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Effect of vanillin-conjugated chitosan-stabilized emulsions on dough and bread characteristics

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ABSTRACT

In this study, the effect of chitosan-vanillin Schiff base emulsions (CSVAEs) on dough and bread characteristics was investigated. The results revealed that CSVAEs were embedded in the gluten and that the viscoelasticity and mechanical strength of the dough gradually increased with increasing CSVAEs concentration, α-helical and β-fold content, and elastic structure in the dough increased with the same patterns. The basic properties of bread were measured, and it was found that low concentrations of CSVAEs were effective in improving the quality of bread and slowing the staling rate. As the storage time increased, CSVAEs had less effect on the rate of moisture loss, hardness and springiness of the bread and more effect on the inhibition of the acidity of the bread. The addition of CSVAEs slowed the increase in bacteria and molds and extended the shelf life of the bread.

1. Introduction

During storage, bread undergoes physical, chemical and microbiological changes that result in a decrease in quality and a shorter shelf life. Physical and chemical changes reduce the freshness of the bread, the crumb gradually hardens, and the taste deteriorates. Microbial spoilage caused by bacteria, yeast and molds can produce toxins with off-flavors that affect people's lives and health (Melini and Melini, 2018). Stale bread is therefore an issue of concern because it causes huge economic losses to the baking industry and consumers, as well as human poisoning due to fungal toxin contamination (Goryńska-Goldmann et al., 2021). To address this economic and food safety issue, the baking industry has been working hard to find ways in which bread can be protected from physical and chemical changes and have a longer shelf life, reducing changes in the organoleptic quality of bread and improving the safety of bread consumption.

Changes in the quality of bread include both staling and microbial contamination. Bread staling is accompanied by crumb hardening, crust softening and loss of the characteristic fresh flavour of the product (Gauchez et al., 2020). The main theories on the mechanism of bread staling include the transfer of the moisture in bread, the retrogradation of starch, and the interaction between starch and gluten in bread (Ju et al., 2020). Some studies have shown that the crust readily absorbs water from the internal crumb, which has a moisture content of approximately 45 %, crust moisture can increase to 28 % when bread is stored for 100 h (Arp et al., 2020). Starch retrogradation is an ongoing process, which initially involves rapid recrystallization of amylose molecules followed by a slow recrystallization of amylopectin molecules. Amylose retrogradation determines the initial hardness of a starch gel and the stickiness and digestibility of processed foods. The long-term development of gel structure and crystallinity of processed starch, which are involved in the staling of bread and cakes, are considered to be due to retrogradation of amylopectin (Wang et al., 2015). The gluten network represents, with leached amylose, the continuous phase of bread, and its proper formation and hydration contributes to the perceived characteristics of a fresh bread. During storage of bread, the gluten network is expected to undergo physicochemical changes (i.e. dehydration and, consequently, loss of plasticity/flexibility; modified interaction with starch), contributing, possibly, to bread staling (Curti et al., 2014). Bread ingredients favor the growth and proliferation of microorganisms at all stages of bread production, processing, packaging and storage (Jideani and Vogt, 2016; Garcia and Copetti, 2019). Mold growth is the most common cause of bread spoilage (Luciana et al., 2009). Bread fresh from the oven is free of molds; and the moisture content and water activity of bread crust are usually too low to permit mold growth; but during storage, moisture moves from the moist crumb to the drier crumb zone, raising the moisture content and water activity of the latter. The changes result in a reduction of the crumb moisture

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content and an increase in that of the crust and raise the potential for mold growth (Cauvain, 2016). In addition, the main microorganism that causes bacterial spoilage is *Bacillus subtilis*, whose spores form endospores that readily survive baking and germinate and grow within the bread within 36~48 h; due to the release of volatile compounds such as diacetyl, ethylene coupling, acetaldehyde and isovaleraldehyde, a characteristic soft, sticky brown color with the smell of ripe pineapple or melon is produced. Bacteria also produce amylase and protease to degrade crumbs and increase the spoilage of bread. Yeast spoilage is the least common of all types of microbial spoilage, and yeast cannot survive the baking process. Molds and bacteria are the main pathogens of microbial spoilage of bread (Axel et al., 2017).

In the field of bread preservation, it is important to use the right methods to address spoilage and rancidity and extend shelf life without affecting the sensory and quality of the product. Physical factors, chemical factors and various packaging methods are all emerging areas of food technology that can provide many preservation benefits for a wide range of food products (Ramaswamy et al., 2012; Jideani and Vogt, 2016). The main objectives are to maintain organoleptic quality and extend the shelf life of food products while maintaining nutritional quality and ensuring microbiological safety. To date, there is little available literature on preservation strategies that meet the consumer's desire for 'natural' products. Chitosan (CS), an antimicrobial material that inhibits the growth and reproduction of a wide range of target organisms, such as bacteria, yeast and fungi, can be used in the preservative action of bread and improves its textural properties (Davidovich-Pinhas et al., 2014). Additionally, vanillin (VA), a flavor enhancer used in bread, has antibacterial and antioxidant activity, but at low concentrations, the antibacterial effect is not significant; at high concentrations, it affects the sensory odor of the product (Banerjee and Chattopadhyay, 2019). Therefore, chitosan-vanillin Schiff base emulsions (CSVAEs), obtained from our previous experiments, were applied to the study of bread staling and preservation (Zhu et al., 2023).

In this work, different concentrations of CSVAEs were selected as natural antibacterial materials to investigate their effects on dough and bread quality. The behavior of CSVAEs on the rheological properties, gluten microstructure and secondary structure of the dough was investigated to understand the mechanism of CSVAEs action on the dough. By measuring the basic properties of bread, the changes in the specific volume, hardness and staling rate of bread containing CSVAEs were investigated. Then, the changes in the physicochemical indicators and microbial population of bread containing CSVAEs under different storage times were investigated. The physical and chemical indicators of bread are moisture, acidity and textural properties. Bread is susceptible to microbial contamination during storage, so it is necessary to observe the changes in the number of colonies, molds and *E. coli*. The results show that CSVAEs we studied have the potential to act as natural bacterial inhibitor materials, both in terms of slowing the staling rate of bread, improving the quality of dough and bread, and delaying the increase in bacterial and mold microorganisms, thus extending the shelf life to a certain extent.

2. Materials and methods

2.1. Materials

Potato dextrose agar was provided by Shanghai Aladdin Reagent Co., Ltd. (Shanghai, China). Tryptone lauryl sulfate and Brilliant Green Lactose Bile Salt Broth were provided by Shanghai Maclean Chemical Reagent Co., Ltd. (Shanghai, China). Chloromycetin was provided by Chengdu Kelong Chemical Co., Ltd. (Chengdu, China). Co. Other reagents were provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Unless otherwise specified, all of the reagents were of analytical grade.

2.2. Preparation of bread containing CSVAEs

Making recipe of bread containing CSVAEs contains 200 g of high-gluten wheat flour, 100 g of CSVAEs, 10 g of sugar, 1.0 g of salt, 2.0 g of yeast, and 10 g of butter. Different concentrations of VA + CS (0 % + 0 %, 0.025 % + 0.125 %, 0.050 % + 0.250 %, 0.100 % + 0.500 %, 0.200 % + 1.000 %, relative to wheat flour weight, w/w) CSVAEs were formulated to study their effects on dough and bread when added as ingredients.

The process was as follows:

Ingredients → dough preparation → dough formation → butter added → dough mixing → first rise → primary rise, shaping → secondary rise → baking → finished product.

Operational points:

(1) Activation of dry yeast: Sugar was dissolved in CSVAEs, and then yeast was added, stirred and placed in a water bath at 30 °C for 30 min before use.
(2) To make the dough: The high gluten flour, salt and yeast-sugar mixture were poured into the dough mixing machine and mixed for 20 min. Then, softened butter was added, and the dough was kneaded for 10 min.
(3) Primary fermentation: If the hollow inside the dough was small and dense. The dough had not retracted around a hole poked in the middle of the dough, indicating that the primary fermentation was completed. The dough was covered with cling film and placed in an incubator with a fermentation function (25–28 °C) for 60 min to increase the volume of the dough to 2 to 2.5 times its size.
(4) Letting and shaping: The dough was deflated, divided, covered with cling film, allowed to rise for 15 min, and then shaped to the desired shape.
(5) Second rise: The shaped loaves were placed in an oven with a fermentation function (35–38 °C) and left to rise for approximately 40 min.
(6) Bread baking: Oven upper temperature 140 °C, lower temperature 150 °C, baking time 20 min.
(7) Packaging: The cooled bread was sealed and packaged in cling bags.

2.3. Determination of dough rheological properties

Sample preparation: The dough after the second rise was prepared in a mixer with different concentration concentrations. The rheological properties of the dough were tested using a Thermo HAAKE rotational rheometer (HAAKE MARS 60, Shanghai, China) with a frequency scan in oscillation mode. A certain amount of sample was taken from the center of the dough and laid flat evenly on the test bench of the rheometer, followed by a dynamic rheological test. Specific operating conditions: temperature 25 °C, plate diameter 20 mm, slit distance 1 mm, strain 0.1 %, scanning frequency 10~0.1 Hz. The modulus of elasticity (G') and the modulus of viscosity (G'') of the dough samples were then recorded (Liu et al., 2016).

2.4. Determination of dough microstructure

The treated samples were taken, and their cross-sectional structures were observed at 1.0 k magnification using a scanning electron microscope (S-8020, Hitachi, Japan). Then, the effect of different concentrations of CSVAEs on the microstructure of the dough gluten was analyzed (Upadhyay et al., 2012).

2.5. Determination of dough secondary structure

The dough was freeze-dried, and the samples were analyzed for secondary structure using FTIR spectroscopy (IRPrestige-21, Shimadzu,

Japan). The sample was weighed at 2 mg of powder and 200 mg of KBr and then pressed into thin slices using a tablet press. Scanning was performed on an FTIR spectrometer, and KBr was used as a control group. The test parameters were as follows: resolution 4.0 cm^{-1}, number of waves scanned 4000~400 cm^{-1}, and number of scans 32. Protein amide I band 1700-1600 cm^{-1} spectra were extracted using Omnic 8.2 software and PeakFit 4.12 software. The data were baseline corrected, Fourier deconvoluted and second-order derivative fitted until $R^2 \geq 0.99$. The protein secondary structure changes were then analyzed based on the peaks and areas of the designations (Liu et al., 2017).

2.6. Determination of bread specific volume

A sample of bread to be tested was weighed and placed in a container of a certain volume. Small particles of filler (millet or rapeseed) were added to the container, completely covering the bread sample, and the container was shaken. When the bread was removed and the filler was poured into a measuring cylinder to measure the volume, the volume of the bread was the volume of the container minus the volume of the filler (Ding et al., 2019).

The bread specific volume was calculated as follows:

$$P = V/m \quad (1)$$

where P is the specific volume of bread, V is the volume of bread, and m is the mass of the bread.

2.7. Determination of bread texture

After baking, the bread was left to cool at room temperature for 2 h. The bread was then cut into 2 × 2 cm pieces, and the core portion was taken for textural determination using a texture meter (TA-XT Plus, Stable Micro System, UK). Test parameters: Probe P/36R, pretest speed 2.00 mm/s, test speed 1.00 mm/s, posttest speed 2.00 mm/s; compression ratio 50 %, time interval 5 s, trigger force 5.0 g, 3 repetitions per sample (Encina-zelada et al., 2018).

2.8. Determination of the staling rate of bread

The samples were stored in a refrigerator at 4 °C for 5 days, and their hardness was measured using a texture meter. Rate of staling = (5 days hardness - 0 days hardness)/5 days (Zhang et al., 2018).

2.9. Determination of bread moisture content

The clean flat weighing bottles were placed in a drying oven at 101 °C–105 °C and heated to a constant weight. Samples (2 g–10 g) were weighed, put into this weighing bottle, capped, weighed precisely and placed in a drying oven at 101 °C–105 °C. The samples were dried for 2 h–4 h, capped and removed, put into a desiccator to cool for 0.5 h and weighed. Then, the samples were placed into a drying oven at 101 °C–105 °C for approximately 1 h, removed, placed into a desiccator to cool for 0.5 h and weighed again. This process was repeated until the difference between the two masses did not exceed 2 mg, which is the constant weight.

The moisture content of the bread was calculated as follows:

$$X = \frac{m_1 - m_2}{m_1 - m_3} \times 100 \quad (2)$$

where X is the moisture content of the samples, m_1 is the weighing of the bottles and samples, m_2 is the weighing of the bottles and samples after drying, and m_3 is the weighing bottle mass.

2.10. Determination of bread acidity

The center portion of the bread was weighed to 25 g, added to 60 mL of CO_2-free distilled water, pounded with a glass rod, transferred to a 250 mL volumetric flask, set to scale and shaken well. The volumetric flask was allowed to stand for 10 min, shaken for 2 min and then allowed to stand for an additional 10 min. The filtrate (25 mL) was transferred into a triangular flask (200 mL), and 2 to 8 drops of phenolphthalein indicator solution were added and titrated with NaOH standard solution (0.1 mol/L) until slightly red for 30 s without fading. The volume of NaOH standard solution consumed was recorded. A blank test was also performed with distilled water (Sandvik et al., 2016).

The bread acidity T was calculated as follows:

$$T = \frac{c \times (V_1 - V_2)}{m} \times 1000 \quad (3)$$

where c is the concentration of the standard solution of NaOH, V_1 is the volume of NaOH standard solution consumed in the titration of the test solution, V_2 is the volume of NaOH standard solution consumed in the blank test, and m is the mass of the sample.

2.11. Determination of the total number of bacterial colonies in bread

The total number of bacteriological colonies per g of sample was obtained after incubation in plate counting agar medium (Arienzo et al., 2020).

2.12. Determination of total molds in bread

The total number of molds per g of sample was obtained after incubation in potato dextrose agar medium (Chaste et al., 2019).

2.13. Determination of coliform counts in bread

The total number of coliforms per g of sample obtained after incubation in Lauryl Sulfate Tryptone (LST) broth with Brilliant Green Lactose Bile Salt (BGLB) broth (Khanom et al., 2016).

2.14. Statistical analysis

The significance of the acquired findings was determined through one-way analysis of variance (ANOVA) with 95 % confidence intervals. SPSS software version 24.0 was employed for statistical analysis. Figures were made by Origin 8.6.

3. Results and discussion

3.1. Effect of CSVAEs on the rheological properties of dough

A dynamic rheometer was used to determine the viscoelastic quality of the dough. The frequency scan was performed at 25 °C to obtain the variation in the modulus of elasticity (G′) and the modulus of viscosity (G″) with dough. G′ was the amount of energy stored in the dough as a result of elastic deformation when it was deformed, reflecting the amount of material elasticity. G″ was the amount of energy lost due to the viscous deformation of the dough when it was deformed, reflecting the fact that the material was too viscous. The loss angle tangent tanδ = G″/G′, when tanδ <1, the material was similar to a solid; conversely, when tanδ >1, the material was similar to a fluid. However, when G′ was comparable to G″, the material was semisolid, and the gel was a typical semisolid substance (Álison et al., 2020).

As shown in Fig. 1A and B, the trends in G′ and G″ of the dough after the addition of CSVAEs were the same, both increasing with frequency. As the concentration of CSVAEs increased, both G′ and G″ of the dough gradually increased, indicating that the addition of CSVAEs enhanced the viscoelasticity and mechanical strength of the dough. The tanδ values were all less than 1, indicating that the dough was more elastic than viscous and had solid-like properties. It was shown that CS has a

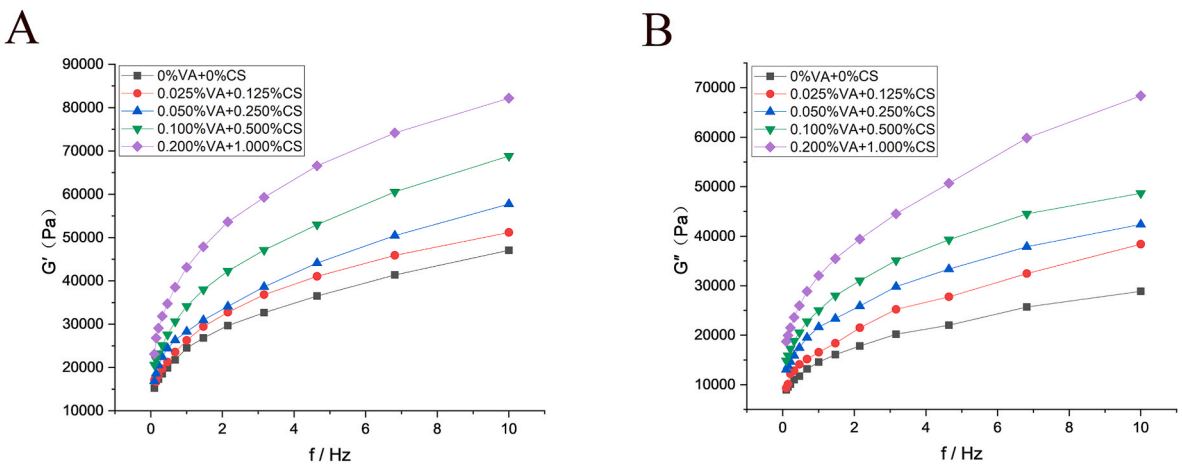

Fig. 1. Effect of CSVAEs on the rheological properties (the elastic modulus (G') (A) and the viscos modulus (G") (B)) of dough.

swelling effect, filling the gluten network structure during dough formation and enhancing the water holding capacity of the dough. The increase in the water-holding capacity of the dough contributed to its viscoelasticity, and therefore, the addition of CSVAEs was able to increase the viscoelasticity of the dough (Sansano et al., 2018).

3.2. Effect of CSVAEs on the microstructure of dough

Scanning electron microscopy enabled a more visual observation of the microstructural changes in the dough. Fig. 2A shows that the blank group gluten network was relatively loose, with spherical and irregularly shaped starch granules of varying sizes floating outside the gluten network. As the concentration of CSVAEs increased, the number of independently present starch granules gradually decreased. The internal structure of the dough gradually became tighter, the number of free spaces decreased and the viscoelasticity increased, which were consistent with the above rheological test results. The gluten network of the dough was best formed when the concentration of CSVAEs was 0.050 % VA + 0.250 % CS, as shown in Fig. 2Ac. The starch granules were almost completely encapsulated, and a thin film-like closed structure was formed in the dough. When the concentration of CSVAEs continued to increase, the spatial network structure within the gluten of wheat flour began to collapse in large patches. The starch granules were irregularly crowded together, and the surface became rough. The reason for this may have been that the high concentration of CSVAEs had certain emulsifying properties that held the starch particles in the gluten network together and made the gluten proteins less ordered (Gómez et al., 2013).

3.3. Effect of CSVAEs on the secondary structure of dough

Protein IR interferograms mainly reflected the protein amide I band (mainly C═O stretching vibrations, 1700-1600 cm^{-1}), which contains information on α-helix, β-fold, β-turn and random curl structures; therefore, it is commonly used to resolve the secondary structure of proteins. The amide I band corresponded to the glutenin secondary structure as follows: α-helix: 1646~1664 cm^{-1}; β-fold: 1615~1637 cm^{-1} & 1682~1700 cm^{-1}; β-turn: 1664~1681 cm^{-1}; random curl: 1637~l645 cm^{-1}. The α-helix and β-fold were more ordered protein secondary structures with high stability, while the β-turn and random curl were disordered structures with less stability (Ouyang et al., 2023). As shown in Fig. 2B, there was no significant change in the location of the IR characteristic peaks for the different CSVAE concentrations of dough, but there were some differences in peak areas. As shown in Fig. 2C, the protein secondary structure in the dough was dominated by the content of β-turns and random curls. The blank group contained 15.11 %, 30.92 %, 36.57 % and 17.40 % α-helices, β-folds, β-turns and irregular curls, respectively. The α-helix content gradually increased with increasing concentrations of CSVAEs, and there was no significant change in the β-fold content. When the concentration of CSVAEs was 0.050 % VA + 0.250 % CS, the α-helix content was 19.81 %, which was the highest in the group. CSVAEs improve the orderliness of the protein structure and help promote the formation of gluten protein network structures. However, when the concentration of CSVAEs was 0.200 % VA + 1.000 % CS, the α-helix content and β-fold content were reduced by 8.34 % and 3.40 %, respectively. It is suggested that high concentrations of CSVAEs reduce the orderliness of protein secondary structure and hinder the formation of gluten protein network structure. Low concentrations of CSVAEs enhanced the electros-b tatic interactions between amino acids and contributed to the stabilization of the protein secondary structure.

3.4. Effect of CSVAEs on the specific volume of bread

The bread specific volume results are shown in Fig. 3A, where the bread specific volume increased as the concentration of CSVAEs increased from 0 % to 0.050 % VA + 0.250 % CS. When the concentration reached 0.200 % VA + 1.000 % CS, the specific volume of bread dropped considerably. CSVAEs changed the volume of the bread; the higher the specific volume, the fluffier the tissue and the higher the number of internal air pockets. The reason for this phenomenon could be the increased content of highly hydrophilic CSVAEs, which caused that the action of noncovalent bonds such as hydrogen bonds among the molecular backbones created a form of continuous gluten network structure with a certain degree of viscoelasticity. The reasons for the decrease in the specific volume of bread as the cs concentration continued to increase require further exploration.

3.5. Effect of CSVAEs on the hardness of bread cores

The determination of bread core hardness is one of the most common methods used to evaluate the quality of bread. The results of the bread hardness measurements are shown in Fig. 3B. As the concentration of CSVAEs increased, the hardness of the bread showed a small decrease followed by an increase, in contrast to the specific volume curve of the bread. The lower the specific volume of the bread, the harder it was. The change in hardness and specific volume of bread was small for the low concentration of CSVAEs, while the change in hardness and specific volume of bread was larger for the high concentration of CSVAEs. The reason for this could be that as the concentration of CSVAEs increases, the aggregation of starch particles in the gluten network increases, resulting in a progressively stiffer bread with a harder texture.

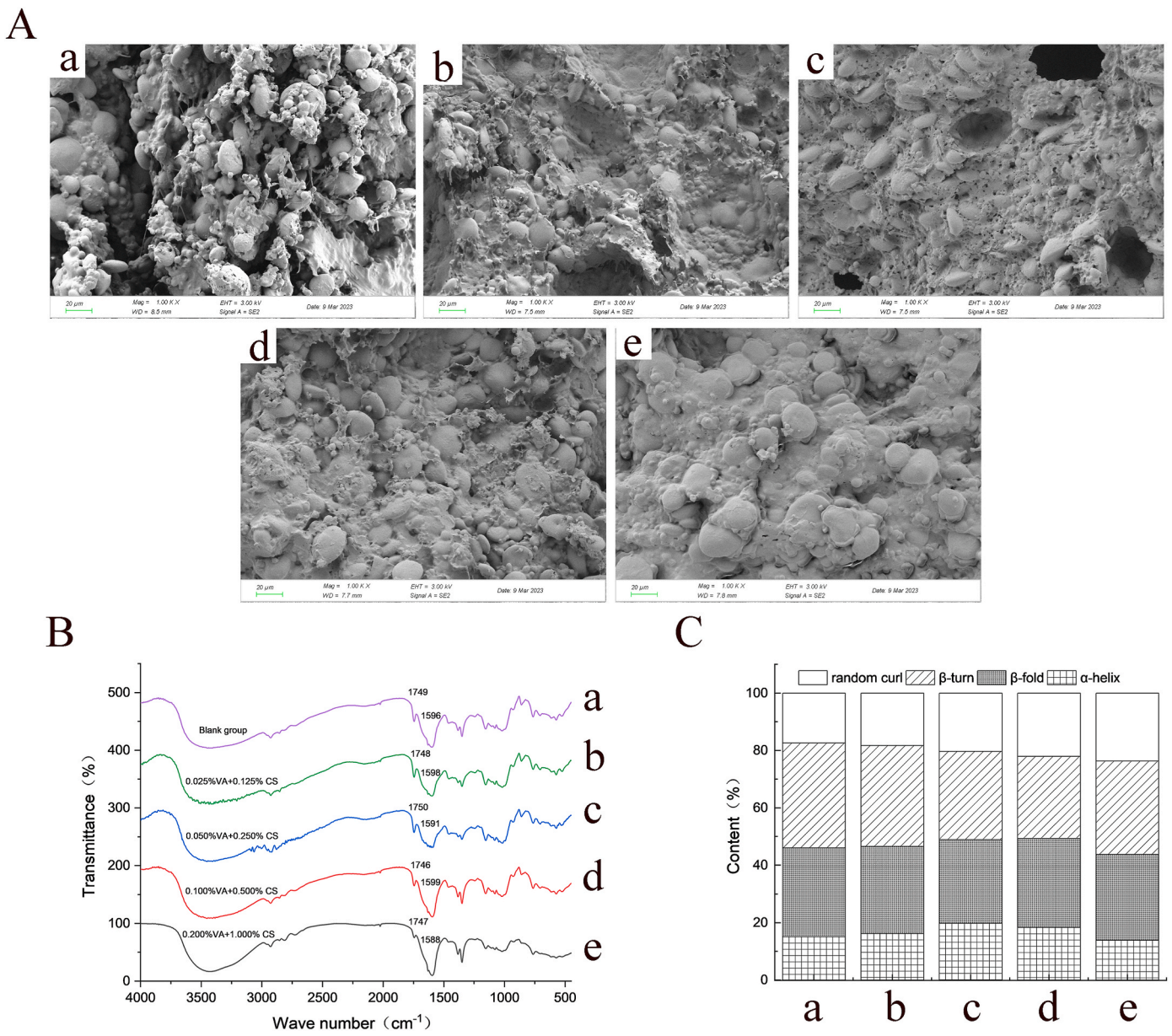

Fig. 2. Microstructure (A) and FTIR spectra (B) of dough at different CSVAE concentrations (a: 0 %VA+0 %CS; b: 0.025 %VA+0.125 % CS; c: 0.050 %VA+0.250 % CS; d: 0.100 %VA+0.500 % CS; e: 0.200 %VA+1.000 % CS).

3.6. Effect of CSVAEs on the staling rate of bread

The staling back of starch was the main cause of bread hardening, so the higher the rate of bread staling, the faster the rate of bread hardening (Korus et al., 2020). As shown in Fig. 3C, the staling rate of bread increased as the concentration of CSVAEs increased; the rate of bread staling was slower at lower concentrations of CSVAEs than at higher concentrations. From this study, the low concentration of CSVAEs did not accelerate the staling phenomenon, while the addition of a high concentration of CSVAEs may have accelerated the recrystallization of branched starch and the water migration process, which agglomerated the starch particles in the gluten network, thus increasing the staling rate of bread.

3.7. Changes in the moisture content of bread at different storage time

The variation in the moisture content of bread with storage time is shown in Fig. 4A. As seen from the graph, the moisture content of the bread gradually decreased as the storage time increased. The rate of moisture content reduction was greater in the first period and slowed from Day 10 onward. In the blank group, the moisture content decreased from 39.0 % to 18.2 %, a decrease of 53.33 %, while in the CSVAE group, it decreased from 40.8 % to 18.5 %, a decrease of 54.65 %. There was no significant ($p < 0.05$) difference between the blank group and the CSVAE group. These results indicate that the addition of CSVAEs did not change the rate of moisture content reduction in the bread. Although the moisture content of the blank bread containing CSVAEs was substantially reduced, according to Chinese national standard GB/T 20981-2021 ″General Rules for Bread Quality”, the moisture content of dried bread products is ≤ 6 % to effectively inhibit microbial contamination, indicating that the reduction in moisture activity in this experiment was not a major factor in inhibiting microbial growth in bread.

3.8. Changes in the acidity of bread at different storage times

The change in acidity of the bread with storage time is shown in

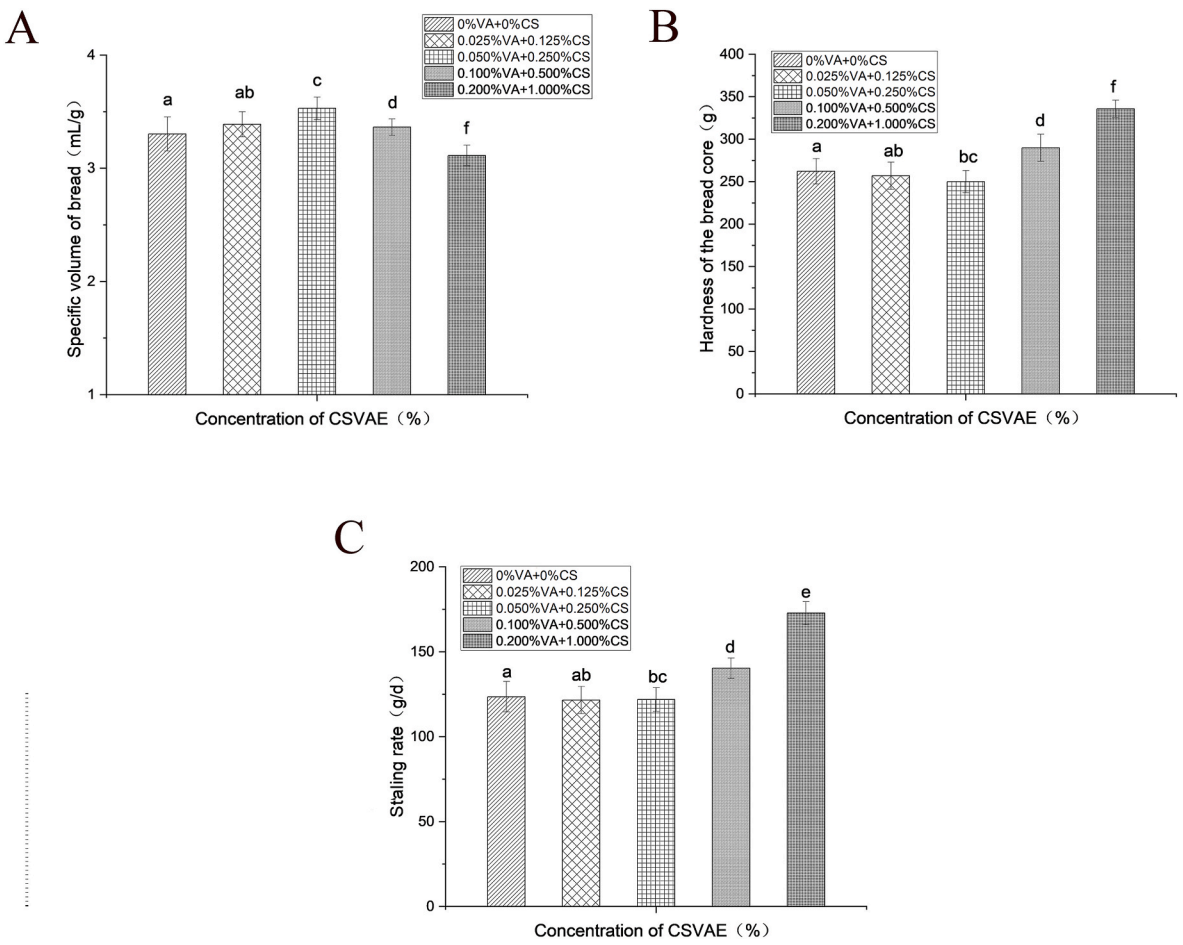

Fig. 3. Effect of CSVAE on the specific volume (A), the hardness (B), and the aging rate (C) of breads.

Fig. 4B. As seen from the figure, the acidity of the blank group and the bread containing CSVAEs gradually increased as the storage time increased, with the rate of increase in the blank group being higher than that of the bread containing CSVAEs. The rate of increase of the blank group was higher than that of the bread containing CSVAEs. The initial acidity of the blank group increased from 1.23°T to 8.50°T, with an increase of 85.5 %, while that of the bread containing CSVAEs increased from 1.20°T to 3.06°T, with an increase of 60.7 %. According to GB/T 20981-2021 ″General rules for bread quality”, the acidity of bread must be $\leq 6°$ T. The acidity of ordinary bread exceeded the standard on Days 20∼30; the acidity of the bread containing CSVAEs did not exceed the standard for the duration of the experimental range. Three reasons have been suggested in the study thus far for the rise in bread acidity: 1) contamination by stray bacteria, where microorganisms grow and multiply and carry out metabolic acid production, leading to an increase in acidity; 2) rancidity of fats and oils, where fats and oils are used in bread and rancidity of fats and oils occurs, which may also result in a sour taste in bread; and 3) yeast dosage and fermentation time, where failure to control the amount of yeast and fermentation time within reasonable limits results in overfermentation and bread acidity. If the amount of yeast used and the fermentation time are not controlled within a reasonable range, overfermentation will result, and the sourness of the bread will increase. Under the same fermentation parameters, the increase in acidity of the bread containing CSVAEs was smaller than that of the blank group, indicating that bread containing CSVAEs has a certain effect on inhibiting the growth of weedy bacteria and oil rancidity, which can effectively delay the spoilage of bread.

3.9. Changes in the hardness and springiness of bread at different storage times

The changes in hardness of the bread with storage time are shown in Fig. 4C, which shows that the hardness of both the blank group and the bread containing CSVAEs gradually increased, as the storage time increased. Fig. 4D shows the changes in springiness of the bread with storage time, but both the blank group and the bread containing CSVAEs gradually decreased with increasing storage time. The changes in the textural properties of the bread containing CSVAEs or not were similar, indicating that the addition of CSVAEs had a small effect on the textural properties of the bread.

3.10. Changes in the microbial population of bread at different storage times

The bread was placed at 37 °C and 50 % RH, and the bacterial colony count was measured during storage. According to the requirements for microbiological indicators of bread in Chinese national standard GB 7099-2015 ″Sanitary Standard for Confectionery and Bread”, the total bacterial count of bread must be less than or equal to 1.5×10^3 CFU/g, and the total mold count should not exceed 1.0×10^2 CFU/g. As shown in Table 1, the total bacterial count of ordinary bread exceeded the standard on Day 8 at 1.9×10^3 CFU/g, and the mold count exceeded the standard on Day 6 at 1.2×10^2 CFU/g. The total bacterial count of the bread containing CSVAEs exceeded the standard on Day 30 at 1.5×10^3 CFU/g, and the total mold count exceeded the standard on Day 15 at 1.0×10^2 CFU/g. It can be concluded that the microbial count of the bread containing CSVAEs was much lower than that of ordinary bread during

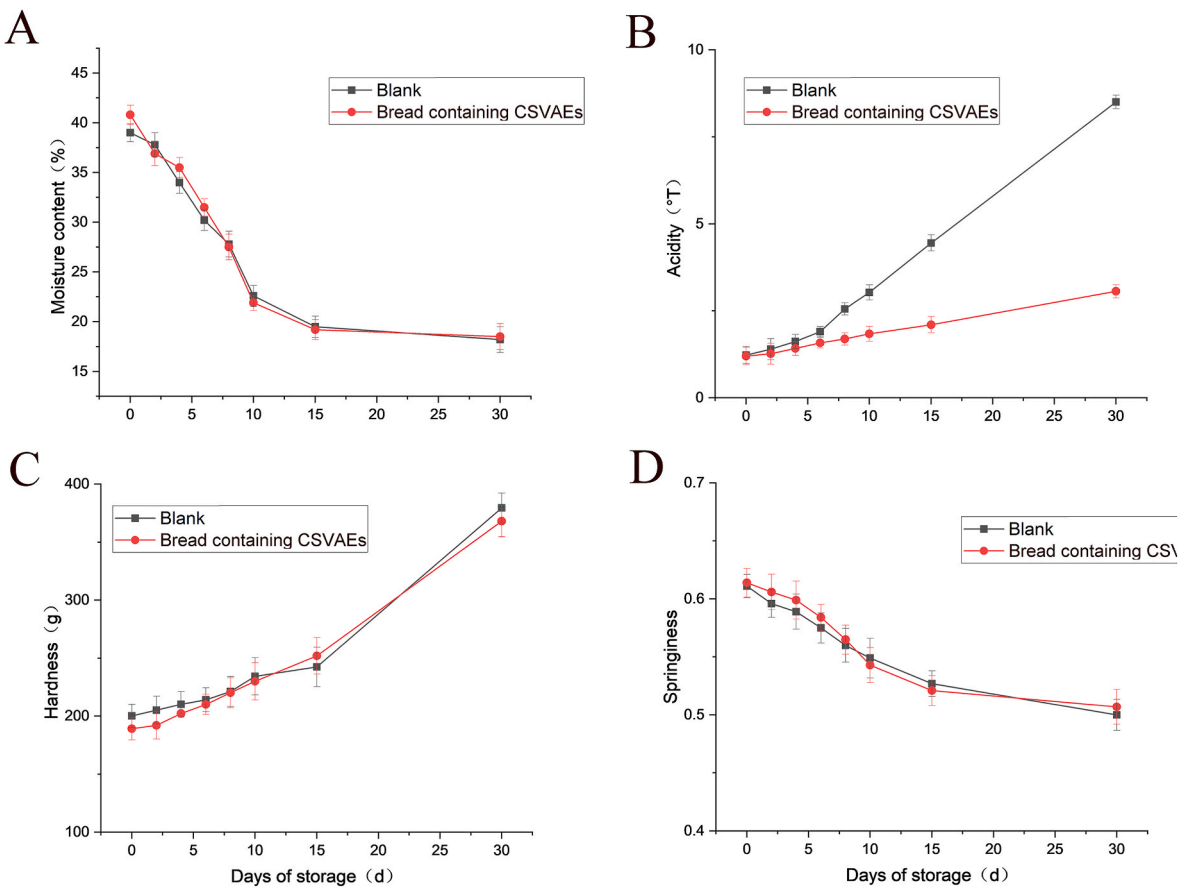

Fig. 4. Changes in bread moisture (A), acidity (B), hardness (C), and springiness (D) during storage.

Table 1
Microbial changes in bread during storage.

Storage days/d	Total colony count/(CFU/g)		Total mold/(CFU/g)		Total coliform/(MPN/100 g)	
	Blank	CSVAE	Blank	CSVAE	Blank	CSVAE
0	<10	<10	<10	<10	<10	<10
2	90 ± 2.77^{a}	<10	<10	<10	<10	<10
4	$2.8 \times 10^{2} \pm 20.77^{b}$	16 ± 1.32^{a}	25 ± 1.32^{a}	<10	<10	<10
6	$8.5 \times 10^{2} \pm 12.61^{c}$	80 ± 5.52^{b}	$1.2 \times 10^{2} \pm 10.28^{b}$	21 ± 3.32^{a}	<10	<10
8	$1.9 \times 10^{3} \pm 17.60^{d}$	$1.4 \times 10^{2} \pm 10.52^{c}$	$3.8 \times 10^{2} \pm 10.62^{c}$	45 ± 8.52^{b}	<10	<10
10	$3.2 \times 10^{4} \pm 50.68^{e}$	$3.3 \times 10^{2} \pm 20.26^{d}$	$7.2 \times 10^{2} \pm 50.32^{d}$	90 ± 10.52^{b}	<10	<10
15	$2.5 \times 10^{5} \pm 45.59^{f}$	$5.4 \times 10^{2} \pm 20.37^{e}$	$1.1 \times 10^{3} \pm 70.67^{e}$	$1.2 \times 10^{2} \pm 10.00^{d}$	<10	<10
30	–	$1.8 \times 10^{3} \pm 10.56^{f}$	$8.7 \times 10^{3} \pm 90.32^{f}$	$4.3 \times 10^{2} \pm 20.30^{e}$	<10	<10

Note: Different letters in the same column in the table indicate significance at the $p < 0.05$ level, and the same letter indicates nonsignificance.

the same period; this indicates that the addition of CSVAEs can delay the increase in bacteria and molds, thus extending the shelf life to a certain extent. In addition, *E. coli* flora was not detected in any of the sample breads. As seen in Fig. 5, the bread containing CSVAEs had a yellowish attractive shine, which was due to the different degrees of the Maillard reaction and caramelization between CS and VA and gluten protein at high temperature. Additionally, no visible spoilage was observed by the naked eye at Day 30 for the bread supplemented with CSVAEs, while the blank group had deteriorated severely, which indicated that CSVAEs inhibited the growth of microorganisms during the storage process.

In conclusion, CSVAEs were used in the preparation of bread to improve the quality of dough and bread, to inhibit the growth and reproduction of microorganisms and to extend the shelf life of bread. CSVAEs were added to bread making in the form of raw materials and can effectively improve the quality of dough and bread. As the concentration of CSVAEs increased, both G′ and G″ of the dough gradually increased, and viscoelasticity and mechanical strength gradually increased. Electron microscopy visualisation showed that the CSVAEs were embedded in the gluten. The gluten network of the bread dough was well formed at low concentrations of CSVAE, but there was an obvious collapse of the gluten structure at high concentrations of CSVAEs. The results of gluten secondary structure showed that the α-helix and β-fold content of dough increased and then decreased with the increase of CSVAEs concentration. High concentrations of CSVAEs decreased the order of protein secondary structure and affected the formation of gluten protein network structure. The basic properties of the bread were determined, and it was found that low concentrations of CSVAEs were effective in improving the quality of the bread and slowing the staling rate. Therefore, low concentrations of CSVAEs (0.050 % VA + 0.250 % CS) were chosen to prepare bread and to study the preservative effect of bread containing CSVAEs. As the storage time increased, CSVAEs had less effect on the rate of moisture loss, hardness and

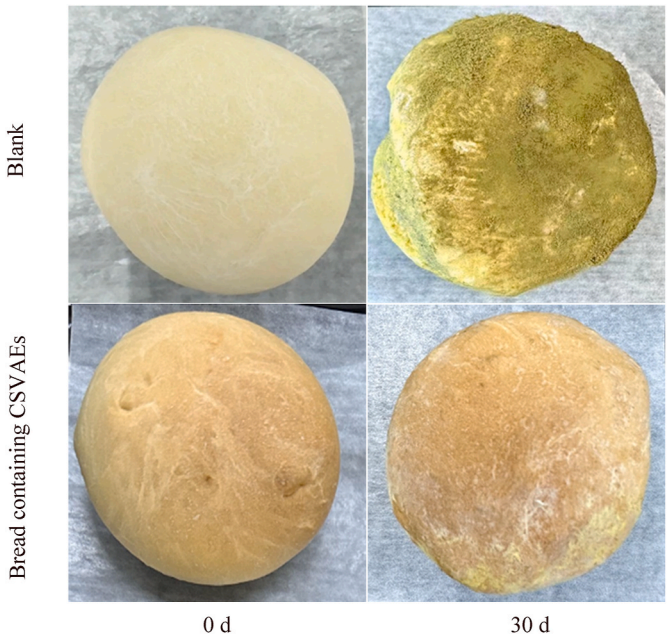

Fig. 5. Appearance of breads with different storage time.

elasticity of the bread and a greater inhibitory effect on the acidity of the bread. The acidity of bread gradually increased, and the rate of increase in the blank group was much higher than that in the CSVAEs group. The increase in acidity may be due to microbial metabolism, and the inhibition of acidity increase by CSVAEs was consistent with the inhibition of microbial growth in the study. The change in colony count of bread was measured at 37 °C and 50 % RH storage conditions at different storage times. The total bacterial count of ordinary bread exceeded the standard on Day 8, and the mold count exceeded the standard on Day 6, while the total bacterial count of the bread containing CSVAEs exceeded the standard on Day 30 and the total mold count on Day 15. Therefore, the addition of CSVAEs can delay the increase in bacteria and molds, thus extending the shelf life of bread to a certain extent.

CRediT authorship contribution statement

Jianfei Zhu: Conceptualization, Methodology, Writing – review & editing, Resources, Supervision, Funding acquisition. **Tingting Huang:** Investigation, Writing – original draft. **Xiaomei Chen:** Formal analysis, Validation. **Dongling Tian:** Software.

Declaration of competing interest

No potential conflict of interest was reported by the authors.

Data availability

Data will be made available on request.

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