Effect of ultrasound-assisted dough fermentation on the quality of dough and steamed bread with 50% sweet potato pulp

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ABSTRACT

Ultrasound at an intensity of 17.5, 20.0, 22.5, 25.0 and 27.5 W/L was used to assist dough fermentation to prepare steamed bread with 50% sweet potato pulp (SB-50% SPP), which was compared with SB-50% SPP without ultrasonic treatment. The dough rheology, starch-gluten network, texture characteristics and sensory quality of steamed bread with different ultrasonic power densities (UPDs) were investigated. Dough samples at UPD of 22.5 W/L showed optimal viscoelasticity. The microstructure images exhibited that the content of starch particles wrapped in the gluten network increased significantly after sonication. In addition, the reduction in free sulphydryl (SH) content and increase in wet gluten content after ultrasonic treatment led to significantly improved dough extensibility (p < 0.05). Results exhibited that the specific volume of SB-50% SPP increased by 13.93% and the hardness decreased by 21.96% compared with the control under UPD of 22.5 W/L. This study suggested that the application of ultrasound as a green technology to dough fermentation could lead to SB-50% SPP with good quality and sensory characteristics.

1. Introduction

Steamed bread is one of the traditional staple foods in central China, which occupies a vital position in their daily diet [1]. Traditional white steamed bread (WSB) mainly contains refined wheat flour, yeast and water, which is popular with consumers due to its soft texture, good sensory, and flavour characteristics [2]. The nutritional deficiency and high glycaemic index of WSB increased the risk of diseases such as obesity and cancer, which attributed to that the main component of wheat flour was starch [3,4]. The diversity of staple foods and the convenience of life made consumers pay close attention to the nutrition and function of their diet. Therefore, methods to improve nutritional characteristics of steamed bread had become a research hotspot. Relevant studies have shown that high nutritional grains (HNGs; such as potato, purple sweet potato, cassava, sorghum, and buckwheat) were added to wheat flour was a feasible way to solve the nutritional problem of steamed bread [4–9]. Sweet potato (Ipomoea batatas [L.] Lam) is native to South America and widely cultivated in tropical and subtropical areas all over the world. [10]. It is an important vegetable and grain crop with high yield and strong adaptability. Moreover, sweet potatoes have high nutritional value, which protein, β-carotene, minerals (phosphorus, potassium, calcium), vitamins and other dietary bioactive substances [11]. In recent years, studies have shown that bread and steamed bread prepared by adding an appropriate amount of sweet potato flour into wheat flour exhibited increased antioxidant activity in vitro and decreased glycaemic index [4,12]. Therefore, it is feasible to add sweet potato into wheat products to improve its nutritional value. However, the quality of WSB decreased significantly after adding sweet potato flour in terms of poor taste, poor quality and skin shrinkage. Thus, appropriate processing methods are sought to ensure that HNG can increase the nutritional value and reduce its adverse influence on the overall attributes of steamed bread, which will be the key to promote the research progress of HNG steamed bread.

Ultrasound has been extensively applied in the field of food processing due to its green and environmental protection characteristics [13]. Cavitation and shear force induced by ultrasound can affect the quality of food [14]. Ultrasonic treatment can significantly influence the structure of ingredients in food, resulting in changes in food colour, flavour, texture and safety [15–17]. Research showed that the structure and properties of proteins and enzymes changed after sonication, which

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can alter food texture and quality [18,19]. Studies have shown that 400 W ultrasonic treatment of soybean protein isolate could significantly enhance the solubility, emulsifying stability, emulsifying activity, surface hydrophobicity and turbidity of soybean protein isolate [20]. Meanwhile, ultrasound also played a vital role in improving the enzymatic reaction kinetics and thermodynamics of alkaline protease. Thus, ultrasound treatment could improve the enzymatic hydrolysis efficiency [18]. Moreover, ultrasound has been applied to flour products. Ultrasound-assisted freezing dough improved the water distribution in frozen dough and enhanced the stability of the protein molecular structure [21]. In the tempering process of flour processing, the application of ultrasound could prolong the stabilisation time of flour, increase the softening resistance and elevate the specific volume of bread by 7.53% [15].

At present, there were few reports on the application of ultrasonic technology to improve the quality of HNG steamed bread. However, studies have shown that the processing characteristics and product quality of flour products could be improved by ultrasound. Therefore, this work mainly investigated the application of ultrasound-assisted dough fermentation to prepare dough and SB-50% SPP. The dough rheology, starch-gluten network, microstructure, specific volume, texture characteristics, colour and sensory properties of steamed bread were analysed. This study can offer a reference basis for improving the quality characteristics of steamed bread containing HNG.

2. Materials and methods

2.1. Materials

Wheat flour with protein and moisture of 14.89 g/100 g and 13.54 g/100 g (on a dry basis), respectively, was obtained from Zibo Yunhai Flour Factory (Zibo, China). Fresh sweet potatoes and active dry yeast (Angle Yeast Co., Ltd., China) were obtained from the local market (Zibo, China). Sweet potatoes were peeled and cut into small pieces after washing. The sweet potatoes were mixed with water in proportion (the ratio of water to sweet potato is 15:1), and the mixture was crushed in a blender (Zhongshan Dizan Electric Appliance Co., Ltd, China, 5000 r/min) for 2 min. All other reagents and chemicals used were of analytical reagent grade.

2.2. Preparation of dough and steamed bread

The basic recipe of WSB was according to previous experiments: 300.0 g of wheat flour, 171.6 g of water and 3.0 g of active dry yeast were mixed together [22]. Wheat flour was replaced by SPP at 50% (w/w) level to make steamed bread and the amount of water added was 0%. WSB and the steamed bread formulated with 50% SPP were used as control. All the ingredients containing wheat flour, SPP, yeast and water were mixed and kneaded manually for 5 min. The dough was fermented for the first time in a fermentation tank (BRF-18C, Guangzhou, China) for 60 min at 32 °C and 80% relative humidity. After the first fermentation, the dough was sheeted seven times and cut into several portions of about 100 g of dough. Subsequently, the dough was rounded, formed manually and fermented under the same conditions as the first fermentation for 15 min. Finally, the fermented dough was steamed for 20 min and the steamed bread was cooled for 60 min at 25 °C with 50% relative humidity. The production process of the dough with ultrasound was consistent with the control. The prepared dough was placed in a fresh-keeping bag. Then, a 15 cm-long straw was inserted into the bag and tied tightly to keep the dough in contact with the outside air and prevent the infiltration of external moisture. The bag with dough was put into an ultrasonic bath (KQ-500DE, Kun Shan Ultrasonic Instrument Co., Ltd, Kun Shan, 40 kHz of frequency) for ultrasonic treatment for 40 min. The bag was located at 3 cm below the water level and 7 cm from the bottom as described in the literature [23]. The water temperature in ultrasonic tank was maintained at 32 °C, which was controlled by a cooling water circulation system. According to the previous experiment, the ultrasonic time was fixed at 40 min, and the UPD was 17.5, 20.0, 22.5, 25.0 and 27.5 W/L. The dough after sonication was treated the same as the control.

2.3. Determination of wet gluten content in the dough

Wet gluten content was determined following the method of Approved Method 38–10 (AACC, 2000) [24]. The determination starts after the first fermentation of dough. All the above analyses were repeated three times.

2.4. Rheological properties

A KNX2110 rheometer (Malvern instrument, UK) installed with a parallel plate (40 mm diameter) was applied to analyse the rheological properties of dough according to previous experiments [25]. Dough samples after that first fermentation were determined at 25 °C and a little silicone oil was used to the exposed surface of the dough to avoid drying the dough surface. The frequency scanning range of dough was 0.01–10 Hz. The strain amplitude and gap size were set to 0.1% and 2 mm respectively. The elastic modulus (G’) and viscous modulus (G’″) related to dough viscoelasticity were obtained. All the above analyses were repeated three times.

2.5. Determination of free SH content

The content of free SH in gluten was determined by spectrophotometry according to the method based on a spectrophotometric assay [26]. The freeze-dried dough sample (75 mg) was mixed with 1 mL Tris-Glycine buffer solution (86 mmol/L Tris, 90 mmol/L Glycine, 4 mmol/L EDTA, pH 8.0) and then 4.7 g guanidine hydrochloride was added to the mixture. The mixture was diluted with Tris-Glycine buffer solution to 10 mL and immediately stirred for 30 min. The mixed liquor was centrifuged at 4000 r/min for 10 min. The supernatant (1 mL) was added with 5 mL of urea solution (8 mol/L urea, configured with Tris-Glycine buffer) and 0.04 mL of Ellman’s solution (4 mg/ml DTNB (Dithiobis-2-nitrobenzoic acid), configured with Tris-Glycine buffer). The absorbance value of the dough sample at 412 nm was read after incubation in a water bath (25 °C) for 30 min. Distilled water was used as the blank group. All the above analyses were repeated three times. The content of free SH was calculated through the following formula:

$$Free\;SH\;(\mu\;mol/g) = 73.53\times A_{412}/D\times C$$

where A412 means the absorbance at 412 nm, D means the dilution factor (D is 5.02), and C denotes the sample concentration in mg/mL.

2.6. Elongation properties

The elongation characteristics of dough treated with different ultrasonic power densities were determined by using A/KIE equipped with texture analyser ( Stable Micro Systems, Goldalming, UK). The dough was pressed into the mould. The tests were conducted at a distance of 75.0 mm and the trigger force was set to 5.0 g. The pretest, testing and posttest speeds were 2.0, 3.3 and 10.0 mm/s. The resistance to extension (g) and extensibility (mm) of dough were obtained. All the above analyses were repeated three times.

2.7. Scanning electron microscopy (SEM)

SEM (Sirion200, FEI, USA) was conducted to observe the microstructure of fermented dough. The dough was freeze-dried and cut into cubes with dimensions of 1 cm × 1 cm × 1 cm. The dough samples were coated with sputtered gold and observed by SEM under 10 KV energy.
2.8. Specific volume evaluation

The specific volume of the cooled steamed bread was determined through the method of millet replacement, and the bread was weighed. The specific volume was expressed in mL/g. All the above analyses were repeated three times.

2.9. Texture profile analysis (TPA)

The TA-XT2i texture analyser (Stable Micro Systems, Goldalming, UK) equipped with 5 kg weighing sensor and a probe of P/36R can be used to determine the texture profile of steamed bread. Two 20 mm thick slices were cut from the middle of steamed bread for analysis. The pretest, testing and posttest speeds were 1.0, 1.0 and 2.0 mm/s, respectively. All the above analyses were repeated six times. The hardness (g), springiness, cohesiveness, and chewiness were obtained. All the above analyses were repeated three times.

2.10. Colour measurements

The colorimeter (CM-3600A, Konica Minolta, Osaka, Japan) was used to measure the colour of crust and crumb of steamed bread, and the colorimeter was calibrated with white and black standard tiles. Lightness, redness/greenness and yellowness/blueness were represented by colour parameters L*, a* and b*, respectively. All the above analyses were repeated six times. The hard,ness (g), springiness, cohesiveness, and chewiness were obtained. All the above analyses were repeated six times.

2.11. Sensory evaluation

Sensory evaluation was performed according to the Chinese standard method SB/T 10,139-1993 with some modifications. The evaluation was performed by trained panellists from Shandong University of Technology. The related information is shown in Table 1.

2.12. Statistical analysis

All the analyses were performed in triplicate. Results were expressed as mean ± standard deviation. The statistical analysis and one-way analysis of ANOVA followed by the Duncan’s test was performed with the SPSS 23.0 software. Differences were regarded as significant at p < 0.05.

3. Results and discussion

3.1. Effect of different UPDs on the dough quality

3.1.1. Contents of wet gluten in dough

Gluten protein is the basis of dough formation, which plays a vital role in the attributes of steamed bread [27]. The contents of wet gluten in the dough added with SPP declined significantly compared with that of WD (p < 0.05; Table 2). SPP did not contain gluten protein, and the addition of SPP reduced the proportion of gluten protein in dough. Ultrasound treatment did not cause the change in wet gluten contents in the dough within the range of UPD. However, the wet gluten content of the dough decreased significantly when the UPD reached 27.5 W/L compared with the control. High UPDs might lead to the destruction of disulphide bonds between protein molecules in dough, resulting in protein denaturation and secondary structure changes [28].

3.1.2. Influence of UPD on rheological properties of dough

The viscoelastic behaviour interrelated with the stability of gluten network is closely related to the rheological properties of dough, which ultimately affect the fermentation properties of dough and product quality [25]. Fig. 1 shows the changes in G′ and G″ with frequency of dough containing 50% SPP under different UPDs. G′ was lower than G″ in the total frequency scanning range for all the dough samples (G′ > G″), indicating that the elastic modulus was dominant. Studies have shown that SPP applied to dough could reduce the gluten content in the dough, further destroy the construction of gluten network structure and ultimately cause the viscosity modulus and elastic modulus diminish significantly [3]. However, G′ and G″ of dough with sonication increased compared with the control, which indicated that sonication could enhance the rheological properties of dough containing 50% SPP. Similar results were observed for gluten-free dumplings prepared by ultrasonic treatment of dough. The application of ultrasound during the preparation of gluten-free dough changed its rheological characteristics and homogenised the dough structure [29]. That study further declared that dough with ultrasonic treatment exhibits excellent rheology and elasticity compared with the control, and could be used to form dumplings. In addition, the viscoelastic modulus of dough with sonication at the UPD of 22.5 W/L was higher than that of other dough. This result might be attributed to the low SH contents in dough under the action of low UPDs, which enhanced the network structure of gluten and improved the dough rheological properties [30]. However, the effect of sonication on G′ and G″ of dough decreased significantly when the UPD was above 22.5 W/L. Therefore, high UPDs could lead to the unfolding of the wheat glutenin structure, weaken the interaction between glutenin and gliadin, and cause the destruction of chemical bonds between stable protein molecules such as polar hydrogen bonds and S-S [23].

3.1.3. Content of free SH in dough

Free SH can be oxidised by molecular oxygen to form disulphide for Table 2

| Table 2 | Free sulphydryl groups, wet gluten and elongation properties in dough with sonication. |
|---------------------------------|----------------------------------------------------------|
| Ultrasonic power density (W/L) | Free SH (μmol/g) | Wet gluten (%) | Resistance to extension (g) | Extensibility (mm) |
| WD | 3.05 ± 0.07a | 27.05 ± 0.08a | 45.74 ± 1.82a | 18.04 ± 1.12a |
| Control | 4.67 ± 0.11ab | 18.16 ± 0.47ab | 35.16 ± 1.69ab | 12.54 ± 0.14ab |
| 17.5 | 4.36 ± 0.11ac | 18.02 ± 0.19ac | 38.70 ± 0.09ac | 12.45 ± 0.58ac |
| 20.0 | 4.23 ± 0.05cd | 18.79 ± 0.33cd | 40.45 ± 1.49bd | 14.51 ± 0.60bd |
| 22.5 | 4.00 ± 0.23de | 19.25 ± 0.86de | 43.39 ± 1.72de | 15.50 ± 0.73de |
| 25.0 | 4.37 ± 0.10ef | 18.94 ± 0.85ef | 32.87 ± 0.85ef | 13.21 ± 0.16ef |
| 27.5 | 4.94 ± 0.31f | 17.11 ± 0.30f | 30.12 ± 2.40f | 11.79 ± 0.34f |

Means with different lowercase letters in the same column are significantly different (p < 0.05).
bonds, which stabilise the conformation of protein molecules [21]. Table 2 shows that the content of free SH in dough increased by 53.11% after the addition of SPP. The contents of free SH in wheat dough (WD) were significantly lower than that of the control \((p < 0.05)\), because the structure of the gluten network was diluted by the addition of SPP and further reduced the dough stability. Nonetheless, the contents of free SH in dough declined initially and then increased with the increase in UPD. Furthermore, free SH contents decreased significantly by 14.35% compared with the control at the UPD of 22.5 W/L \((p < 0.05)\). That might be because sonication under low UPDs could enhance the interaction between protein molecules and make the cross-linking between glutenin molecules more orderly, thus reducing the content of free SH provided for gluten protein cross-linking, and ultimately promoting the construction of gluten network structure [31]. In addition, hydroxyl radicals generated by cavitation effect of ultrasonic in water could oxidize free SH into S-S and finally enhance the network structure of gluten protein [32]. The free SH contents increased when the UPD was above 25.0 W/L. This was consistent with previous studies that the increase of SH content might be due to the cavitation effect of ultrasound, which caused protein folding and exposes the internal free SH to the protein surface. In general, the interruption of S-S in protein molecules could be reflected by an increase in free SH content in the protein [33]. These results indirectly proved the destruction of S-S bonds by sonication when the UPD was high, which caused that fracture to be converted into free SH [34]. Thus, the molecular structures and S-S of gluten were damaged by the cavitation induced by the application of high UPDs [23].

3.1.5. Effect of ultrasonic treatment on the microstructure of dough

The microstructure of dough samples with different UPDs was observed by SEM (Fig. 2). Wheat flour dough exhibited starch granules that were tightly embedded in a compact and well-established gluten network (Fig. 2A) [25]. The starch granules in the control were bonded to one another, and some starch granules were exposed compared with WD because that could not be wrapped by the gluten network (Fig. 2C). Results indicate that the addition of SPP reduced the content of gluten protein, hindered the construction of gluten network structure, and ultimately destroyed the continuity and integrity of WD microstructure. However, the continuity of the gluten network structure with sonication significantly improved compared with the control, and starch granules from SPP could be evenly filled into the gluten starch matrix. Sonication could significantly improve the damage of the dough gluten network structure caused by the addition of SPP, and finally enhance the quality of dough. Moreover, the starch granules in the dough were most evenly distributed at the UPD of 22.5 W/L (Fig. 2B). The improvement effect of ultrasonic treatment on dough began to decline under UPD above 22.5 W/L (Fig. 2F and 2G). On the other hand, this phenomenon might be due to the cavitation effect caused by high UPDs, which destroyed the S-S and hydrogen bond of gluten and ultimately resulted in the reduction of the dough gluten network’s strength [18]. On the other hand, high UPDs led to excessive fermentation of dough, and the carbon dioxide gas produced by yeast would be released prematurely from the pores of the discontinuous gluten protein membrane, thereby reducing the ability of dough to hold gas and worsening the microstructure of dough [23].

3.2. Effect of ultrasound on steamed bread quality

3.2.1. Specific volume and texture

Specific volume and texture characteristics reflect the cooking characteristics, softness and chewing characteristics of steamed bread, which are important indicators to measure the steamed bread attributes and consumer acceptability [3]. The specific volume and cohesiveness of the control decreased significantly, whilst the hardness and chewiness increased compared with WSB (Table 3). For one thing, the addition of SPP weakened the dough strength. For another, SPP contained a large amount of dietary fibre with strong water absorption ability, which affected the moisture of some gluten proteins and interfered with the formation of the gluten network, resulting in the deterioration of the texture characteristics of steamed bread [41]. Compared with the
control, the specific volume of SB-50% SPP with UPD of 22.5 W/L increased by 13.93%, which was closest to that of WSB. This growth might be attributed to the hydrogen bond between protein polymers being broken by ultrasound with low UPD, which led to the formation of small soluble protein aggregates, and decline in the hardness and volume of steamed bread [28]. Generally, good gluten network would enhance the elasticity and hardness of flour products [42]. When the UPD was 22.5 W/L, SB-50% SPP showed excellent hardness, elasticity, cohesion and chewiness. Consistent with the results of dough rheological properties, ultrasonic treatment made the dough more uniform and enhanced the gluten network structure, which finally led to the improvement of dough processing characteristics and quality characteristics. However, the dough treated by high UPDs might lead to excessive fermentation, resulting in the collapse of the gluten structure. Similarly, the volume of dough could be doubled by ultrasonic treatment in the process of forming dumplings [29]. Ultrasonic-assisted stirring of sponge cake batter would significantly increase the volume of sponge cake and make the cake obtain lower hardness, higher springiness, cohesiveness and resilience [43].

### 3.2.2. Colour

The colour of SB-50% SPP was dark as the L* value of crust and crumbs decreased and the a* and b* value increased compared with WSB (Table 4). Results illustrated that the addition of SPP made a notable difference to the colour of steamed bread, making it appear obviously dark yellow. Similarly, the addition of potato pulp significantly darkened the colour of steamed bread, which might be due to the browning reaction of phenolic substances and polyphenol oxidase (PPO) under the action of oxygen due to the cutting of potato into small pieces during the preparation of steamed bread [25]. Different UPDs had significant effects on the L*, a* and b* values of SB-50% SPP crust and crumbs compared with the control (p < 0.05). This colour change was mainly caused by sweet potato browning, which was caused by PPO played. Studies have shown that the activity of PPO was high in the browning area of sweet potato, which led to the accumulation of brown and reduced the sensory quality [44]. The L* value of SB-50% SPP increased significantly with ultrasonic treatment, and first increased and
slightly stickiness. Moreover, the colour of control changed from bright
3.2.3. Sensory evaluation
activity of PPO [46]. Moreover, the colour of product significantly
improved due to the bleaching ability and the homogenisation of ul-
treatment has no adverse effect on the colour of the sample [48].
mechanical vibration produced by ultrasound, which changed the confor-
This was due to the effects of cavitation, magnetostriction and me-
then decreased with the increase of UPD. In contrast, the values of a*
Table 4
White bread was prepared under 25% ultrasonic power density,

| Ultrasonic power density (W/L) | Crust color | Crumb color |
|-------------------------------|-------------|-------------|
|                              | L*          | a*          | b*          | L*          | a*          | b*          |
| WSB                           | 82.72±0.64 | −1.02±0.10 | 15.04±1.33 | 85.05±0.29 | −0.21±0.04 | 15.26±0.10 |
| Control                        | 69.29±0.91 | 10.61±0.36 | 43.05±0.65 | 68.27±0.73 | 4.56±0.36 | 44.08±0.51 |
| 17.5                          | 68.61±0.45 | 4.56±0.17  | 33.79±0.38 | 70.97±0.38 | 4.72±0.29 | 33.16±0.63 |
| 20.0                          | 69.95±1.08 | 4.86±0.47  | 34.22±1.05 | 71.24±1.05 | 4.69±0.04 | 33.01±0.23 |
| 22.5                          | 73.34±0.52 | 3.21±1.08  | 32.73±1.05 | 71.95±1.05 | 5.15±0.04 | 34.41±0.53 |
| 25.0                          | 70.03±0.32 | 4.17±0.17  | 34.53±0.47 | 69.98±0.21 | 5.24±0.19 | 34.23±0.19 |
| 27.5                          | 69.83±0.22 | 5.25±0.68  | 34.06±0.42 | 69.41±0.21 | 5.27±0.42 | 33.65±0.96 |

Means with different lowercase letters in the same column are significantly different (p < 0.05).

4. Conclusions
The quality of dough containing 50% SPP and SB-50% SPP was significantly improved by ultrasound assisted dough fermentation. Results demonstrated that the optimum attribute of SB-50% SPP was obtained under the UPD of 22.5 W/L, which was closest to WSB and more easily accepted by consumers. Ultrasonic treatment at low power density (< 25.0 W/L) could reduce the content of free SH in dough, and significantly promote the formation of disulphide bond and wet gluten, which resulted in an improvement in the rheological properties of dough containing 50% SPP and the quality characteristics of SB-50% SPP. SEM analysis exhibited that the dough with sonication had better continuity, and the starch particles were evenly filled into the gluten starch matrix, which made the gluten network structure more compact. Compared with the control, ultrasound had a remarkable impact on the specific volume, hardness, and chewiness of SB-50% SPP (p < 0.05). Moreover, the colour change of steamed bread caused by adding SPP improved by ultrasonic treatment, which exhibited that the colour of SB-50% SPP after sonication was lighter and closer to WSB. This study provided a new reference scheme for the development of steamed bread with high addition of SPP, further enriched the theoretical system of ultrasonic modification of flour products, and provided a theoretical basis for the application of ultrasonic technology in the field of steamed bread production.

Fig. 3. Sensory scores of quality attributes of steamed bread with SPP at different ultrasound power density.
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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