. This difference in structural distribution suggests that β-turn structures are an important aspect in bread structure and loaf volume. Bock and Damodaran (2013) and Bock et al. (2013) pointed to the ability of hard wheat doughs to maintain β-turn structures as potentially critical to the quality of bread, and these findings would appear to support that hypothesis.

The addition of bran to hard wheat dough led to behavior that was intermediate to that of refined hard and soft wheat doughs (Figure 5c). Hard wheat whole wheat doughs exhibited a structural redistribution in the first 5 min of baking that
FIGURE 6  Sheets:turns ratio of doughs at various points in the proofing and baking stages of bread making: (a) refined hard wheat doughs; (b) refined soft wheat doughs; (c) whole wheat hard wheat doughs; and (d) whole wheat soft wheat doughs [Color figure can be viewed at wileyonlinelibrary.com]
was similar to that for hard wheat dough: β-sheets = 36.6%, β-turns = 30.6%, and aperiodic/α-helical structures = 32.7%. However, the final structural distribution of hard wheat whole wheat breads, similarly to refined soft wheat breads, more heavily favored β-sheet and aperiodic/α-helical structures: β-sheets = 60.9%, β-turns = 11.6%, and aperiodic/α-helical structures = 27.5%. Again, this points to a loss of β-turns as being a critical aspect in loss of loaf volume. As noted in Figure 3c, major water alterations occur at 16 min of baking in hard whole wheat doughs as compared to the refined counterpart. This may explain the lack of structural reorganization in hard whole wheat doughs before baking is finished.

Soft wheat whole wheat doughs closely followed structural distribution patterns for refined soft wheat doughs as seen in Figure 5d. Secondary structural redistribution within the first 5 min of baking resulted in values that were close to those observed for the refined soft wheat counterpart: β-sheets = 42.1%, β-turns = 20.7%, and aperiodic/α-helical structures = 37.2%. However, by the end of baking β-turns were reduced to the almost negligible level of 0.5% of total secondary structures with the redistribution accruing exclusively to β-sheets. Aperiodic/α-helical structures exhibited a comparatively minor loss of ~ 4%. Thus, it is apparent in both hard and soft wheat doughs that the presence of bran results in a significant reduction in β-turns that negatively influences final loaf volume, and it appears that water redistribution is a major driver (Bock et al., 2013; Bock & Damodaran, 2013) as opposed to physical interference (Gan et al., 1989) when combined with the observed timing and pattern of spectral changes.

One final aspect of secondary structure to consider is the ratio of different secondary structures to one another to understand structural transformations taking place during processing (Marti, Bock, Pagani, Ismail, & Seetharaman, 2016; Quayson, Marti, Bonomi, Atwell, & Seetharaman, 2016). The sheets:turns ratio of each dough is plotted throughout the bread making process in Figure 6. As shown in Figure 6a, sheets:turns never exceed 5.0 throughout the entire bread making process for hard wheat doughs, and there is a significant structural reorganization that takes place between 16 and 24 min of baking (fully baked bread) that drops the ratio to 3.6. Soft wheat doughs, on the other hand, exhibit a continued build-up in the sheets:turns ratio through the end of baking (Figure 6b). The final sheets:turns ratio is 21.3 in the final baked product. Figure 6c shows how structural reorganization at the end of baking is impeded in hard wheat doughs when bran is added to the system. The sheets:turns ratio reaches a maximum at 16 min of baking and remains relatively stable at ~5.1 through the end of baking. Adding bran to soft wheat doughs exaggerates the tendency to build β-sheets at the expense of β-turns (Figure 6d). The final sheets:turns ratio in soft wheat whole wheat doughs is 129.

Examination of the sheets:turns ratio casts the role of β-turns in a different light. Although bread is structurally stabilized by starch in the final product, these results show that proteins have a role to play in maintaining loaf volume through the ability to maintain a certain critical threshold of β-turn structures. Hard wheat seems to have a greater propensity to maintain these structures as observed in this study and others (Bock et al., 2013; Bock & Damodaran, 2013; Quayson et al., 2016), and thus, this may explain why it is superior to soft wheat in bread making in both refined and whole wheat formats.

4 | CONCLUSIONS

Fourier transform infrared spectroscopic study of refined and whole wheat doughs has provided insight on the structural evolution of gluten through the bread making process. Specifically, gluten structural relaxation takes place during proofing in optimally hydrated doughs and largely follows water relaxation trends. The extent of relaxation is greater in hard wheat doughs, and the addition of bran appears to interfere with this relaxation phenomenon through stronger partitioning of water although it should be noted that some degree of physical interference cannot be discounted. Perturbations in water state and structure after mechanical energy input (i.e., punching and panning) appear to be too transient to have any impact on the progression of relaxation.

The ratio of sheets:turns increases rapidly as water is lost during baking. The extent of this build-up in the ratio is dependent on the wheat source for the flour (hard vs. soft wheat), presence/absence of bran, and the water loss (i.e., denaturation). Lower sheets:turns ratios favor better bread loaf volume, most likely through a better ability to contribute to structural integrity and retain gas cells during the initial stages of baking.

ACKNOWLEDGMENTS

I would like to acknowledge the assistance of Dr. Shane Walker in capturing the internal dough temperature profile during baking and the late Dr. Koushik Seetharaman for the encouragement to pursue the study.

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How to cite this article: Bock JE. The structural evolution of water and gluten in refined and whole grain breads: A study of soft and hard wheat breads from postmixing to final product. *Cereal Chem.*, 2019;96:520–531. https://doi.org/10.1002/cche.10152