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Effects of Storage Time on Steamed Bread Quality and Gluten Protein Structure

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Abstract. The effects of storage time on the quality of steamed bread and the structure of gluten protein were investigated by determining the specific volume, the texture characteristics, the microstructure, the secondary structure of gluten and the moisture distribution. The results showed that with the increase of storage time, the specific volume of steamed bread gradually decreased. The hardness, stickiness and chewiness of steamed bread increased gradually, elasticity, cohesiveness and recovery decreased gradually. The contents of α-helix and β-turn in gliadin decreased, the contents of β-sheet and random coil increased. The contents of β-fold and random coils in glutenin increased, the contents of α-helix and β-turn gradually decreased. Both T_{21} and T_{22} decreased, the proportion of bound water gradually increased. The gluten protein and starch granules in steamed bread gradually break off, the gluten network also gradually collapsed and surface structure of steamed bread also changed from smooth to uneven.

1. Introduction

Wheat flour mainly consists starch, protein and moisture, a small amount of fat and non-starch polysaccharides. It's the main material of steamed bread. During the production and storage of steamed bread, there are lots of reactions of the various components in wheat flour. These reactions have essential effect on the quality of steamed bread, protein is considered to be one of the most critical factors in the quality of steamed bread. Gluten protein has ductility and viscoelasticity, which plays an irreplaceable role in the steamed bread.

2. Materials and Methods

2.1. Materials and reagents

Wheat flour (Xinjiang Jiahong Group) and Angel Yeast (commercially available). Sodium Hydroxide (Analytical Pure), Tianjin Kemiou Chemical Reagent Co., Ltd. Ethanol (Analytical Pure), Tianjin Sailboat Chemical Reagent Technology Co., Ltd. Trichloromethane, Tianjin Jingqiang Chemical co. LTD.

2.2. Steamed bread processing

The wheat flour, water and yeast were placed in a dough mixer for 10 minutes, and then dough was placed in a proofing box at 33°C and relative humidity 70% for 100 minutes. Next, the dough was divided into sub-agents(70g) and shaped rapidly, these sub-agents were placed in the proofing box for 20 minutes. Finally, they were steamed for 20 minutes. The steamed breads were cooled for 30 minutes and they were stored at 4°C. The steamed breads were divided into six groups: stored for one, two, three,

four, five and six days.

2.3. Determination of specific volume
The specific volume of steamed bread was measured by a BVM6630 volumetric analyzer. Six samples were measured and average value was gained.

2.4. Measurement of texture
The sensory quality of steamed bread was detected by texture analyzer. The steamed bread was cut into cubes of 2cm*2cm*2cm. The measured parameters were: pre-test rate 1.0 mm/s, test mid-rate 1.0 mm/s, post-test rate 1.0 mm/s, compression ratio 50%, two compression intervals 5s. Each sample was measured six times, hardness, elasticity, cohesiveness, stickiness, chewiness, resilience and other indicators were measured.

2.5. Determination of microstructure
The microstructure of steamed bread was measured by a scanning electron microscope. The steamed bread was cut into strips of 30 mm*8 mm*8 mm. After vacuum freeze-drying, the samples were cut into cubes of 5mm*5mm*5mm. Scanning electron microscope parameters were set, pictures were taken.

2.6. Degreasing of steamed bread
The method of MacRitchie et al[1] were slightly modified. The steamed bread pieces were mixed with trichloromethane in 1:2 (w/w) and then it was stirred on a magnetic stirrer for 10 min. This operation was repeated 3 times.

2.7. Extraction of gluten protein

2.7.1 Extraction of gliadin. According to the Osbron method[2], skim steamed bread(100g) was added to 70% ethanol solution(1000ml). The mixture was stirred on a magnetic stirrer for 6 h at room temperature. After adjusting pH to 8, it was placed in the darkness for 1h. Then the precipitate was collected by centrifuge(7000rpm, 30min), it was washed three times with distilled water. The protein was freeze-dried to reserve.

2.7.2 Extraction of glutenin. Similarly, according to the Osbron method, steamed bread(100g) was added to distilled water(1000ml) and then adjusted pH to 8.5. The mixture was stirred on a magnetic stirrer for 6h at room temperature, the supernatant was collected by centrifuge(7000rpm,20 min). Adjusted the isoelectric point and placed in the darkness for 1h. Then the precipitate was collected by centrifuge(7000rpm, 40min), it was washed three times with distilled water. The protein was freeze-dried to reserve.

2.8. Infra-red spectrum assay
The free-drying(1mg) sample was completely mixed with KBr(150mg), ground to powder and compressed. The scanning was performed on a Nicolet iS50 FT-IR Fourier Transform Infrared Spectrometer. The range of scanning was 4000-400cm-1, the number of scans was 16, and the resolution was 4cm-1.

2.9. Determination of moisture distribution
The moisture distribution of steamed bread was measured by the CPMG experiment. Steamed bread(5g) was put into nuclear magnetic tubes and wrapped with plastic wrap, which was placed in the center of radio frequency coil at the permanent magnetic field at 30℃. The spin-spin relaxation time T2 of the sample was measured by the CPMG pulse sequence.

CPMG experimental parameters: the number of sampling points TD =22290, the number of repetition scans NS =16, the number of echoes C0NH =1000, relaxation decay time D0 =1.2s. The T_2 was calculated by the T2-CPMG fitting program. The parameters were: the number of participating

inversion points were 400, and the relaxation time points were 200.

2.10. Data Processing

Excel 2016 was used for data analysis and processing, and Spss 18.0 software was used to analyze the differences in test data. The infrared data was processed by Omni 5.0 software, a Fourier infrared spectrophoto self-prepared software. The image was drawn by origin 9.0.

3. Results and discussion

3.1. Effect of storage time on the specific volume

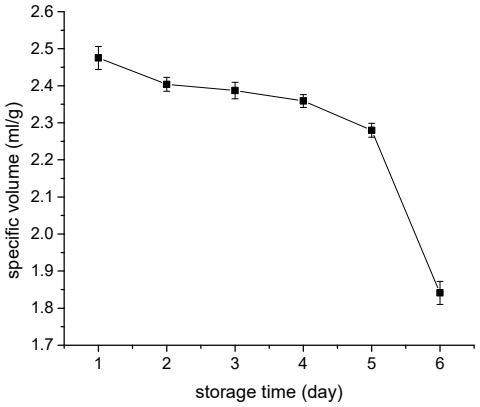


Figure 1. Effect of storage time on the specific volume of steamed bread

With the increasing of the storage time, the specific volume of steamed bread gradually decreased(figure.1), which is probably because the moisture in the steamed bread gradually decreased, some hydrogen bonds between water and gluten protein were broken, it caused the holding capacity of gas impaired and the volume reduced.

3.2. Effect of storage time on texture characteristics

Table 1. Effect of fermentation temperature on texture characteristics of storage time

storage time(d) (min)	hardness(N)	flexibility	cohesiveness	gumminess	chewiness	springiness
1	7.53±0.53^{a}	0.92±0.00^{a}	0.77±0.01^{a}	5.82±0.38^{a}	5.37±0.34^{a}	0.41±0.00^{a}
2	13.40±0.39^{b}	0.85±0.01^{b}	0.54±0.01^{b}	7.25±0.21^{b}	6.17±0.17^{a}	0.21±0.01^{b}
3	26.71±1.42^{c}	0.72±0.01^{c}	0.40±0.01^{c}	10.70±0.71^{c}	7.70±0.45^{b}	0.12±0.00^{cd}
4	39.53±0.71^{d}	0.69±0.01^{d}	0.41±0.01^{c}	16.28±0.49^{d}	11.16±0.37^{c}	0.12±0.00^{cd}
5	41.15±1.32^{de}	0.69±0.00^{d}	0.40±0.00^{c}	16.47±0.42^{d}	11.32±0.24^{cd}	0.12±0.00^{c}
6	43.40±1.00^{e}	0.69±0.01^{d}	0.44±0.01^{d}	18.92±0.34^{e}	13.00±0.37^{d}	0.13±0.00^{d}

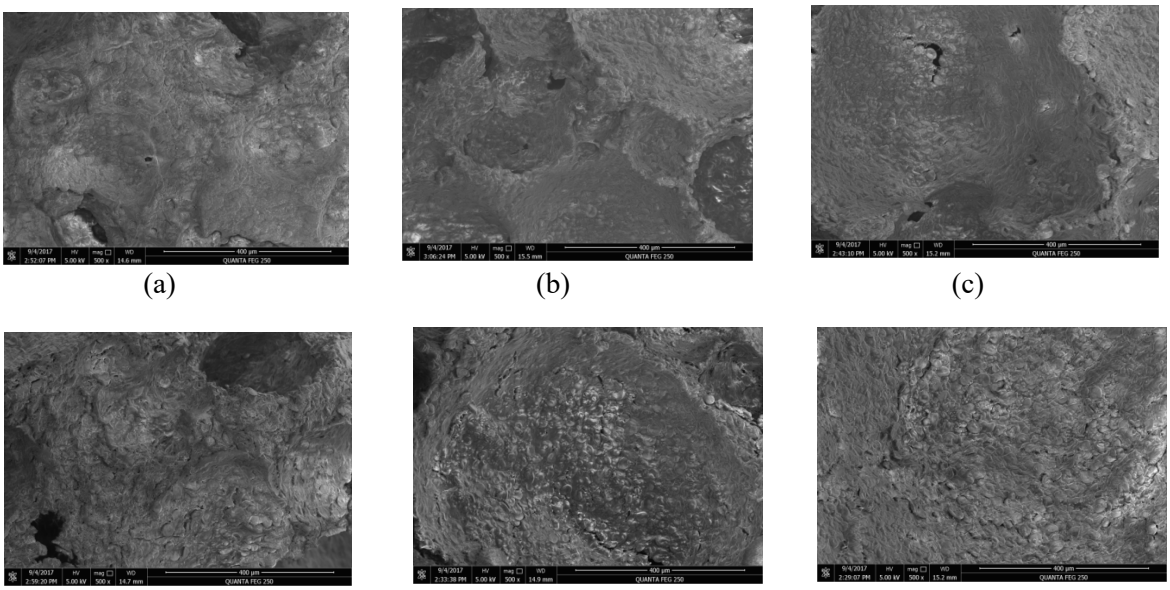
Different letters within columns indicate significant differences between the at $p<0.05$.

The results of the texture characteristics of steamed bread are presented in Table 1. With the increase of storage time, the hardness, gumminess and chewiness of steamed bread increased gradually, the flexibility, cohesiveness and springiness decreased gradually. As storage time went by, the water content reduced, the gluten network structure was broken as well as denser in the steamed bread. Besides, the hardness, gumminess and chewiness of the steamed bread increased. Due to the breakage of

the gluten network structure, the gas holding capacity impaired, the elasticity, cohesiveness and recovery reduced. The elasticity and resilience were significantly related to the quality of steamed bread. The higher the elasticity and resilience of values were, the better the quality of steamed bread was. With the prolongation of storage time, the quality of steamed bread was getting worse.

3.3. Effect of storage time on the microstructure

The effect of different storage time on the microstructure of steamed bread was observed by scanning electron microscopy. The results are shown in Figure 2.



(a) (b) (c)

(d) (e) (f)

Figure 2. Effect of storage time on microstructure of steamed bread

Photos of the microstructure of steamed bread for different storage time were shown in figure 2. Figure(a) is a photograph of steamed bread stored for one day, in the same way, figure(f) is a photograph of steamed bread stored for six days.

When steamed bread stored for one day, the starch granules were fully encapsulated by the surrounding gluten protein and it fused into a single body, the pores were also delicate. But when it stored for six days, the junction between starch granules and gluten protein broken and the gluten protein continuous net structure collapsed. The starch granules accumulated and the pores were bigger than before. The surface structure of the steamed bread also changed from uniform to uneven, which means that the microstructure of the steamed bread was destroyed.

3.4. Effect of storage time on the secondary structure of gliadin

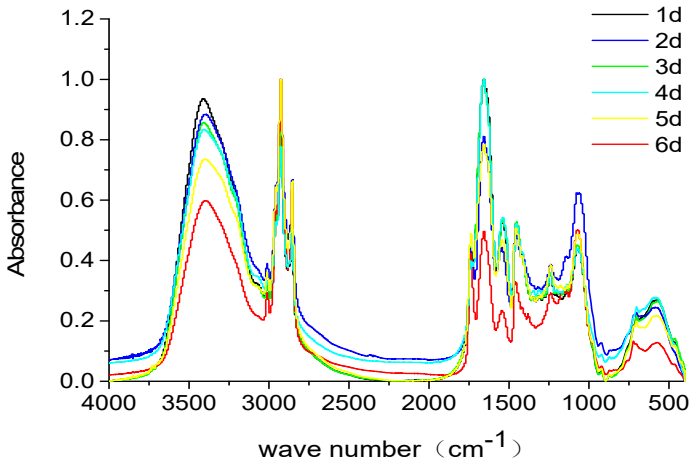


Figure 3. FTIR spectra of gliadin with various storage time

Table 2. Secondary structure and content of gliadin with various storage time

	1d		2d		3d		4d		5d		6d	
assignment	peak position (cm^{-1})	content (%)	peak position (cm^{-1})	content (%)	peak position (cm^{-1})	content (%)	peak position (cm^{-1})	content (%)	peak position (cm^{-1})	content (%)	peak position (cm^{-1})	content (%)
	1606		1606		1606		1606		1606		1606	
	1618		1617		1618		1618		1618		1617	
β-sheet	1629	52.30	1628	52.72	1629	53.43	1628	53.52	1629	53.54	1628	53.84
	1684		1684		1682		1683		1683		1684	
	1694		1694		1694		1694		1694		1694	
random coil	1638	9.01	1639	9.75	1638	10.37	1638	11.04	1638	12.23	1641	14.09
α-helical	1647	19.41	1654	18.80	1647	18.04	1647	17.56	1647	17.01	1654	15.47
	1656				1656		1656		1656			
β-turn	1665	19.28	1665	18.73	1665	18.16	1665	17.88	1665	17.22	1665	16.60
	1674		1675		1673		1674		1673		1675	

The data (table 2) were obtained by the graphic processing of figure 3. It can be seen that with the increasing of storage time, the contents of α-helix and β-turn in gliadin showed a downward trend; the contents of β-fold and random coils showed an upward trend. Which is probably because the misture in the steamed bread gradually decreases. Some hydrogen bonds between water and gliadin were broken and reduced. The structure of helix and corner was destroyed[3]. In this process, small molecule substances were produced and it aggregated with each other under the action of non-covalent bonds. Thus, the contents of the β-sheet and Random coil increased.

3.5. Effect of storage time on secondary structure of glutenin

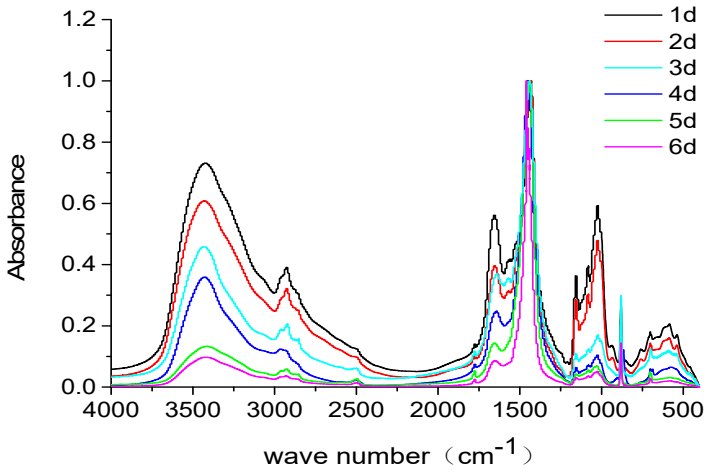


Figure 4. FTIR spectra of glutenin with various storage time

Table 3. Secondary structure and content of glutenin with various storage time

	1d		2d		3d		4d		5d		6d	
assignment	peak position (cm^{-1})	content (%)	peak position (cm^{-1})	content (%)	peak position (cm^{-1})	content (%)	peak position (cm^{-1})	content (%)	peak position (cm^{-1})	content (%)	peak position (cm^{-1})	content (%)
β-sheet	1607 1618 1629 1684 1695	48.92	1607 1618 1629 1684 1695	49.18	1607 1618 1629 1684 1695	49.32	1607 1618 1628 1684 1695	49.43	1607 1618 1628 1683 1695	49.79	1607 1617 1628 1684 1695	50.04
random coil	1638	9.01	1638	9.18	1638	10.02	1638	11.05	1639	11.43	1639	13.10
α-helical	1647 1656	20.45	1648 1656	20.06	1648 1656	19.72	1648 1656	18.80	1648 1656	18.35	1648 1656	17.16
β-turn	1665 1674	21.62	1665 1674	21.58	1665 1674	20.94	1665 1674	20.72	1665 1674	20.43	1665 1674	19.70

The data(table 3) were obtained by the graphic processing of figure 4. It can be seen that with the increase of storage time, the contents of β-sheets and random coils in glutenin had an increasing trend, the contents of α-helix and β-turns decreased. Which is probably because the misture in the steamed bread gradually decreases. Some hydrogen bonds between water and gliadin were broken and reduced, the alpha helix unwound and the hydrogen bond in the β-turn ruptured. In this process, small molecule substances were produced and it aggregated with each other under the action of non-covalent bonds, it combined into β-sheets. Thus, the contents of the β-sheet and Random coil increased.

3.6. Effect of Storage Time on Water Distribution

The inversion map of the sample was obtained by CPMG-T_2 pulse sequence detection, and the lateral relaxation time (T_2) table of the steamed bread was shown in Table 4.

Table 4. The changes of T_2 of the bread with the storage time

	1d		2d		3d		4d		5d		6d	
	peaks time	peaks /%	peaks time	peaks /%	peaks time	peaks /%	peaks time	peaks /%	peaks time	peaks /%	peaks time	peaks /%
T_{21} (ms)	10.75	4.73	9.27	6.36	8.59	10.23	7.81	13.65	7.02	17.5	6.73	18.07
T_{22} (ms)	60.54	95.27	55.81	93.64	48.03	89.77	40.23	86.35	34.21	82.50	29.49	81.93

Through the detection of CPMG pulse sequence, there were two main types of water in the steamed bread(T_{21} and T_{22}). T_2 indicates the spin - relaxation time of water in the steamed bread. The size of T_2 characterizes the fluidity of the dough. The smaller the value of T_2 is, the weaker the fluidity of this part is. The smaller the value of T_2 is, the more closely it binds to the other components in the steamed bread. The larger the value of T_2 is, the stronger the fluidity of the part is. The larger the value of T_2 is, the weaker the binding force is. (T_2 is divided into T_{21} and T_{22}, $T_{21}<T_{22}$)

The range of T_{21} is within 1~19ms, which is considered to be a combination of hydrogen bonding with amino carbonyl groups such as starch and protein. The scope of T_{22} is above 19ms, which is considered to be the free water in the steamed bread with the maximum liquidity. As it can be seen from table 4, with the extension of storage time, both T_{21} and T_{22} of steamed bread reduced. This may be due to the fact that with the extension of storage time, the non-crystalline starch became the ordered crystalline structure, the colloidal stability was destroyed and the water dissipated gradually. The moisture that was initially uniformly combined with the non-crystalline regions of the starch gradually entered into the crystal structure of the starch where the retrogradation has occurred[4]. In the end, the water holding capacity of the steamed bread reduced, the mobility of molecules decreased. It may also be that the loss of free water during the storage process led to the redistribution of water. Some hydrogen bonds between water and gliadin were broken, and the unbroken part had stronger binding force, which reduced the mobility of water molecules[5].

As can be seen from the table, the proportion of free water is much higher than that of the combined water. However, as the storage time was prolonged, the proportion of free water continued to decrease, the proportion of combined water increased. In the process of storage, part of the water was lost in steamed bread. There was a dynamic balance on the moisture both internally and externally[6]. The amount of water in steamed bread decreased, the proportion of free water in the lost water increased, which made the water holding capacity of steamed bread decreased.

3.7. Correlation analysis of macro and micro indicators of steamed bread

The macro-indicators and micro-indicators of the steamed bread section were analyzed for correlation. The results are shown in Tables 5 and 6.

Table 5. Correlation coefficients of the texture of the bread, the content of the secondary structure of the gliadin amide I，T_{21}，T_{22}

	hardness	flexibility	cohesiveness	gumminess	chewiness	springiness	β-sheet	random coil	α-helical	β-turn	T_{21}	T_{22}
hardness	1											
flexibility	-.958**	1										
cohesiveness	-.837*	.941**	1									
gumminess	.988**	-.909*	-.76	1								
chewiness	.976**	-.881*	-.727	.998**	1							
springiness	-.845*	.937**	.992**	-.783	-.756	1						
β-sheet	.959**	-.981**	-.906*	.931**	.912*	-.926**	1					
random coil	.877*	-.772	-.632	.919**	.932**	-.692	.861*	1				
α-helical	-.904*	.822*	.687	-.938**	-.945**	.744	-.905*	-.993**	1			
β-turn	-.937**	.870*	.755	-.952**	-.954**	.797	-.929**	-.983**	.987**	1		
T_{21}	-.966**	.931**	.867*	-.956**	-.950**	.890*	-.954**	-.919**	.933**	.972**	1	
T_{22}	-.976**	.902*	.769	-.986**	-.985**	.797	-.937**	-.957**	.965**	.987**	.978**	1

** correlation was set as statistically significant at p≤0.01. * correlation was set as statistically significant at p≤0.05(two-tailed distribution).

From Table 5, it can be seen that the chewiness was significantly correlated with the β-sheet content; random curl, α-helix, β turn, T_{21} and T_{22} were correlated significantly with chewiness. Hardness was correlated significantly with random coil and α-helix, there was a highly significant difference found for steamed bread with hardness and β-fold, β-turn, T_{21}, T_{22}. Flexibility was significantly correlated with all of micro-indicators. There is on significant difference found between cohesiveness and springiness of macro-indicators or β-fold, random curl, α-helix, β-turn, T_{21} and T_{22} of micro-indicators.

Table 6. Correlation coefficients of the texture of the bread, the content of the secondary structure of the glutenin amide I，T_{21}，T_{22}

	hardness	flexibility	cohesiveness	gumminess	chewiness	springiness	β-sheet	random coil	α-helical	β-turn	T_{21}	T_{22}
hardness	1											
flexibility	-.958**	1										
cohesiveness	-.837*	.941**	1									
gumminess	.988**	-.909*	-0.76	1								
chewiness	.976**	-.881*	-0.727	.998**	1							
springiness	-.845*	.937**	.992**	-0.783	-0.756	1						
β-sheet	.904*	-.820*	-0.712	.927**	.934**	-0.758	1					
Random coil	.913*	-0.805	-0.631	.956**	.967**	-0.685	.963**	1				
α-helical	-.909*	0.793	0.638	-.954**	-.967**	0.692	-.976**	-.995**	1			
β-turn	-.918**	.836*	0.669	-.948**	-.952**	0.716	-.966**	-.991**	.980**	1		
T_{21}	-.966**	.931**	.867*	-.956**	-.950**	.890*	-.958**	-.913*	.927**	.918**	1	
T_{22}	-.976**	.902*	0.769	-.986**	-.985**	0.797	-.972**	-.967**	.970**	.969**	.978**	1

** correlation was set as statistically significant at p≤0.01. * correlation was set as statistically significant at p≤0.05(two-tailed distribution).

Gumminess and chewiness were correlated signficantly with β-fold, random curl, α-helix, β-turn, T21 and T22. Hardness was significantly correlated with β-sheet, random coil, and alpha helix. There

was a highly significant correlation between hardness and β-turn, T21, T22. The flexibility was significantly correlated with β-sheet, β-turn and T22, there was highly significant correlation between flxibility and T21. Cohesiveness was significantly correlated with T21.

4. Conclusion

In this paper, the structure of gluten protein during the storage of steamed bread was studied. The correlation between the structure of gluten protein and the quality of steamed bread was analyzed, the intrinsic mechanism of gluten protein structure on steamed bread quality was explored. It provides the direction and theoretical basis for regulating the quality of steamed bread with the structure of gluten protein. It is possible to solve the practical problems in production, the development process of industrialization on steamed bread can be accelerated. This will be helpful to enrich the science system of the gluten protein network structure, it will provide a theoretical basis for quality stability and product transportation.

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