Rapid analysis of Chinese steamed bread staling using Raman Spectroscopy

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Abstract

Staling is an important issue that Chinese steamed bread (CSB) may encounter during storage, which significantly affects their taste, flavor, and nutritional value. The monitoring technology for rapid aging is particularly important to effectively control the aging process of CSB, reduce quality deterioration, and promote the industrial production of CSB. Raman spectroscopy has been widely used in the study of food structure and properties due to its non-destructive and high-sensitivity characteristics, particularly demonstrating unique advantages in the analysis of starch structure. This study explored the possibility of analyzing the staling of CSB using Raman spectroscopy based on hardness and moisture content. Analysis of the correlation between the hardness of CSB and the full width at half maximum (FWHM) at 480 cm−1 during staling was conducted, and a significant positive correlation between them was found, with R² above 0.8. Besides, nine characteristic peaks of CSB samples related to starch were selected for analysis. As the moisture content of CSB decreased, the peak intensities and areas of showed an upward trend during storage, with the best correlation coefficient above 0.8 revealed by linear regression analysis. Therefore, Raman spectra could be used as a potential method for the fast prediction of CSB staling.

Keywords: Chinese steamed bread; staling; hardness; moisture content; Raman spectroscopy
**Introduction**

Chinese steamed bread (CSB), made from fermented wheat flour, is a traditional staple food in China for many centuries. It's predominately crafted in Northern China, where small-scale artisans and home kitchens have traditionally been the custodians of this culinary tradition. However, the swift pace of urbanization has necessitated a shift towards more efficient, large-scale, and productive methods of CSB manufacturing [1, 2]. The primary challenge faced in the transition from small-scale to large-scale production is the rapid staling of CSB during storage, since staling leads to a reduction in the aroma, deterioration in texture, increased hardness, and a shortened shelf life, which in turn affects their commercial value and consumer acceptance [3]. Therefore, rapid staling monitoring of CSB is particularly important, to better control the staling process of CSB.

Common methods used for CSB staling analysis include moisture analysis and texture profile analysis, which are accurate and reliable, but time-consuming and require destructive pre-treatment [4, 5]. Besides, there are methods for directly monitoring the staling process of starch in CSB based on the multi-scale structural characteristics of starch, such as Electron Microscope (EM) visualization and X-ray diffraction (XRD) [6, 7]. Starch structural characterization methods can be mainly classified into three categories of long-range crystalline structure, double-helical short-range ordered structure, and starch granule structure. Electron microscopy is an important tool for observing the starch granule structure, with commonly used techniques including optical microscopy (LM), scanning electron microscopy (SEM), and confocal laser scanning microscopy (CLSM).

Among them, SEM is primarily used to reveal morphological features of starch, such as granule shape, surface pores, and cracks. In contrast, CLSM requires staining of the samples to observe the internal pore structures and surface proteins of starch granules [8, 9]. XRD is widely used to characterize the long-range crystalline structure of starch, but it tends to mistake lattice/crystal defects or diffuse scattering of small crystals as amorphous backgrounds, understimating the actual crystal content in starch, which subsequently leads to lower calculated starch crystallinity [10, 11]. In contrast, Raman spectroscopy reflect the vibrational and rotational energy levels of the molecular structure of the sample, which has also been used for detecting the short-range order of starch, with advantages such as non-destructive detection of small samples, high sensitivity, low signal-to-noise ratio, and resistance to moisture interference [12, 13]. Liu et al. applied Raman spectroscopy to quantitatively characterize the short-range order in non-crystalline starch, and the results indicated that the ratio of the Raman spectrum area for the short-range ordered phase in gelatinized starch to that in just-gelatinized starch can be used to quantify the degree of short-range order in amorphous starch [14]. Lu et al. applied Raman spectroscopy to evaluate the long- and short-range order of retrograded starch, and the Full Width at Half Maximum (FWHM) of the peak at 480 cm⁻¹ is an important indicator for assessing the short-range order of the double helix, which have been proven to be closely related to the characterization of hardness and moisture content associated with CSB staling [4, 15, 16]. To the best of our knowledge, the characteristic peak of Raman spectrum combined with hardness and moisture content to analyze the staling of CSB hasn’t been investigated.

Therefore, the purpose of this study is to explore the feasibility of Raman in evaluating the staling of CSB using based on hardness and moisture content. The specific objectives are (1) to determine the change law of hardness and moisture content of CSB stored at room temperature; (2) to select the the Raman characteristic peaks related to hardness and moisture content; (3) to establish the relationship between Raman characteristic peak and hardness and moisture content. The result provides a new method for comprehensive and rapid detection of CSB deterioration, and also providing a theoretical basis for online monitoring of food safety for wheat flour products.

**Materials and Methods**

**CSB samples**

Fresh CSB samples were purchased from Yongwang Supermarket in Dongxiu District, Wuhan City, Hubei Province, and then transported directly to the laboratory. The samples were stored at room temperature (a average temperature of 24.7°C) for six different sampling times (1, 2, 3, 4, 5, and 6 days). At each sampling time, three samples were taken from the batch, and their core sections were used directly for spectral analysis. Subsequently, the hardness and moisture content of these core sections were measured.

**Determination of texture properties**

CSB samples were cut into uniform slices with thickness of 15 mm, and two slices in the center of the sample were measured by a physical property analyzer (TMS-PRO, Bosin Tech, Shanghai, CHN) using a cylindrical probe with a diameter of 12.7 mm. The deformation level was 50% of the original sample height and a double compression test was conducted. Before the test, the testing speed and trigger force were set to 180 mm/min and 1N, respectively. The probe height was 15 mm higher than the thickness of the CSB sample, and the first compression peak was defined as the hardness.

**Determination of moisture content**

In order to mitigate the influence of external factors, samples with thickness of 1 cm at the top and bottom of CSB were removed [17], and the remaining part was used for the determination of moisture content according to approved method 44-15A (AACC, 2000) [18].

**Collection of Raman spectra**

Place the bulk CSB sample on the slide, with the flatter side facing up. Raman spectra of samples were collected on a confocal micro-Raman system (model in Via Qontor; Renishaw Co., London, UK) equipped with a 785 nm laser, a 1200 lines/mm diffraction grating and a cooled charge-coupled detector under Leica microscope system (a 50 × long-working distance objective and a 10 × eyepiece lens). The WIRE 5.3 software package (Renishaw Co., London, UK) was used to operate the Raman spectral acquisition. The scanning range was established to be from 3200 to 100 cm⁻¹, with a laser power set at 100% and an exposure time of 10 seconds. Each CSB sample was measured three times independently, and the mean spectrum was calculated for subsequent analysis.

**Data processing**

The significance analysis of the data was conducted using IBM SPSS Statistics 19 software. Additionally, the Raman spectra were preprocessed with WIRE5.3 software to obtain high signal-to-noise ratio images. Furthermore, Origin 2021 and Excel were employed to create visualizations and perform analysis of the Raman data.

**Results and Discussion**

**Analysis of changes in hardness and moisture content of CSB during storage**

The hardness of CSBs are the main parameters to characterize the texture properties of CSB, and they could reflect the aging of CSB. Currently, the aging phenomenon of CSB has been widely studied, and the main mechanisms of aging include starch recrystallization, moisture migration and loss, as well as the interaction between starch and protein [2]. In fresh CSB, the gelatinized starch is tightly embedded within the gluten protein network structure, making it difficult to distinguish the boundaries between the two. However, in aged CSB, the volume of starch granules significantly decreases, indicating that the staling of CSB is accompanied by the phenomenon of starch recrystallization [19]. During the storage process, the originally extended branch-chain starches gradually move closer and fold, resulting in increased rigidity of their internal structure and subsequently leading to the hardening of CSB.

As shown in Figure 1(b), there was a noticeable alteration in the hardness of CSB during their storage at room temperature in sealed packaging. The hardness notably increased with the extension of...
storage time. Within the first two days of storage, the hardness indicator underwent a significant change, after which the extent of variation gradually diminished. This suggests that the CSB experienced an aging process, particularly during the initial phase of storage.

At the same time, water also plays an important role in the aging process, which can increase the flexibility and ductility of polymers [3]. The moisture content of CSB decreased as the storage time increased, as shown in Figure 1(a). During this period, the change in moisture content was relatively small, which may be related to the storage method of CSB. The moisture loss of the sealed packaged CSB was relatively slow.

Raman spectral features of CSB during storage

The Raman spectral of CSB samples during storage is shown in Figure 2(a). Raman spectroscopy can characterize the structural vibration information of different molecular groups, and the intensity of its characteristic peaks varied with the concentration of the groups [20]. As shown in Figure 2(a), peaks of CSB were observed at 439, 480, 576, 860, 940, 1080, 1131, 1460, and 2190 cm⁻¹. The peak at 439 cm⁻¹ is skeletal modes of carbohydrate rings (C-CO-) [21], and the peak observed near 480 cm⁻¹ is skeletal mode involving (C-OC) ring mode [22]. The peak at 576 cm⁻¹ may be responsible for skeletal modes CC-stretch and skeletal modes of pyranose ring [23], and the band near 860 cm⁻¹ is related to ν(C-OC) ring mode and C-H-bending α-configuration [23]. The band at 940 cm⁻¹ is due to ν(C-OC) α-1, 4-glycosidic linkage [22], and the peak at 1080 cm⁻¹ may be assigned to the C-OH bending [24, 25]. In addition, the peak of 1131 cm⁻¹ is related to the C-O stretching and COH bending [24]. The peak at 1460 cm⁻¹ is CH₂ bending [24], and the peak observed near 2910 cm⁻¹ is CH-stretching [26]. Changes in Raman spectra during storage are shown in Figure 2(b). Compared to the Raman spectrum of CSB sample on the first day, the Raman peak intensity at 480 cm⁻¹ was significantly enhanced in the sample stored for the sixth day. The band at 480 cm⁻¹ is characteristic peak of starch that is known to be sensitive to starch crystallinity [26]. During storage, as CSB staled, the degree of crystallization continued to increase, resulting in a constant enhancement of the Raman peak intensity at 480 cm⁻¹.

Analysis of Raman Spectroscopy in the Detection of Hardness of CSB

The magnitude of FWHM at 480 cm⁻¹ exhibits a negative correlation with the degree of short-range orderliness. As the starch crystals undergo disruption, the FWHM value demonstrates a strong negative correlation with relative crystallinity. Over time, the peak position of CSB at 480 cm⁻¹ remained statistically insignificant. However, the FWHM at 480 cm⁻¹ significantly decreased with prolonged storage, as shown in Table 1. The results indicated that as the storage duration increased, CSB samples undergo aging, resulting in an increase in crystallinity and a subsequent decrease in the FWHM value at 480 cm⁻¹.

This section focuses primarily on the 480 cm⁻¹ band, where the Raman frequency remains consistently stable throughout the entire storage period, exhibiting no significant trend. However, within the same timeframe, the Full Width at Half Maximum (FWHM) of the 480 cm⁻¹ band consistently demonstrates a decreasing trend (Table 1). As the storage time increased, shifts in the Raman spectra were exclusively ascribed to the retrogradation of amylopectin, rather than the retrogradation of amylose. This could be attributed to the higher prevalence of amylopectin over amylose in wheat starch, coupled with the distinct retrogradation patterns of the two components. Initially, during the staling process, the retrogradation of amylose was predominant, but over time, the retrogradation of amylopectin emerged as the main driver behind the long-term hardening of CSB [26, 27]. In Section 3.1, we monitored the staling of CSB using a texture analyzer. Experimental results indicated that as the storage time increased, the hardness of CSB consistently exhibited an upward trend. Therefore, it is believed that the increase of hardness was primarily due to starch the retrogradation and recrystallization. The correlation between the hardness of CSB and FWHM during storage is depicted in Figure 3.

![Figure 1](https://example.com/fig1.png) (a) Changes in moisture content and (b) hardness of Chinese steamed bread (CSB) during storage

![Figure 2](https://example.com/fig2.png) Characteristic peaks of Raman spectroscopy (a), and Raman spectra changes of CSB during storage (b).
Figure 3 illustrates the correlation between the hardness of CSB and FWHM throughout the entire storage process. As can be seen from Figure 3, there was a negative correlation between the hardness of CSB and FWHM during the entire storage period, with R² reached 0.838. The results demonstrated that using a 785 nm laser, the Raman spectrum of CSB can be obtained in a relatively short time without the need for sample preparation, providing a general understanding of the starch molecules in the bread. In particular, the Raman peak at 480 cm⁻¹ was associated with the retrogradation of amylopectin. During the staling process of CSB, the narrowing of the band at 480 cm⁻¹ correlated well with the hardness data obtained using a texture analyzer. This finding suggested that Raman spectroscopy can be a valuable tool for studying the staling process and starch degradation in CSB.

**Peak area and peak intensity analysis of characteristic peaks of Raman spectra**

To further explore the correlation between Raman spectroscopy and the short-range order of starch, we analyzed the Raman spectra and main characteristic peaks of CSB samples. The results are shown in Figure 4. It was found that during the entire storage period, the intensities and areas of these nine peaks gradually increased with the increase of storage time. During the storage process, the trends of peak area and peak intensity of the selected characteristic peaks in the Raman spectroscopy of CSB both increased over time. For example, the peak area at 480 cm⁻¹ increased from 3.23 × 10⁵ to 9.09 × 10⁵, and the peak intensity increased from 12.90 × 10⁴ to 30.28 × 10⁴. In Section 3.1, the staling of CSB were analyzed by measuring the trend of moisture content during storage. The results showed that the moisture content of CSB consistently decreased as the storage time increased. Meanwhile, studies have indicated that changes in the peak intensity and peak area of starch were related to moisture content. Therefore, we analyzed the correlation between the peak area and intensity of the main Raman peaks and the moisture content in the core of CSB.

To further select representative characteristic peaks, linear regression analysis was performed on the area and intensity of these nine peaks and the moisture content. The results are presented in Figure 5. From the regression analysis data, it was evident that the area and intensity of these nine peaks exhibited a good regression with the moisture content. During storage at room temperature, the regression coefficients between the area and intensity of these nine peaks and the moisture content were all less than -0.699, and the R² values were all greater than 0.488. Among these nine peaks, the peak at 2910 cm⁻¹ exhibited a higher correlation coefficient with the moisture content. Therefore, the peak was selected to characterize the short-range molecular order of gelatinized wheat starch. During storage, the Raman peak area and intensity showed a negative correlation with the moisture content. Consequently, the area and intensity of these nine peaks can be used to characterize differences in the short-range order of starch.

**Table 1** Half-peak width and peak position of the Raman spectroscopy of CSB at 480 cm⁻¹

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>Storage time/d</th>
<th>Peak position</th>
<th>FWHM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>479.86±0.18ab</td>
<td>15.36±0.19bc</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>479.21±0.03ab</td>
<td>14.89±0.04b</td>
<td></td>
</tr>
<tr>
<td>Room temperature</td>
<td>479.14±0.22a</td>
<td>14.61±0.08b</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>479.91±0.10abc</td>
<td>14.46±0.02bc</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>479.70±0.50ab</td>
<td>14.23±0.11b</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>479.63±0.19abc</td>
<td>13.53±0.35bc</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>479.21±0.03ab</td>
<td>14.89±0.04bc</td>
<td></td>
</tr>
</tbody>
</table>

Note: The data is expressed as mean ± standard deviation. The labeling of different letters after the data in the same column indicates significant differences between the same parameters of different samples (P<0.05).

![Figure 3 Correlation between the Hardness of CSB and the Full Width at Half Maximum (FWHM) of the Raman Band at 480 cm⁻¹ during Storage](image)

![Figure 4 Peak area and peak intensity analysis of nine characteristic peaks of Raman spectra](image)
The results showed that during the entire storage period, both the peak intensity and peak area of the main characteristic peaks in the Raman spectra of CSB samples increased with the decrease of moisture content. The linear regression analysis between the peak area and intensity of the main Raman peaks and the moisture content in the core of CSB indicated that the correlation coefficient between the moisture content in the core of CSB and the peak area and intensity of the Raman characteristic peaks was relatively high during storage.

**Conclusion**

In this study, hardness and moisture content of CSB during storage were analyzed. Raman characteristic peaks were selected to analyze the staling degree of CSB according to hardness and moisture content, and the relationship between Raman characteristic peaks and hardness and moisture content of CSB were established. The results showed that there was a negative correlation between FWHM at 480 cm\(^{-1}\) and hardness, with \(R^2\) above 0.8. The peak intensity and peak area of the nine main characteristic peaks of the Raman spectra of CSB samples related to starch structure increased with the decrease of moisture content. At the same time, the correlation coefficient of linear regression analysis results was relatively high, with the best the best correlation coefficient above 0.8. The results indicated that Raman spectra combined with hardness and moisture content could be used as a potential method to fast predict the staling of wheat flour products including CSB.

**References**


