Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.10122

Effects of heating method and refrigerating time on nutritional quality and digestive characteristics of refrigerated Chinese steamed bread

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Abstract
Two re-heating methods and different refrigerating time were carried to compare the re-heating stored, refrigerated bread quality. Compared with the natural thawed steamed, sensory properties of re-heating bread were significantly improved, steam method improved moisture content while that re-heated by microwave decreased, the secondary structure of protein was significantly affected by re-heating. Rapidly digestible starch (RDS) content in bread re-heated by steam and by microwave decreased significantly, compared with fresh bread. With the storage time increasing, the sensory quality of bread showed a downward trend after being re-heated by steam; water content increased and then decreased; β-turn decreased gradually, while random coil increased gradually; starch aging showed a trend of gradual enhancement. RDS content and protein digestibility decreased gradually. Nutritional quality of the bread re-heated by steam was better than that by microwave, optimum cold storage time for the bread re-heated by steam should be no more than 24 h.

Keywords: steamed bread; re-heating method; refrigerating time; nutritional quality; digestive characteristics.

Practical Application: Processing and storage of Chinese steamed bread.

1 Introduction

According to the data of International Diabetes Federation, in 2019, there was 463 million adults aged 20-79 years with diabetes (1 out of 11 people with diabetes), it is estimated that by 2030, the number of patients with diabetes will reach 578.4 million; by 2045, the number of patients with diabetes will reach 7002 million around the world, while in China, there has been about 116.4 million (http://www.diabetesatlas.org/). Diabetes has a strong relationship with the daily diet. In China, Chinese steamed bread (CSB) is a traditional staple food, whose basic formulation includes wheat flour, water and yeast (Wang et al., 2016). CSB is widely eaten in northern China, accounting for about 40% of China's wheat consumption (Wang et al., 2019), but the bread tends to increase postprandial blood glucose, especially for diabetics.

In order to decrease the negative effect of Chinese steamed bread and improve the nutritional quality of Chinese steamed bread (CSB), many studies focus on changing the formula of the bread, for example, FDDG was used to replace 0%-25% all-purpose flour (APF) in CSB formulations (Li et al., 2020), effect of extruded adzuki bean flour (EABF) substitution (10%, 20%, 30%, and 40%) for wheat flour on the quality properties of blended flour and CSB was studied (Chen et al., 2019), bread products were formulated on a French-bread recipe basis and replacing wheat flour by resistant starch at different level (0%, 10%, 20% and 30%) (Gabriel Arp et al., 2020), but most diabetics consumers, are used to treating Chinese steamed bread as their daily staple food, it's not easy to change their eating habits in a short run.

The fresh bread is a kind of food with high glycemic index, which is not health for diabetics. With advantages of long shelf life and high convenience, frozen steamed bread (FSB) is suitable for commercialization and industrialization, so steamed bread was not often consumed fresh, it will be re-heated after being refrigerated (Zhao et al., 2021). According to different digestion time, the starch in steamed bread can be divided into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). High content of RDS could cause a rapid rise in postprandial blood glucose, which has a negative effect on glucose metabolism of diabetic patients, resistant starch has a good effect on improving insulin resistance and reducing blood sugar in type 2 diabetes mellitus (T2DM) patients, different starch ratio in steamed bread had a significant effect on blood glucose content (Zhang et al., 2020). While, there is no fully study on the digestion of starch in fresh and stored steamed bread.

Storage of fresh bread at a high temperature led to significant weight losses and bad changes in taste and smell (Autio & Sinda, 1992). The frozen storage was effective to prevent the loss of volatiles during the first week, which could affect the sensory evaluation of bread (Pico et al., 2017). The quality of the dough was reduced, resulting in the reduced bread loaf volume and firmer texture (Wang et al., 2017). During the storage of breads, the RDS decreased while SDS and RS increased, respectively (Shumoy et al., 2018). Different processing methods will change

Received 03 Jan., 2022
Accepted 18 Feb., 2022
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the physical and chemical characteristics of food, thus affecting human health (Wang & Pang, 2019), the effects of different fermentation (yeast and sourdough) and cooking (steam and baking) methods affect the physicochemical, rheological, and morphological properties of dough, and nutritional property of Chinese steamed/baked bread were investigated (Gao et al., 2021), but studies on the quality of re-heating dough products during different refrigeration time are few reported. In China, steam heating and microwave heating are the most frequent re-heating methods. Steaming heated evenly, had high moisture content and preserved diverse endogenous and added nutrients (Wang et al., 2015a). Microwave method caused nonuniform heating of food (Vadivambal & Jayas, 2010), it also led to the loss of moisture, compared with conventional steam heating (Miller & Hoseney, 2010). Microwave re-heating for baked dough-based products was reported to produce an undesirable leathery tough texture (Uzzan et al., 2007). However, the effect of re-heating method and refrigeration time on the sensory evaluation, moisture content, protein secondary structure, extractable amylose and in vitro starch and protein digestion of refrigerated steamed bread have not been fully studied.

In this study, two re-heating methods (steam and microwave) and different refrigerating time (0 h, 12 h, 24 h, 48 h, 72 h and 128 h at 4 °C) were carried to compare the effect of two re-heating methods and refrigerating time on re-heating stored, refrigerated bread quality, including the sensory evaluation, moisture content, protein secondary structure, extractable amylose and in vitro starch and protein digestion of refrigerated steamed bread. The two re-heating methods were compared to find an appropriate storage time, which will not negatively affect the sensory quality and protein utilization of steamed bread, but decrease the content of RDS, resulting in lower sugar index of steamed bread.

2 Materials and methods

2.1 Materials

The wheat flour was ground from Ai-Kang 58 wheat. Active dry yeast was purchased from Angel Yeast Co., Ltd. Pepsin (149.21 U/mg) and trypsin (52.93 U/mg) were purchased from Sigma (US); α-amylase was purchased from Beijing Aoboxing Biotechnology Co., Ltd.

2.2 Steamed bread making

The dough was composed of flour 200 g, water 96 g and yeast 1.6 g. Firstly, the yeast was activated in 37 °C waters for about 15 min, and then all the ingredients was mixed evenly with a mixer. After mixing, the dough was fermented at 30 ± 0.5 °C, humidity 80 ± 5% for about 50 min. The well-fermented dough was kneaded into smooth one and then divided into 50g dough. The proofed dough was steamed for 30 min in a steamer. The steamed bread was cooled to room temperature, some was sampled as fresh bread and the others was stored in a refrigerator at 4 °C.

2.3 Re-heating of refrigerated steamed bread

Fresh steamed bread was cooled to room temperature as a positive control. After 24 h of storage, steamed bread was removed from the refrigerator and thawed at room temperature. Some steamed bread was heated by steaming in a steamer at 100 °C for 15 min. Other steamed bread was heated at 700 W for 75 s in a closed microwave oven that had been preheated. Another steamed bread was naturally thawed at room temperature as a control.

According to the experimental results of re-heating, the better re-heating method was used to re-heat the steamed bread refrigerated for 12 h, 24 h, 48 h, 72 h and 128 h, while the fresh steamed bread (0 h) was used as a control.

2.4 Sensory evaluation

The fresh steamed bread, natural thawed steamed bread and re-heating steamed bread refrigerated for different time were evaluated, respectively. According to the rules of Ethics Committee of Henan University of Technology, fifty member judges were selected from staff and graduate students of School of Food Science and Technology, Henan University of Technology. Bread was served at room temperature using the more widely used practice of three-digit code during sensory analysis. Just before each test session, a 3-day training was given to the judges on descriptive terms, assessment method and how to using scale in order to reduce between-subject variations. Sensory panelists evaluated the bread according to the Chinese standard GB/T 17320-1998, with some modification as indicated in Table 1. The sensory index included appearance, color, aroma, visual internal texture, elasticity, firmness, stickiness and hard core, and all acceptability were evaluated according to Liu's method (Liu et al., 2019).

2.5 Moisture content

The moisture content of the steamed bread was determined by the oven-drying method. The moisture content of steamed bread was determined according to the national standard GB 50093-2010.

2.6 The secondary structure of protein

The steamed bread was freeze-dried to a constant weight. The potassium bromide particles and the steamed bread powder were separately grounded to a uniform powder under a tungsten lamp. The ground potassium bromide powder was mixed with the steamed bread powder by 100:1 evenly. The appropriate amount of the mixed sample was tableted by a tableting machine and then placed in a Fourier transform infrared spectrometer for full-band scanning (Almutawah et al., 2007). The scanning wavelength is 4000-400^{-1}, of which 1640-1605^{-1} is β-fold, 1650-1640^{-1} is random coil, 1660-1650 is α -helix, and 1700 -1660^{-1} is β-sheet.

2.7 Staling of steamed bread

A colorimetric method was used to determine the amount of extractable amylose (Williams et al., 1970). 5 g pulverized steam

Table 1. Scoring criteria for sensory evaluation of steamed bread.

Project	Score	Evaluation rules
Appearance	15	Good volume and symmetry, good upright, very smooth, bright, no specks (11-15), basic symmetry and upright, smooth, no specks (7–10) No symmetry, low height, and bad shape, rough surface, shrinking skin, specks or bubbles in skin (1–6)
Color	10	White/creamy white (8-10) light yellow (4-7), gray or dark (1-3)
Aroma	5	Good fresh wheat (4-5), not fresh wheat, and musty or abnormal smell (1–3)
Visual internal texture	15	Good crumb structure dense homogenous and spongy (no big holes) (11–15), acceptable crumb, homogenous with few big holes (6–10), uneven large holes, and not homogenous (1–5)
Elasticity	15	Bound back quickly when pressed with finger (11-15) bound back slowly (6-10), and no bounce and hard (1-5)
Firmness	10	A little hard to bite (8-10), bite with a little stress (4-7), hard and crumbly (1-3)
Stickiness	10	Not sticky (8–10), a little sticky (4–7), and very sticky (1–3)
Hard core	20	No hard core, consistent (16-20), a little hard core (11-15), obvious hard (6-10)
Total score	100	

bread was extracted by water in a 10 mL Erlenmeyer flask and stirred using a magnetic stirrer for 1 h, and then centrifuged for 10 min at 5000 rpm. The supernatant (2 mL) and 1 mL iodine reagent were transferred to a 50 mL volumetric flask, mixed with appropriate amount of water and bathed at 35 °C waters for 15 minutes, and then diluted to 50 mL, and the absorbance was measured at 625 nm.

2.8 In vitro starch digestion process

The method of starch digestibility was modified according to Englyst et al. (1992). The steamed bread core (1 g) was ground into homogenate with 20 mL sodium acetate buffer (pH = 5.2, 0.1 M) and then transferred to a 50 mL centrifuge tube. The mixtures were bathed in water at 37 °C for 20 min. Supernatant (0.2 mL) was mixed with 1.8 mL water and 2 mL DNS Reagent. Samples were boiled for 5 min and rapidly cooled, water was added to a 25 mL mark and being shaken, the absorbance of samples was read at 540 nm. The glucose content, G_0, was calculated according to the DNS standard curve.

100 mg α-amylase and 0.5 mL amyloglucosidase were added to the centrifuge tube, and digested for 20 min. The enzymatic hydrolysate (0.2 mL) was mixed with 4.8 mL absolute ethanol to inactivate the enzyme, and then centrifuged at 3000 r/min for 10 min. Supernatant (0.5 mL) was mixed with 1.5 mL water and 2 mL DNS. Samples were boiled for 5 min and rapidly cooled. The absorbance was measured at 540 nm after adding water to a 25 mL and being shaken. The glucose content, G_{20}, was calculated from the DNS standard curve.

After enzymatic hydrolysis for 120 min, enzymatic hydrolysate (0.2 mL) and 4.8 mL absolute ethanol was mixed to denature the enzyme, centrifuged at 3000 r/min for 10 min. Supernatant (0.5 mL) was mixed with 1.5 mL water and 2 mL DNS. Samples were boiled for 5 min and rapidly cooled. Then water was added to a 25 mL and shaken before reading the absorbance at 540 nm. The glucose content, G_{120}, was calculated from the DNS standard curve.

The starch that was digested in the first 20 min of digestion phase is defined as rapidly digestible starch (RDS), whereas the starch that was digested between 20 min and 120 min of digestion phase is defined as slowly digestible starch (SDS). The starch digested more than 120 min is defined as resistant starch (RS). The content of RDS, SDS and RS were calculated by method of Chung (Chung et al., 2009) according to the following Equations 1, 2, 3:

$$RDS\ (\%) = (G20 - G0) \times 0.9 / TS \tag{1}$$

$$SDS\ (\%) = (G120 - G20) \times 0.9 / TS \tag{2}$$

$$RS\ (\%) = 1 - RDS\% - SDS\% \tag{3}$$

Where: G0, G20, G120—representing the glucose content (mg) at 0 min, 20 min, and 120 min, respectively; TS is the crude starch content in 1 g buns of dry bread, 0.9-conversion coefficient in the sample.

2.9 In vitro protein digestibility

The protein digestibility was determined by enzymatic hydrolysis (Yu-ting et al., 2014). 1 g steamed bread core and 20 mL 0.1 mol/L HCl were mixed evenly. And then 10 mg pepsin was added to mixture evenly after water bath at 37 °C for 20 min, and the mixture was incubated in a constant temperature shaker at 37 °C for 240 min. The pepsin hydrolysate was adjusted to pH 7.0 with 1.0 mol/L phosphate buffer (pH 8.0). The mixture was centrifuged at 5000 r/min for 10 min after being boiled in a water bath for 15 min. 10 mL supernatant was sampled, and crude nitrogen content was determined by Kjeldahl method after acid digestion using Kjeltec 8400 Analyzer Unit (FOSS Tecator, Hoganas, Sweden). The in vitro digestibility of the protein was calculated according to the Formula 4.

$$Protein\ in\ vitro\ digestibility\ /\% = N / N_{tot} \times 100 \tag{4}$$

N-enzymatic hydrolysis of the nitrogen content in the supernatant after 480 min;

N_{tot}-the total nitrogen content of steamed bread, nitrogen content mentioned here was the nitrogen content of dry matter.

2.10 Statistical analysis

Data were presented as the mean ± SD. Analysis of variance (ANOVA) was used to compare the differences among treatments. The significance level was analyzed using the Duncan test in SPSS 22 (IBM Corporation, Armonk NY, USA). The values were considered statistically significant at $p < 0.05$.

3 Results and discussion

3.1 Sensory evaluation of Chinese steamed bread

Effect of heating method on sensory evaluation of refrigerated steamed bread was shown in Table 2. In this study, the fresh CSB got higher total scores than the natural thawed CSB for most sensory index, including appearance, color, texture, elasticity, firmness and hard core, which showed that the sensory quality of CSB decreased significantly after being refrigerated for 24 h. In contrast to the naturally thawed CSB, refrigerated CSB re-heated by steam and microwave got higher scores in appearance, texture, elasticity, firmness and hard heart, it showed that the two re-heating methods could improve the sensory quality of the refrigerated steamed bread. While steam re-heating got higher total scores than microwave re-heating, especially in color, texture and hard core. This indicated that compared with microwave re-heating, steam re-heating could improve the sensory quality of steamed bread better after refrigerated and was more acceptable.

Effect of refrigerating time on sensory evaluation of steam bread re-heated by steam was shown in Table 2. The scores had no significant difference between the fresh bread (0 h) and that being refrigerated for 12 h. While there was no significant difference between fresh bread and the re-heated steam bread refrigerated for 24 h in color, aroma, elasticity, stickiness and hard heart. Total scores of sensory evaluation decreased when the bread was refrigerated for more than 24 h. Scores of the bread refrigerated for 128 h was lowest in every sensory index. With the cold refrigerated time increasing, the total score of re-heating stored, refrigerated bread decreased significantly. This might be explained that the moisture content of steam bread affected sensory evaluation to some extent, when the moisture content decreased after 48 h, and the score of sensory index also gradually decreased. According to the total scores of sensory evaluation, optimum cold storage time for the bread re-heated by steam should be no more than 24 h.

The functional properties of starch control the moisture, viscosity and texture of the finished product. Nutritional properties of Chinese steamed bread depended on changes in starch during processing (Wang & Copeland, 2013). These changes included water absorption of starch, granule swelling. Starch underwent retrogradation during the cold storage, which affected sensory evaluation (Zhao et al., 2022). The water loss of FSB and the retrogradation of the starch occurred during the storage process, while starch would form a viscoelastic paste during heating (Chung et al., 2009). After steam re-heating, the moisture content increased and starch retrogradation was retarded, which affected the sensory evaluation.

3.2 Moisture content of Chinese steamed bread

Effect of heating method on moisture content of re-heating stored, refrigerated bread was shown in Figure 1a. There were significant differences in the moisture content between different re-heating method. Compared with the fresh steamed bread, moisture content of the naturally thawed steamed bread decreased significantly after 24 h of cold storage. After steam re-heating, there was no significant difference in water content between the fresh bread and that re-heated by steam, while the moisture content of the bread re-heated by microwave was lowest. Steam re-heating was to bring moisture and energy to the steamed bread from the outside to the inside through the strong penetration of water vapor (Uzzan et al., 2007). In contrast, microwave heating produced inverted temperature gradient, namely it was higher internally and colder at the surface. Resulting in the water inside the steamed bread lost outside due to the temperature difference (Carbonaro et al., 2012). The moisture content of the microwave re-heating steamed bread reduced, resulting in low score of texture and hard core. However, the moisture content of the steamed bread was replenished after steam re-heating, which improved the sensory quality of CSB.

Table 2. Effect of reheating method and refrigeration time on sensory evaluation of steamed bread.

		Sensory Index								
		Appearance	Color	Aroma	Texture	Elasticity	Firmness	Stickiness	Hard Core	Total Score
Reheating method	Fresh (0 h)	13.75 ± 0.70^{c}	8.50 ± 0.53^{b}	4.62 ± 0.51^{a}	11.87 ± 0.83^{c}	11.75 ± 0.71^{c}	6.75 ± 1.28^{b}	7.00 ± 0.75^{b}	18.75 ± 1.04^{c}	84.38 ± 2.26^{d}
	Natural thawed	7.87 ± 0.83^{a}	7.00 ± 0.75^{a}	4.00 ± 0.75^{a}	8.37 ± 1.06^{a}	4.62 ± 0.74^{a}	4.62 ± 0.51^{a}	6.37 ± 1.06^{ab}	8.50 ± 1.19^{a}	51.38 ± 2.56^{a}
	Steam	11.37 ± 1.30^{b}	8.12 ± 0.64^{b}	4.52 ± 0.53^{a}	11.00 ± 0.75^{c}	7.37 ± 1.03^{b}	6.50 ± 0.71^{b}	5.37 ± 1.18^{a}	11.00 ± 0.75^{c}	73.63 ± 3.46^{c}
	Microwave	10.87 ± 2.30^{b}	6.62 ± 0.98^{a}	4.50 ± 0.51^{a}	9.50 ± 1.06^{b}	8.25 ± 1.06^{b}	8.25 ± 0.53^{c}	6.25 ± 0.88^{ab}	9.50 ± 1.30^{b}	68.13 ± 2.53^{b}
Refrigeration time	0 h	13.75 ± 0.70^{c}	8.50 ± 0.53^{b}	4.62 ± 0.51^{a}	11.87 ± 0.83^{c}	11.75 ± 0.71^{c}	6.75 ± 1.28^{b}	7.00 ± 0.75^{b}	18.75 ± 1.04^{c}	84.38 ± 2.26^{d}
	12 h	13.00 ± 0.76^{d}	8.00 ± 0.76^{bc}	4.62 ± 0.52^{b}	11.63 ± 0.74^{cd}	11.63 ± 0.74^{cd}	8.13 ± 1.13^{b}	6.75 ± 0.71^{c}	18.38 ± 0.74^{bc}	82.13 ± 2.30^{e}
	24 h	11.88 ± 0.83^{c}	7.88 ± 0.83^{bc}	4.50 ± 0.53^{ab}	10.88 ± 0.83^{bc}	11.38 ± 1.06^{c}	7.88 ± 1.25^{b}	6.50 ± 0.76^{c}	18.25 ± 1.16^{bc}	79.13 ± 3.48^{d}
	48 h	11.37 ± 1.30^{c}	8.12 ± 0.64^{bc}	4.50 ± 0.53^{ab}	11.00 ± 0.76^{cd}	8.25 ± 1.04^{b}	6.75 ± 0.71^{a}	5.37 ± 1.19^{b}	18.00 ± 0.76^{bc}	73.63 ± 3.47^{c}
	72 h	9.88 ± 0.83^{b}	7.63 ± 0.52^{b}	4.00 ± 0.53^{ab}	10.13 ± 0.83^{b}	7.75 ± 0.71^{ab}	6.37 ± 0.52^{a}	5.00 ± 0.76^{ab}	17.5 ± 0.76^{b}	68.25 ± 2.26^{b}
	128 h	8.25 ± 1.04^{a}	6.00 ± 0.76^{a}	3.88 ± 0.83^{a}	7.88 ± 0.83^{a}	7.00 ± 0.53^{a}	5.75 ± 0.71^{a}	4.38 ± 0.52^{a}	16.25 ± 0.89^{a}	59.38±2.33^{a}

Results are represented as means ± SD, n = 3 and values within each column with the same letters are not significantly different (P > 0.05).

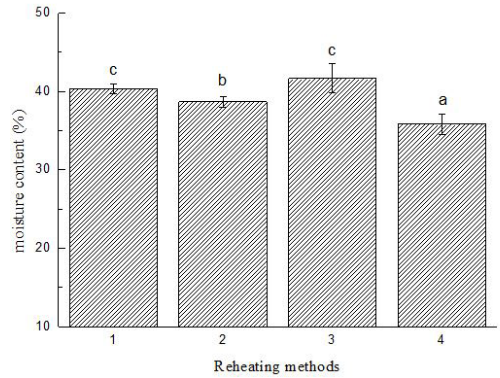

Fig. a.

1,2,3,4 represent the fresh steam bread, naturally thawed steamed bread, steam reheating, microwave reheating.

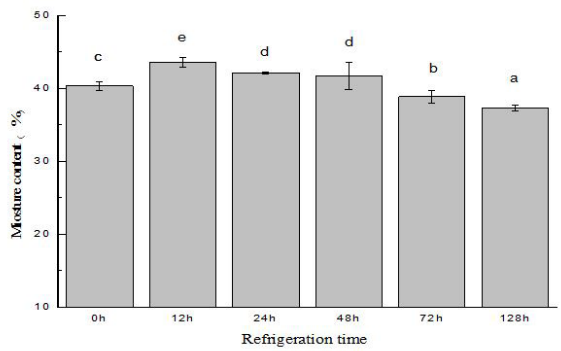

Fig. b.

Figure 1. Effect of reheating method and refrigeration time on moisture content of reheated CSB. Results are represented as means ± SD, n = 3 and values within each column with the same letters are not significantly different (P > 0.05).

Effect of refrigerating time on moisture content of steam bread re-heated by steam was shown in Figure 1b. The moisture content of re-heating steamed bread refrigerated for 12 h, 24 h and 48 h steam was higher than the fresh steamed bread (Figure 1b), while that of 72 h and 128 h was lower than the fresh bread. Moisture content of re-heating steamed bread refrigerated for 12 h was highest, while that of bread refrigerated for 128 h was lowest. There was no significant difference in moisture content between the bread refrigerated for 24 h and 48 h. After 48 h, the moisture content of re-heating steamed bread decreased significantly. Due to the temperature difference between the inside and outside of CSB, the steam began to transfer from the surface to the inside. The steam encountered the low temperature part and condensed into liquid water. The heat of the steam was gradually transferred to the inside of the steamed bread, and the moisture was gradually transferred to the inside of the steamed bread (Uzzan et al., 2007). However, as the storage time increased, starch retrogradation aggravated, and the moisture absorption performance also decreased (Ma et al., 2019). Therefore, the moisture compensation of the steamed bread decreased during re-heating, which affected the sensory evaluation.

3.3 Protein secondary structure of Chinese steamed bread

Effect of heating method on secondary structure of the protein of refrigerated steamed bread was shown in Table 3. Content of α-helix, β-sheet and random coil in the natural thawing bread were higher than those of the fresh bread. After re-heating, the β-turn structures increased significantly compareed with the natural thawing, while α-helix, β-sheet and random coil decreased. The secondary structure of the protein was primarily maintained by non-covalent forces. As conditions changed, protein molecules underwent a conformation rearrangement to achieve the lowest energy to maintain a relatively stable state (Wang et al., 2016). The α-helix structure was more orderly and generally supported the main peptide backbone structure of the polypeptide. The content of the α-helical structure reduced after re-heating. Heating may destroy the skeletal structure of gluten, resulting in decreasing molecular size and lower spatial aggregation order (Mejri et al., 2005).

Effect of refrigerating time on secondary structure of the protein of steam bread re-heated by steam was shown in Table 3. The content of α-helix structure decreased from 0 h to 48 h,

Table 3. Effect of reheating method and refrigeration time on protein secondary structure of protein of CSB.

		structure			
		α-helix (%)	β-turn (%)	β-sheet (%)	Irregular curl (%)
Reheating method	Fresh (0h)	26.45 ± 0.07^{b}	37.56 ± 0.11^{d}	18.74 ± 0.42^{a}	17.23 ± 0.39^{a}
	Natural thawed	29.51 ± 0.05^{c}	28.42 ± 0.07^{a}	24.09 ± 0.28^{b}	20.47 ± 0.56^{b}
	Steam	24.30 ± 0.21^{a}	34.62 ± 0.12^{c}	23.27 ± 0.07^{d}	17.78 ± 0.07^{a}
	Microwave	25.35 ± 0.28^{ab}	31.95 ± 0.07^{b}	22.96 ± 0.14^{c}	19.72 ± 0.23^{b}
Refrigeration time	0 h	26.45 ± 0.07^{e}	37.56 ± 0.11^{d}	18.74 ± 0.42^{a}	17.23 ± 0.39^{b}
	12 h	25.38 ± 0.27^{d}	36.42 ± 0.38^{d}	21.45 ± 0.36^{b}	16.74 ± 0.24^{a}
	24 h	24.30 ± 0.21^{c}	34.63 ± 0.12^{c}	23.27 ± 0.07^{c}	17.79 ± 0.07^{c}
	48 h	22.60 ± 0.36^{a}	30.44 ± 0.49^{b}	27.33 ± 1.14^{d}	19.62 ± 0.27^{d}
	72 h	23.21 ± 0.28^{b}	29.15 ± 0.27^{a}	27.20 ± 0.44^{d}	20.44 ± 0.27^{e}
	128 h	29.83 ± 0.38^{f}	28.70 ± 0.89^{a}	21.00 ± 0.48^{b}	20.46 ± 0.12^{e}

Results are represented as means ± SD, n = 3 and values within each column with the same letters are not significantly different (P > 0.05).

the content of β-turn structure decreased from 0 h to 128 h, while the content of β-sheet structure increased from 0 to 48 h. Changes in the molecular structure of proteins make the spatial structure of proteins tend to attain stable (Wang et al., 2016). Content of random coil increased significantly from 12 h to 72 h, the content of random coil in 72 h and 128 h were higher than other treatments. The secondary structure change of 128 h was irregular due to the multicomponent nature of CSB regime and environmental complexity, which needed further studies.

3.4 Extractable amylose of Chinese steamed bread

Effect of heating method on amylose content of refrigerated steamed bread was shown in Figure 2a. Compared with the fresh steamed bread, the content of amylose in natural thawing bread and the bread re-heated by steam and microwave decreased significantly. Compared with the natural thawed, the content of amylose in the bread re-heated by steam increased significantly, while that of the bread re-heated by microwave significantly reduced. Amylose content of native starch was often determined colorimetrically from the iodine complexation (Wang et al., 2015b), starch retrogradation related to the loss of the ability of starch to combine with iodine to form a colored complex. The absorbance value was positively correlated with starch content and negatively correlated with starch aging. This indicated that steam re-heating could alleviate starch retrogradation, while microwave re-heating could accelerate starch retrogradation. This effect might be explained that different heating methods make the starch change from soluble to insoluble, and the soluble amylose content decreased with the aging of the steamed bread (Morad & D'Appolonia, 1980).

Effect of refrigerating time on amylose content of steam bread re-heated by steam was shown in Figure 2b. As the refrigerating time increased, the amylose content of CSB re-heated by steam decreased significantly. Moisture content and amylose content had an effect on starch retrogradation. The moisture content affected the rate of starch retrogradation, which was mainly affected by amylose content (Zhou et al., 2011). After the steam re-heating, the moisture content of the steamed bread increased, and starch retrogradation was alleviated. The moisture content reduced after the microwave re-heating, and the starch retrogradation accelerated. The amylose content in re-heating bread reduced, the amylose changed from soluble to insoluble during the storage of CSB, which related to the recrystallization of starch (Chung et al., 2009), the content of soluble amylose decreased with the aging of bread (Morad & D'Appolonia, 1980). With the increase of storage time, aging of CSB increased. Although steam re-heating alleviated starch retrogradation, it could not completely eliminate starch retrogradation after re-heating.

3.5 In vitro starch digestion of Chinese steamed bread

Effect of heating method on In vitro starch digestion of refrigerated steamed bread was shown in Table 4. Compared with fresh steamed bread, the content of RDS in natural thawed steamed bread was lower, while the content of SDS and RS was higher; the content of RDS and RS in CSB re-heated by steam and by microwave decreased significantly, while SDS increased significantly. The result showed that the digestibility of starch decreased significantly after cold storage in the refrigerator. This might be due to the fast retrogradation of amylose molecules after 24 h cold storage in the refrigerator (Chung et al., 2006). Compare with natural thawed CSB, the SDS content in CSB re-heated by steam and CSB re-heated by microwave increased significantly; the content of RDS was higher in that re-heated by steam, while that was lower in CSB re-heated by microwave; the content of RS decreased in CSB re-heated by steam and microwave, the result showed that steam re-heating improved the digestibility of starch in the refrigerated bread better than the microwave method. In the storage and heating process of CSB, the more the starch structure was disrupted during the process, the greater was the susceptibility of the starch to enzymatic digestion (Wang & Copeland, 2013). In the storage and heating process of CSB, temperature of microwave heating was higher than that of steam heating, which was destructive to starch structure, and the sensitivity of starch to enzyme was also increased (Wang & Copeland, 2013). Extractable amylose content in CSB re-heated by microwave was lowest, while the fresh steam was highest in fresh steamed bread, which might explain the result. As for In vitro starch digestibility, steam re-heating was better than microwave re-heating. The fresh steamed bread was frozen and then reheated, the digestibility of fast digestible starch can be significantly reduced, which was helpful for the regulation of blood glucose

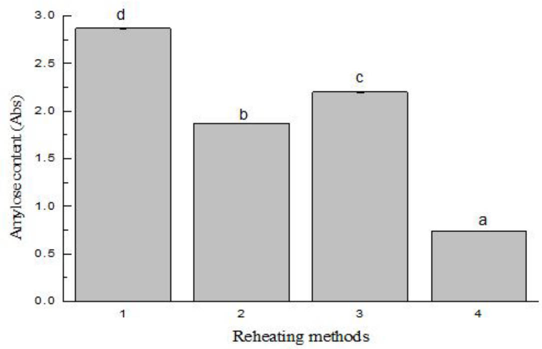

Fig. a.

1–4 represent the fresh steam bread, naturally thawed steamed bread, steam reheating, microwave reheating, respectively.

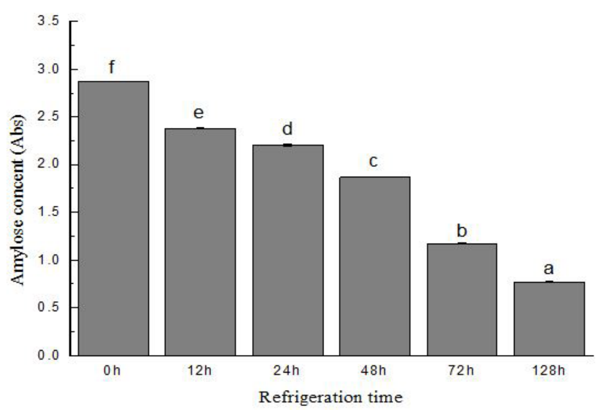

Fig. b.

Figure 2. Effect of reheating method and refrigeration time on Amylose content of reheated CSB. Results are represented as means ± SD, n = 3 and values within each column with the same letters are not significantly different (P > 0.05).

Table 4. Effect of reheating method and refrigeration time on in vitro starch and protein digestibility of CSB.

		RDS	SDS	RS	Protein Digestion (%)
	Fresh (0 h)	54.80 ± 2.70^{d}	9.40 ± 2.44^{a}	37.71 ± 0.05^{c}	88.92 ± 9.42^{d}
	Natural thawed	44.54 ± 1.52^{b}	17.29 ± 1.18^{b}	38.17 ± 0.03^{d}	55.73 ± 3.33^{a}
	Steam	48.46 ± 0.10^{c}	18.30 ± 1.01^{c}	33.24 ± 0.01^{a}	60.15 ± 10.07^{b}
Reheating method	Microwave	44.11 ± 2.03^{a}	18.36 ± 0.75^{d}	37.53 ± 0.03^{b}	61.53 ± 2.87^{c}
	0h	54.80 ± 2.70^{d}	9.40 ± 2.44^{a}	37.71 ± 0.05^{c}	88.92 ± 9.42^{d}
	12h	46.99 ± 8.46^{ab}	15.45 ± 2.35^{c}	37.56 ± 0.11^{ab}	62.34 ± 0.78^{a}
	24h	44.11 ± 2.03^{ab}	17.36 ± 0.75^{c}	38.53 ± 0.03^{ab}	60.66 ± 10.08^{a}
Refrigeration time	48h	42.46 ± 7.31^{ab}	24.85 ± 2.73^{d}	32.69 ± 0.10^{a}	59.40 ± 3.02^{a}
	72h	40.02 ± 2.22^{a}	4.94 ± 1.03^{a}	55.07 ± 0.03^{c}	55.58 ± 6.48^{a}
	128h	40.31 ± 4.72^{a}	8.62 ± 0.00^{ab}	51.07 ± 0.05^{bc}	53.40 ± 2.52^{a}

Results are represented as means ± SD, n = 3 and values within each column with the same letters are not significantly different (P > 0.05).

Effect of refrigerating time on In vitro starch digestion of steam bread re-heated by steam was shown in Table 4. Content of RDS significantly decreased from 0 h to 128 h, content of SDS increased from 0 h to 48 h and then decreased, while the RS content had a trend to increase from 0 h to 128 h. Starch digestibility had positive correlation with RDS content. It showed that in vitro starch digestion in steam re-heating stored, refrigerated bread decreased with refrigerated time. In vitro starch digestion had a certain correlation with starch retrogradation. Cold storage induced retrogradation of amylose molecules at constant low temperatures, which might explain the reducing digestibility of starch (Chung et al., 2006; Zhou & Lim, 2012).

3.6 In vitro protein digestion of Chinese steamed bread

Effect of heating method on In vitro protein digestion of refrigerated steamed bread was shown in Table 4. Compared with fresh steamed bread, naturally thawed steamed bread had a lower In vitro protein digestibility. Compared with the natural thawed steamed, In vitro protein digestibility was significantly improved after steam and microwave re-heating. This indicated that re-heating could improve In vitro protein digestibility. As for In vitro protein digestion, microwave re-heating was better than steam re-heating. There was an inverse correlation between β-sheet content and the protein digestibility (Carbonaro et al., 2012). Compared with natural thawing, the content of β-sheet after re-heating decreased, which could explain that the re-heating improved the In vitro digestibility of protein.

Effect of refrigerating time on In vitro protein digestion of steam bread re-heated by steam was shown in Table 4. With the storage time increasing, the protein digestibility of steamed bread gradually decreased after being re-heated by steam. The changes of protein secondary structure affected protein digestion (Zhang & Yu, 2012). The content of the α-helix and β-sheet structures in the protein secondary structure influences the nutritional value and digestion behavior of the protein (Yu, 2005). Content of β-sheet increased from 0 to 128 h, which could explain that the in vitro protein digestion decreased after steam re-heating as the storage time increased.

4 Conclusions

Re-heating could increase moisture content of reheated bread especially in steam re-heating treatment. Steam re-heating could promote starch gelatinization and alleviate starch retrogradation, while microwave re-heating could accelerate starch retrogradation. Reheating treatment of being stored, refrigerated bread can significantly reduce the digestion of starch. Steam re-heating could improve digestibility of protein but the protein digestibility of steamed bread gradually decreased after being re-heated by steam with the storage time increasing.

Microwave and steam re-heating significantly improved the nutritional quality and digestive characteristics of CSB, steam re-heating was better than microwave re-heating. The digestibility of fast digestible starch in bread reheated can be significantly reduced, which was helpful for the regulation of blood glucose. Optimum cold storage time for the bread re-heated by steam should be no more than 24 h.

Acknowledgements

The study was funded by the Fund of National Engineering Laboratory/ Provincal Key Laboratory of food Science Discipline, Henan University of Technology (NL2021007), Key Scientific Research Projects of Higher Education Institutions of Henan Province (2018QNJH24) and Research Fund for High-level Talents of Henan University of Technology (2017BS019).

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