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Fortification of Wheat Bread with Edible Chrysanthemum (Chrysanthemum morifolium Ramat.): Unraveling the Mechanisms of Dough Rheology and Bread Quality Changes

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Abstract

The inclusion of plant material in bread formulations is a global trend, but research on the mechanisms of its effect on the structural and baking performance of the dough is lacking. Herein, edible chrysanthemum (*Chrysanthemum morifolium* Ramat.) powder (CP) was substituted for wheat flour at 3–15 wt% and its effects on dough structure and rheology, bread physicochemical properties, and aroma profile were investigated. The inclusion of CP reduced the relative crystallinity and structure orderliness of starch in terms of X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy analysis. Steady-state shear and oscillation tests showed that the inclusion of CP resulted in an enhanced viscoelastic behavior of dough, implying an increase in the resistance of the dough system to flow and deformation, which caused a decrease in specific volume and softness of bread compared to the control (bread without CP). The large oscillatory amplitude oscillation shear tests (LAOS) indicated that doughs exhibited behavior (strain thinning). The scanning electron microscopy (SEM) observation further confirmed these findings. Significantly, formulations with 3 and 6 wt% CP not only improved the rheological and structural properties of doughs but also enriched the aroma profile of bread compared to the control. Furthermore, sensory evaluators reported that bread enriched with 6% CP scored highest in appearance, texture, and overall acceptability, meaning a product with a high sensory appeal to consumers. This work provides valuable information for an in-depth study of the relationship between bread quality and the rheological behavior and structural properties of the dough.

1 Introduction

Globally, there is a continuing increase in the prevalence of chronic diseases among the population. One means of addressing this issue is the incorporation of plant materials rich in phenolics and dietary fiber into highly consumed traditional staples such as bread (Klopsch et al., 2019). Epidemiology studies have shown that a plant-rich diet can promote the physiological activity of the body, thus effectively preventing chronic diseases such as diabetes, obesity, and cardiovascular disease (Ma et al., 2022). Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is recognized as a medicinal food homology plant that is rich in dietary fiber, polyphenols, amino acids, micronutrients, and other functional ingredients, all of which have shown positive efficacy in the prevention and treatment of chronic diseases (Sun et al., 2021). Previous studies have reported the incorporation of processed plant powders into bread as an added functional ingredient, such as coriander leaf (Das, Raychaudhuri, & Chakraborty, 2012), green tea (Seidel et al., 2007), grape peels (Mironeasa et al. 2018), and maqui berry powder (Bianchi et al. 2022). Based on this, the incorporation of processed chrysanthemum powder (CP) in bread formulations may be an innovative and promising approach to increasing the intake of health-promoting ingredients in traditional staple foods such as bread. Therefore, there is a need to elucidate the mechanisms by which chrysanthemum powder as a food additive ingredient affects dough structure and bread quality, which would provide a theoretical guide for the commercial application of chrysanthemum powder in bread processing.

The structure of dough was reported to deform by up to 50,000% strain during the mixing and fermentation process, which affected its rheological behavior and thus changes the texture of bread (Yildirim-Mavis, Yilmaz, Dertli, Arici, & Ozmen, 2019). Therefore, the dough prepared with wheat-chrysanthemum composite flour should be assessed for its rheological properties in the non-linear viscoelastic region. However, traditional small amplitude oscillatory shear (SAOS) tests are inappropriate to characterize the non-linear mechanical responses. Currently, large amplitude oscillatory shear (LAOS) has gained considerable attention because it provides information about microstructural changes in complex food systems. Compared to SAOS, LAOS could better simulate the actual processing conditions and correlates with the sensory attributes collected during the chewing phase (Joyner, 2018). However, the rheological behavior of wheat-chrysanthemum composite flour-based dough has not been studied so far. Liu,

Chen, Zheng, Lu, and Chen (2020) investigated the rheology of doughs containing matcha powder, but they focused only on the rheological behavior in the linear viscoelastic region (LVR) that could not provide deeper insights into the link between structural properties and rheological behavior of the dough when it was subjected to large deformations. Other than rheological behavior, the crystallinity and microstructure of the dough may also be affected by CP. However, there has been no systematic investigation on how CP affects the structure of the dough at the molecular level.

For the assessment of the final quality of the bread, the specific volume and textural properties are known to be important indicators determining its baking performance. Of particular interest is the fact that the aroma of bread is actually an important feature that could be directly perceived by consumers, but it is often overlooked in the industry chain. Thus, a detailed understanding of the effect of CP incorporation on the aroma profile of bread is the key to improving consumer sensory acceptability. In addition to the physicochemical characteristics of bread, consumers' preferences and choices are extremely dependent on their own sensory and psychological conditions (Pu et al., 2019). With this in mind, it is necessary to clarify the relationship between the sensory evaluation of chrysanthemum-fortified bread and its quality information obtained through multi-instrumental analysis.

In this study, we hypothesized that freeze-dried chrysanthemum powder would be suitable as an additive ingredient to improve the texture and organoleptic properties of bread without compromising the rheological and structural properties of the dough. Therefore, we assessed the effect of different CP substitution levels (3–15 wt%) on linear/nonlinear rheological behavior and associated with the changes in the microstructure and crystallization state of dough, which were determined using rheometry, X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM). Furthermore, the texture, antioxidant capacity, and aroma characteristics of the bread were quantified by employing texture profile analysis (TPA), C-cell image analysis, and gas chromatography-mass spectrometer (GC-MS) methods. Finally, with the help of Mantel’s test, the effect of dough and bread characteristics on sensory evaluation was proved. These findings are important guidelines for the application of chrysanthemum in the innovation and development of functional plant-rich foods.

2 Material And Methods

2.1 Raw material

Fresh chrysanthemum (*Chrysanthemum morifolium* Ramat.) was harvested in the experimental field of China Agricultural University (Beijing, China). Samples with homogeneous physical properties were freeze-dried for 48 h using a freeze-dryer (LGJ-18C, Beijing Sihuan Scientific Instruments Factory Co., Ltd.) at -60 ^{o}C. The samples obtained were ground and sieved through a 100-mesh sieve. High-gluten wheat flour, dry yeast, butter, egg, salt, and sugar were purchased from the located supermarket (Beijing, China).

2.2 Dough preparation and bread baking

The formulation of bread-making was as follows: 200 g wheat flour, 20 g butter, 30 g sugar, 2 g salt, and 3 g dry yeast. Wheat flour was replaced by CP at 0, 3, 6, 9, 12, and 15g/100g levels, respectively, and doughs and bread prepared from these formulations were named as control, CP3, CP6, CP9, CP12, and CP15, respectively. The preparation process of dough and bread referred to previous work published by Özcan (2022) with slight modifications. Briefly, pre-mix the wheat flour, butter, sugar, salt, and yeast for 5 min, then add 90 mL of ultra-pure water and mix twice in a professional spiral mixer (MM-ESC1510, Midea, Foshan, China). The times of the first and second stirring lasted 10 min and 30 min, respectively, with an interval of 10 min. Subsequently, the dough was

placed in a temperature-humility-controlled chamber (37°C, 80% relative humidity) and fermented for 60 min. After finishing fermentation, the dough was baked at an upper temperature of 180°C for 30 min. The bread preparation process for each formula was repeated three times.

2.3 Structural properties of dough

2.3.1 X-ray diffraction (XRD)

The crystal structure of doughs was determined using X-ray diffraction (XD-2, PG Instruments Ltd., Beijing, China). The diffraction intensities were scanned from 10° to 40° (2θ) with a speed of 2°/min and a step size of 0.02°. The relative crystallinity (%) was determined according to the method of Liu et al. (2021).

2.3.2 Fourier-transform infrared (FTIR) spectroscopy

To determine the changes in the functional group of doughs, FTIR spectra were collected using an FTIR spectrometer (PerkinElmer, Waltham, MA, USA). Briefly, the dough was mixed thoroughly with potassium bromide and pressed into flakes. The FTIR measurement was performed from 4000 to 400 cm^{-1} in 32 scans, with a resolution of 4 cm^{-1}.

2.3.3 Scanning electron microscopy (SEM)

The cross-section morphology of the doughs was observed using a scanning electron microscope (SU3500, Hitachi, Tokyo, Japan). Before observation, the dough was mounted on a metal specimen stub with double-sided conductive tapes and coated with gold for 60 s. The prepared dough was then allowed to be observed under an acceleration voltage of 10 kV and photographed at magnifications of ×500 and ×1000.

2.4 Rheological behavior of dough

The dough was prepared without the addition of yeast to prevent the fermentation from interfering with the experimental process. All tests were performed at 25 ^{o}C using an ARSE G2 rheometer (TA Instruments, New Castle, DE, USA) equipped with a 50-mm parallel plate at a 1.5 mm gap. Before each test, the sample was placed on a Peltier plater to relax stress for more than 1 min, and a layer of silicone oil was then applied to the edge of the sample to prevent moisture from evaporating during the experiment. Each test was repeated in triplicate.

2.4.1 Steady-state shear tests

The variation of apparent viscosity (η) with shear rate (γ) was determined under a shear rate from 1 to 100 s^{-1}. To further evaluate the differences in flow characteristics, the Power Law model (Eq. (1)) and the Cross model (Eq. (2)) were employed to describe the flow behavior of the samples, respectively.

$$\eta = K \cdot \gamma^{n-1}$$

1

$$\frac{\eta - \eta_{\infty}}{\eta_o - \eta_{\infty}} = \frac{1}{1 + (\lambda\gamma)^m}$$

2

where K is viscosity index (Pažs^n); n is flow index; η_0 and η_∞ stand for viscosity value at $\gamma \to 0$ and $\gamma \to \infty$, respectively; λ is the time constant (s); m is the dimensionless exponent, indicating the dependence of viscosity on shear rate.

2.4.2 Small amplitude oscillation shear (SAOS) tests

The SAOS measurement was performed with the angular frequency range from 0.1 to 100 rad/s at 0.01% of constant deformation amplitude (within the LVR). The experiment data (storage/elastic modulus (G'), loss/viscous modulus (G''), and loss factor (tan δ)) were recorded. The phase angle (δ) described the phase lag between the viscous and elastic components of the complex modulus, calculated by Eq. (3).

$$\tan \delta = \frac{G''}{G'}$$

3

2.4.3 Large amplitude oscillation shear (LAOS) tests

The non-linear rheological properties of samples were determined by strain sweep at 1 Hz over a strain amplitude range of 0.01–100%. The experimental data were obtained from raw waveform data collected by the TRIOS V5.5.0 and then used for the further analysis of the Lissajous curve using MITlaos software.

2.5 Baking performance of bread

2.5.1 Texture profile analysis (TPA)

TPA was conducted by using a texture analyzer (TA-XT plus, Stable Micro System, UK) equipped with a P/25 cylindrical probe. A bread slice was cut in a cubic shape with the dimensions of 20 mm × 20 mm × 20 mm (approximately) and used to perform a double-compression test. Test parameters were set as follows: pre-test speed 1.00 mm/s, test speed 1.00 mm/s, post-test speed 1.00 mm/s, strain deformation 50%, and the interval between two compressions was 30 s. For all assays, 10 bread chunks were randomly chosen from each formula.

2.5.2 Specific volume

Bread cooled to room temperature was weighted and its volume was measured by applying the millet displacement method. Specific volume (mL/g) of bread was obtained by calculating the ratio of its volume and weight.

2.5.3 Color properties

Digital images with 1024 × 1024 – pixel resolution of bread slices was acquired by using an image scanner (MRS-2400A48U, Zhongjing Technology Co., Ltd., China), and stored in TIFF format. The CIE Lab color parameters (L^*, a^*, and b^*) of all captured images were obtained using the image processing toolbox of MATLAB software (MATLAB 2015a, The Mathworks Inc., Natick, MA, USA), where L^*, a^*, and b^* indicate lightness, redness/greenness, and yellowness/blueness, respectively. Chroma (C) and color difference (ΔE) were calculated as Eqs. (4) and (5), respectively.

$$C = \sqrt{a^* + b^*}$$

4

$$\triangle E = \sqrt{(L^* - {L_0}^*)^2 + (a^* - {a_0}^*)^2 + (b^* - {b_0}^*)^2}$$

5

where C indicates the saturation of the color. ΔE indicates the color difference between samples with chrysanthemum powder and samples without chrysanthemum powder (the control). ${L_0}^*$, ${a_0}^*$, and ${b_0}^*$ are the color parameters of the control, while L^*, a^*, and b^* are the color parameters of composite bread.

2.5.4 C-cell image analysis

A C-cell image system (Calibre Control International Ltd., Warrington, UK) was used to evaluate the quality of bread. The instrument used dedicated image analysis software to analyze the structural and surface properties of sliced bread, including the size and shape of the sample, cell structure, etc.

2.6 Antioxidant activity of bread

2.6.1 Extraction procedure

Briefly, samples (1 g) were extracted with 10 mL of 80% methanol in an ultrasonic water bath (40 kHz and 200 W) at 37 ^{o}C for 60 min. Then the mixture was centrifuged at 10000 rpm for 15 min. The supernatant obtained was collected and the above procedure was repeated to extract the residue. Finally, the supernatant obtained twice was mixed and stored in the refrigerator (4 ^{o}C) protected from light for further analysis. The total phenolic content and antioxidant capacity of the extracts were measured according to the method described by Zhu, Sakulnak, and Wang (2016) with minor modifications.

2.6.2 Total phenolic content (TPC)

For TPC assay, 300 µL of the methanolic extract, 1 mL of Folin-Ciocalteau reagent, and 2 mL of 15% Na_2CO_3 were mixed in the 10 mL centrifuge tube. After diluting the reaction solution to 10 mL, transfer it to a constant temperature bath and incubated in the dark for 30 min. The absorbance of the extract was determined at 725 nm and gallic acid was used as a standard. All results were expressed in mg gallic acid equivalent per gram dry-weight basis (mg GAE/g DW).

2.6.3 DPPH radical scavenging capacity

For the determination of the scavenging ability of the extract against $DPPH^{\cdot}$, 2.5 mL of freshly prepared $DPPH^{\cdot}$ ethanol solution (0.04 mg/mL) was mixed with 1.5 mL of a 12.5-fold dilution of the extract in a 10 mL centrifuge tube. After the obtained mixture was incubated in the darkness for 30 min, the absorbance was measured at 515 nm and Trolox was used as the standard. The free radical scavenging capacity of extract was expressed as mg Trolox equivalent (TE) per gram sample dry-weight basis (mg TE/g DW).

2.6.4 ABTS radical scavenging capacity

In the ABTS assay, the $ABTS^{+\cdot}$ stock solution consisted of ABTS solution (7.4 mmol/L) and potassium persulphate (2.6 mmol/L), and the mixture was allowed to stand in the dark at room temperature for 12 h before use. To obtain the $ABTS^{+\cdot}$ working solution, the $ABTS^{+\cdot}$ stock solution was diluted about 20 times until the absorbance reached 0.7 ± 0.02 at 734 nm. Then the extract (0.4 mL) was mixed with 3.6 mL of $ABTS^{+\cdot}$ working solution and incubated in the

dark for 30 min. The ABTS radical scavenging capacity was measured at 734 nm and expressed as mg Trolox equivalent (TE) per gram sample dry-weight basis (mg TE/g DW).

2.7 Solid-phase microextraction and gas chromatography/mass spectrometry (SPME-GC/MS)

The volatile compounds in bread were extracted using the solid phase micro-extraction (SPME) method (Xu et al., 2022). Briefly, the sample added with 10 μL internal standard (cyclohexanone, 95.3 μg/L) was transferred to a headspace vial (10 mL) and equilibrated at 50 ^{o}C for 10 min. The SPME fiber (75 μm CAR/PDMS) was then exposed to the sample for 40 min and inserted into the injection port of GC and desorbed at 250 ^{o}C for 5 min.

The separation and identification of volatile compounds were performed on a GCMS-QP2020 system (Shimadzu Corporation, Kyoto, Japan) equipped with a DB-Waxetr capillary column (30.0 m × 0.25 μm × 0.25 mm, Agilent, USA). The temperature of the column was kept at 40 ^{o}C. The temperature rise program was set as follows: 40 ^{o}C for 2 min, programmed at 6 ^{o}C/min to 70 ^{o}C, then at 4 ^{o}C/min to 120 ^{o}C, hold for 3 min, and finally at 6 ^{o}C/min to 230 ^{o}C, hold for 5 min. GCMS condition: injector, interface, and ion source temperature, 250 ^{o}C, 250 ^{o}C, and 230 ^{o}C; ionization energy, 70 eV; mass range, 40 to 500 amu.

2.8 Sensory evaluation

Sensory evaluations were conducted with freshly prepared bread on the day of the experiment using the method of Parenti, Guerrini, Cavallini, Baldi, and Zanoni (2020). Specifically, 2 cm thick slices of bread were offered in random order to 15 panelists (8 females and 7 males) with experience in bread evaluation. Panelists were asked to first assess the appearance of the samples before tasting and then eating them. Before the next sample test, panelists were served water to cleanse their taste buds and rested for 3 min to prevent sensory fatigue. A 9-point scale ranging from 1 (strongly disliked) to 9 (strongly liked) was used to estimate the overall acceptability, crust texture, grain structure, softness, and mouthfeel.

2.9 Statistical analysis

Data from three independent tests were expressed as mean ± standard deviation, and subjected to Duncan's test with a significant level of $p < 0.05$ using SPSS 22.0 (SPSS Inc., Chicago, USA). Hierarchical Cluster Analysis (HCA) was performed using *R* Studio 4.1.2 (*R*-vision, New Zealand).

3 Discussion

3.1 Crystallinity and molecular order of starch

The crystalline structure of food is closely related to its physical and machinal properties. Generally, foods with higher crystallinity possess greater structural stability and are less susceptible to external forces. XRD analysis was conducted to investigate the impact of CP incorporation on the crystalline structure of the dough. Figure 1A shows the XRD patterns of doughs with different CP substitution levels. It could be observed that the control exhibited prominent 2θ peaks at 15^{o}, 17^{o}, and 23^{o}, which was consistent with that reported by Guo, Yu, Copeland, Wang, and Wang (2018). The dough containing CP exhibited a similar X-ray diffraction pattern to that of the control. When the substitution level of CP was 3% and 6%, no obvious differences were found in the major diffraction peaks between control, CP3, and CP6, although the RC values reduced by 0.06% for CP3 (17.96%) and 4.95% for CP6 (17.08%), compared to the control (17.97%). However, when the CP substitution level was increased from 9–15%, the RC

values decreased substantially and the intensity of some diffraction peaks diminish. This phenomenon may be explained that the excessive substitution level of CP resulting in the disruption of the hydrogen bond between the double helix structure of starch. Similar results of reduced relative crystallinity of freeze-dried dough powder were also reported by Jia et al. (2021).

The obtained FTIR spectra in the band of 4000 – 400 cm^{-1} were used to analyze the changes in chemical composition and molecule structure of the dough (Fig. 1B). A broad band around 3295 cm^{-1} caused by intermolecular H-bonding (O-H stretching vibration) was detected in all spectra, a representative characteristic of the starch structure. A sharp peak at 2927 cm^{-1} was attributed to the stretching vibration of C-H bonds. The other two characteristic peaks appeared at 1022 and 1047 cm^{-1}, corresponding to the C-O and C-H bending vibration, respectively. Compared to the control, no variation in the types of main peaks was found in the FTIR spectra of the composite dough, suggesting that the incorporation of CP did not produce or disrupt the chemical groups. Furthermore, the differences in the intensity and location of all detected peaks between the control and five composite doughs may be associated with the hydrogen bond breaking effect caused by the interaction between the starch and CP particles. Significantly, the absorption bands at 1022 and 1047 cm^{-1} were characteristic of crystalline and amorphous regions of starch, respectively, and the relative absorbance ratio of 1047/1022 can be used to indicate the orderliness of the starch structure. As shown in Fig. 1B, it was observed that the absorbance ratio of composite doughs reduced as the CP substitution levels increased, with 0.973, 0.926, 0.920, 0.913, 0.888, 0.869 for control, CP3, CP6, CP9, CP12, and CP15, respectively, explaining that the hydrogen bond between the double helix structure of starch was destroyed (Ma et al., 2021). Similar observations were reported by Tao, Zhu, Nan, Jiang, and Wang (2021), who attributed the reduction in the relative absorbance ratio of 1047/1022 to a disruption of the crystal structure of the starch, causing its reduced orderliness (fewer crystalline areas). This result was consistent with the changes in RC values determined by XRD analysis.

3.2 Rheological behavior

3.2.1 Flow behavior

The flow behavior of doughs with different CP substitution levels was studied by a steady-state shear test. As shown in Fig. 2A, the apparent viscosity of all doughs decreased with the shear rate increased, which was a typical feature of the non-Newton shear-thinning liquids. For doughs, the reduction in the appearance viscosity may be related to structural disruption and rearrangement (Tang, Lei, Wang, Li, & Wang, 2021). Compared to the control, an increase in the apparent viscosity of the composite dough as the increase of CP substitution level from 3–15% was observed. Significantly, the slope of the curve for doughs with lower levels of CP substitution (CP3 and CP6) became small as the shear rate increased, and even after the shear rate above 30 s^{-1}, CP3 and CP6 presented higher appearance viscosity than that of CP9, CP12, and CP15. This phenomenon may be explained that the excess substitution level of CP reduced the interactions between adjacent polymer chains, thus weakening the cross-linking of the gluten network and chrysanthemum powder particles, which resulted in the loosening of the structure (Ma et al., 2012). Table 1 summarized the statistical results of the Power Law and Cross models, which fitted well ($0.984 < r^2 < 0.999$) in describing the flow behavior of doughs. A significant ($p < 0.05$) increase in the consistency index (K) was observed with the increasing CP substitution levels, implying an increase in the viscosity of the doughs. This was consistent with the results of apparent viscosity. In contrast, the flow index (n) reduced significantly ($p < 0.05$), with an increase in the substitution level of CP. Furthermore, in the Cross model, an increase in m values was observed, indicating a greater dependence of viscosity on the shear rater. The η_0 was associated with the

entanglement and aggregation of starch and gluten. The increase in the η_0 value indicated that the composite doughs possessed a higher resistance to deformation compared to the control.

Table 1
The model parameters fitting results of steady flow for doughs with different chrysanthemum powder substitution levels.

	Control	CP3	CP6	CP9	CP12	CP15
K (Pa·s^n)	363.6 ± 68.4^c	1533.2 ± 5.0^c	1772.0 ± 13.4^b	1785.5 ± 12.0^b	2799.2 ± 43.8^a	2750.5 ± 48.8^a
n (dimensionless)	-0.790 ± 0.022^a	-1.490 ± 0.030^c	-1.510 ± 0.061^c	-1.390 ± 0.020^b	-1.580 ± 0.046^c	-1.480 ± 0.040^c
r^2 >	0.984	0.995	0.994	0.997	0.998	0.998
η_0 (Pa·s)	876.4 ± 70.9^c	5621.6 ± 677.4^b	5452.2 ± 598.4^b	6253.3 ± 529.6^b	10399.7 ± 735.4^a	10550.5 ± 650.5^a
η_∞ (Pa·s)	25.92 ± 1.91^c	40.06 ± 2.28^b	50.20 ± 3.55^a	34.15 ± 2.37^c	40.64 ± 7.86^b	31.32 ± 1.26^c
λ (s)	1.73 ± 0.39^a	1.69 ± 0.17^a	1.45 ± 0.20^a	1.63 ± 0.27^a	1.90 ± 0.07^a	1.59 ± 0.34^a
m (dimensionless)	1.29 ± 0.04^c	1.91 ± 0.07^{ab}	2.02 ± 0.02^a	1.72 ± 0.17^b	1.78 ± 0.20^{ab}	1.85 ± 0.05^{ab}
r^2 >	0.999	0.999	0.999	0.999	0.999	0.999

[a] Mean value with different superscripts in the same row is significantly (p < 0.05) different by Duncan's test.

3.2.2 Linear rheology behavior

The effect of the ingredients in a bread formulation on the dough properties can be analyzed by monitoring its rheological behavior. In this study, keeping the amount of water in the formulation constant allowed for assessing the influence of replacing wheat flour with CP on the dough properties for producing wheat-chrysanthemum composite flour-based bread. Figure 2B shows the frequency sweeps of doughs with different CP substitution levels. The storage (G') and loss modulus (G'') increased when the angular frequency increased from 0.1 to 100 rad/s. The G' values were higher than G'' values regardless of the CP substitution level, implying that the elastic behavior of the dough is more prevalent than viscous behavior. These findings were consistent with those previously reported by Tao et al. (2016) for wheat dough. Compared to the control, replacing part of wheat flour with CP caused a significant (p < 0.05) increase in G' and G'' values, which suggested an increase in dough viscosity and elasticity. This increased viscoelasticity of the dough might be because the dietary fiber and phenolic acid contained in the CP form a polymer with gluten proteins through a cross-linking reaction. In addition, the water in the dough has a plasticizing effect, but the combination of the large number of water-absorbing groups of dietary fiber in CP with water molecules through hydrogen bonds might alter the water distribution in the dough, which weakened the plasticizing effect of water and enhanced the viscoelasticity of the dough (Kotsiou, Sacharidis, Matsakidou, Biliaderis, & Lazaridou, 2022). These results were further demonstrated by the tanδ values (< 1, Fig. 2C). A slight decrease in tan δ was observed with the increase of CP substitution level (3–15 wt%), indicating a

solid-like behavior of the dough. This phenomenon might be because the incorporation of CP increased the filler-like effect in the dough matrix (Bonnand-Ducasse et al., 2010), thus improving the mechanical properties of the dough and reducing tan δ value.

3.2.3 Nonlinear rheology behavior

LAOS characteristics of food provide a theoretical basis for producing high-quality products and optimizing processing parameters. Figure 2D presents the variation of G' and G'' with strain amplitudes from 0.01 to 100%. In the LVR, the G' and G'' remain almost constant regardless of the strain amplitude. However, as the increase in strain amplitude, the G' and G'' decreased rapidly, and all samples exhibited type (strain thinning) behavior (Hyun, Kim, Ahn, & Lee, 2002). In type network, the network formation rate is less than its decomposition rate and the network structure is aligned along the flow field. As a result, the gluten network structure was susceptible to disruption, resulting in a decrease in G' and G'' values. Notably, the G' was observed to be higher than G'' for all samples in the LVR, implying that the dough system exhibited an elastic-based gel form. Furthermore, both G' and G'' values within LVR increased as the substitution level increased, which may be due to the enhanced dough strength as a result of its intermolecular interactions. The distribution of components such as protein and starch cellulose in the dough was reported to be closely related to the strength of the dough network (Turksoy, Erturk, & Kokini, 2021). Although the structural integrity of the gluten network deteriorated due to the inability to withstand the deformation generated by high strain amplitude, polyphenols, dietary fibers, and other components enriched in chrysanthemum has been reported to be beneficial in improving the nonlinear rheological properties of doughs (Ozyigit, Eren, Kumcuoglu, & Tavman, 2020).

3.2.4 Lissajous curves analysis

Lissajous curves provide a visual means of analysis that helps to understand the amplitude dependence of the viscoelastic behavior of foods during large oscillatory shear. A purely elliptical Lissajous loop at small strain amplitudes was observed, which represented a linear viscoelastic response of the dough. This implied that the energy exerted by the low strain was not sufficient to cause irreversible damage to the intermolecular interactions in the dough. The shape of the Lissajous curve is associated with the evolution of the viscoelastic network of gluten. As shown in Fig. 2E & F, a distorted ellipse was observed as the strain amplitude increased, which implied a transition from the linear to the non-linear region of the system (Zhao, Li, Wang, & Wang, 2022). The elastic Lissajous curve progressively changed from a narrow ellipse to a wide ellipse with strain increasing, accompanied by an increase in their area, which was related to the increased viscous dissipation. Compared to the viscous stress, a larger deviation between elastic stress (red line) and total stress (black line) was observed, suggesting a transition from elastic to viscous-dominated behavior of the dough response. This phenomenon may be attributed to structural damage to dough samples caused by the disruption of microstructure (Yildirim-Mavis et al., 2019). Furthermore, as the level of CP substitution increased, the total stress response loop exhibited a wider ellipse shape compared to the control, indicating a great improvement in the ability of the dough to resist stress. In the viscous Lissajous curves (Fig. 2F), the deviations from the red (viscous stress) to black lines (total stress) in the viscosity Lissajous curve were observed to become apparent as the substitution level increased from 3 to 15%, indicating that the dough possessed more elastic properties. However, as the strain amplitude increased, the deviation progressively decreased and the loops behaved as a narrower ellipse. This further confirmed the situation revealed by the elastic Lissajous curve, where the non-linear rheological properties of dough under large strain amplitude were dominated by viscosity behavior. Furthermore, in terms of the Lissajous curve shape, the increased level of CP substitution led to larger-sized ellipses compared to the control, implying rigid and solid-like properties of dough samples. These findings have described in detail the effect of replacing wheat flour with CP on the non-rheological

properties of the dough, while the elasticity and viscosity data corroborate each other and help us to further understand and explore the nonlinear viscoelastic behavior of the dough.

3.3 Microstructure

The SEM allows to intuitively observe the microstructure of dough, which helps to reveal the underlying mechanisms that chrysanthemum powder affects the baking performance of bread. Figure 3A presents the microstructure of the dough with different levels of CP substitution. The control exhibited a relatively uniform and compact gluten network in which most of the irregularly shaped starch granules were firmly embedded, and a few floated outside the gluten network structure. The 3% of CP substitution level gave the dough a denser gluten network and a more defined gluten lamellar structure than the control. This phenomenon was probably because CP contained various biological macromolecules, such as polysaccharides and protein, which could act as fillers in the gluten network to further improve the stability of the gluten network structure. Moreover, the inclusion of polyphenolic substances such as tannins preferentially bound the side chain groups of gluten proteins to phenolic hydroxyl groups, and the interaction between the polyphenols and gluten proteins led to the polymerization of proteins, thus enhancing the network structure of gluten (Han, Ma, Zhang, Li, & Sun, 2020). At a 6% substitution level of CP, a compact gluten network structure was still observed covering the surface of the starch granules in a reticulated manner. However, when the substitution level exceeded 9%, the structural stability of the dough decreased and the cross-linked gluten network started to break, showing an uneven gluten film. The number of irregular holes increased, and starch granules and non-gluten components contained in the CP clump together. Especially, the gluten network of CP15 was looser with deep fractures and filamentous structure. This may be due to the interference effect of exogenous proteins and the dilution effect of dietary fiber on the gluten network. Furthermore, some authors have reported that dietary fiber competing with gluten proteins for water may also prevent gluten development (Hemdane et al., 2016). Notably, the retention and expansion of CO_2 gas play an essential role in determining the softness, specific volume, and porosity of the bread during dough fermentation. A loose and porous gluten network structure is not conducive to CO_2 gas retention and thus results in poor baking properties of the bread.

3.4 Textural analysis

Textural properties are an essential factor, closely related to the mouthfeel of food. As presented in Table 2, replacing wheat flour with CP caused an increase in the hardness of bread. Compared to the control, wheat flour substitutions with CP at 3%, 6%, and 9% levels have no significant ($p < 0.05$) effect on the hardness of bread, especially CP3 obtained the lowest hardness value (1.35 ± 0.29 N). However, a significant ($p < 0.05$) increase in hardness was observed in CP12 and CP15, which may be attributed to the enhancement of the dough strength. This was confirmed by the rheological behavior of dough in Section 3.2. Similar findings were also reported by Cao et al. (2021). In addition to hardness, the effect of the interaction between protein, starch, and CP on the structural properties was also evident through changes in cohesiveness, resilience, and springiness values. These parameters showed an increasing trend at first then reduced as the level of CP substitution increased. Notably, 3% and 6% CP substitution levels resulted in a significant ($p < 0.05$) increase in the cohesiveness values, implying that the internal structure of the bread had enhanced resistance to high stress-strain, which would improve its gas retention capacity. Similar trends were observed for springiness and resilience values. The springiness and resilience were correlated with the elasticity of the bread and they depended on the gluten network in the dough system. CP3 and CP6 had similar springiness and resilience to the control, while the springiness and resilience values of bread with 12% and 15% of CP showed significantly ($p < 0.05$) lower than the control. Paciulli, Rinaldi, Cirlini, Scazzina, and Chiavaro

(2016) reported that bread with lower springiness and resilience may be prone to crumble, which would have a negative effect on consumers' acceptance.

Table 2
Textural properties, color parameters, crumb structure, total phenolic content (TPC), and antioxidant capacity of wheat bread with different substitution levels of chrysanthemum powder.

	Control	CP3	CP6	CP9	CP12	CP15
Hardness (N)	1.55±0.13^{cd}	1.35±0.29^{d}	1.67±0.26^{d}	1.9±0.42^{c}	2.68±0.28^{b}	3.85±0.37^{a}
Resilience (%)	30.34±1.22^{c}	33.02±0.93^{a}	32.14±0.77^{ab}	31.22±1.53^{bc}	30.95±1.73^{bc}	27.84±1.38^{d}
Cohesiveness	0.77±0.06^{b}	0.83±0.04^{a}	0.80±0.04^{ab}	0.77±0.03^{b}	0.76±0.04^{b}	0.70±0.02^{c}
Springiness (%)	86.26±1.75^{a}	84.52±0.76^{ab}	84.42±0.99^{ab}	83.96±0.98^{b}	80.69±1.79^{c}	80.56±1.86^{c}
Special volume (ml/g)	4.49 ± 0.10^{ab}	4.64 ± 0.10^{a}	4.54 ± 0.20^{a}	4.06 ± 0.20^{b}	3.18 ± 0.20^{c}	3.10 ± 0.19^{c}
L^*	79.01 ± 1.40^{a}	72.26 ± 0.47^{b}	63.32 ± 0.64^{c}	56.32 ± 0.22^{d}	51.94 ± 0.22^{e}	48.52 ± 0.38^{f}
a^*	-4.35 ± 0.47^{c}	-4.77 ± 0.02^{c}	-4.43 ± 0.16^{c}	-3.79 ± 0.07^{b}	-3.41 ± 0.06^{b}	-2.91 ± 0.10^{a}
b^*	23.58 ± 0.97^{d}	30.63 ± 0.89^{c}	35.31 ± 0.50^{b}	37.33 ± 0.67^{ab}	37.78 ± 0.01^{a}	37.23 ± 1.28^{ab}
ΔE	-	9.79 ± 0.31^{e}	19.60 ± 0.21^{d}	26.54 ± 0.54^{c}	30.58 ± 0.19^{b}	33.45 ± 0.18^{a}
C*	23.99 ± 0.86^{d}	31.00 ± 0.87^{c}	35.59 ± 0.52^{b}	37.52 ± 0.66^{ab}	37.93 ± 0.01^{a}	37.34 ± 1.27^{ab}
Number of cells	6543.5 ± 122.3^{a}	6409.0 ± 32.5^{ab}	6156.2 ± 187.4^{b}	5581.0 ± 107.5^{c}	5087.8 ± 200.8^{d}	4629.4 ± 75.7^{e}
Cell diameter (mm)	2.53 ± 0.04^{d}	3.22 ± 0.32^{c}	3.75 ± 0.01^{b}	4.17 ± 0.09^{a}	4.38 ± 0.09^{a}	4.49 ± 0.04^{a}
Cell wall thickness (mm)	0.476 ± 0.008^{d}	0.507 ± 0.001^{c}	0.508 ± 0.003^{c}	0.513 ± 0.004^{c}	0.529 ± 0.001^{b}	0.544 ± 001^{a}
Coarse/fine clustering	0.047 ± 0.001^{d}	0.050 ± 0.002^{d}	0.054 ± 0.001^{c}	0.064 ± 0.001^{b}	0.066 ± 0.001^{b}	0.098 ± 0.002^{a}
TPC (mg GAE/g DW)	1.30 ± 0.15^{e}	2.57 ± 0.01^{d}	2.69 ± 0.13^{d}	3.40 ± 0.03^{c}	4.00 ± 0.06^{b}	4.87 ± 0.02^{a}
DPPH (mg TE/g DW)	0.31 ± 0.09^{f}	1.23 ± 0.06^{e}	1.67 ± 0.05^{d}	2.73 ± 0.01^{c}	3.04 ± 0.04^{b}	3.41 ± 0.01^{a}
ABTS (mg TE/g DW)	1.28 ± 0.14^{e}	2.28 ± 0.10^{d}	2.67 ± 0.01^{c}	3.50 ± 0.07^{b}	4.39 ± 0.12^{a}	4.61 ± 0.10^{a}

[a] Mean value with different superscripts in the same row is significantly ($p < 0.05$) different by Duncan's test.

[b] L^*, a^* and b^*: Color parameters of the CIE system; ΔE: Total color difference; C*: Chroma.

3.5 Physical properties

The specific volume (SV) results of bread are shown in Table 2 and range from 3.10 to 4.49 ml/g. The substitution levels of CP at 3% and 6% had little influence on the SV values, while the SV values of CP9, CP12, and CP15 were significantly ($p < 0.05$) lower than those of the control, suggesting that the excessive substitution levels of CP had a negative effect on the volume of the bread. This probably was due to the increased level of CP substitution resulting in a decrease in the concentration of gliadin and glutenin, resulting in the formation of a gluten structure with poor gas retention ability and thus reducing the bread volume (Amini Khoozani, Kebede, & El-Din Ahmed Bekhit, 2020). As a rule, the bread with high SV values was preferred by consumers, which implied that CP3 and CP6 would be more acceptable to consumers than other formulations in terms of specific volume.

Color is a critical visual attribute that directly affects consumer preferences along with specific volume. As shown in Table 2, the incorporation of CP reduced the lightness (L^*) and increased the greenness (a^*), yellowness (b^*), and chroma (C) values. The color variation in bread mainly depended on the color of the ingredients in its formulation. Furthermore, the total color difference values (ΔE) were found to gradually increase with the increase of chrysanthemum powder share in bread formulation, and the highest ΔE value was obtained for CP15. Notably, when $\Delta E > 5$, the color difference could be perceptible to the naked eye, meaning that the effect of CP incorporation on consumer sensory was significant (Kowalski, Mikulec, Mickowska, Skotnicka, & Mazurek, 2022).

The quality of breadcrumbs was governed by the number of cells, cell diameter, cell wall thickness, and coarse/fine clustering. Generally, an ideal crumb structure is characterized by a large number of cells, small cell diameters, and thin cell walls. As shown in the brightness correction chart obtained from C-cell image analysis (Fig. $3B_1$), there was no obvious difference between CP3 and the control. However, an increase in crumb texture roughness was observed with increasing levels of CP substitution. Furthermore, a gradual lightening of the blue color and the appearance of a red border line were observed in the stomatal diagram (Fig. $3B_2$), which indicated that the cellular aperture of the breadcrumbs became progressively lager and was accompanied by the formation of pores. These observations were further validated by the calculated results of the C-cell image analysis. No significant ($p < 0.05$) differences were observed between the control and CP3 in terms of the number of cells and coarse/fine clustering (Table 2). However, when the CP substitution level exceeded 9%, a significant ($p < 0.05$) reduction in cell number and a significant increase in cell diameter and thickness were observed. The phenomenon may be explained that the dilution effect of CP on the gluten protein was not conducive to the formation of gluten film, leading to the escape of the CO_2 gas, which caused the cells to merge into coarse cells or even form holes during the fermentation and baking process (Ning, Hou, Sun, Wan, & Dubat, 2017).

3.6 Total phenolic content and antioxidant activity

Phenolics in plants are an important source of natural antioxidants and have attracted considerable attention for their nutritional value and therapeutic potential. As shown in Table 2, compared to the control, the inclusion of CP significantly ($p < 0.05$) increased the total phenolic content from 2.57 to 4.87 mg/g. Similar trends were also found in the DPPH and ABTS assays of bread (Table 2), and significant improvement in free radical scavenging capacity against $DPPH^{ž}$ and $ABTS^{ž+}$ was found with increasing substitution level of CP. Compared to the control, CP15 showed a 10-fold increase in DPPH radical scavenging capacity and a 2.80-fold in ABTS radical scavenging capacity. The increase in the antioxidant capacity of bread could be attributed to an increase in the phenolic content in bread, as phenolics have been established as natural antioxidants with the ability to scavenge free radicals. Excess free radicals in the body can lead to cell and organ damage, which induces the development of various

chronic diseases such as coronary heart disease, hyperlipidemia, hypertension, diabetes, and cancer, and accelerates the aging of the body. Therefore, the daily consumption of wheat bread containing chrysanthemum powder may have a health-promoting impact in preventing chronic diseases caused by free radicals.

3.7 GC-MS

A total of 33 volatile components were detected in bread with different CP substitution levels, including terpenes (12), alcohols (6), esters (4), ketones (3), aldehydes (3), hydrocarbons (2), phenols (1), acids (1). The dominant volatile components (ethyl caprylate, santolina triene, and 1-heptyn-3-ol) in the control showed the native odor of wheat flour, that is, a pleasant scent of with plant. Compared to the control, replacing part of wheat flour with CP resulted in a considerable enrichment of the bread in terms of the total number and content of volatile compounds. Among detected volatile compounds, 13 common volatiles were shared by all samples, while 3 volatiles (terpinene-4-ol, 4-oxatricyclo [4.3.1.1(3,8)] undecan-5-one) and 6-methyl-3,5-heptadiene-2-one) were unique to only a few samples (Table S1). More terpenes were found than alcohols, esters, and other volatiles, whereas esters were considered the predominant group due to the highest concentration of ethyl caprylate (Fig. 4A). To further assess the diversity of volatile compounds detected in bread with different CP substitution levels, a hierarchical clustering analysis was performed. Figure 4B sufficiently showed that bread with lower CP substitution levels and higher CP substitution levels were distinguished. Notably, CP3 and CP6 were clustered together with the control, i.e., there was no significant difference in the aroma profile of the control, CP3, and CP6.

Considering the effect of CP incorporation on the terpenes, it was found that only humulene, β-farnesene, isoborneol were present in the control, which implied that most of the terpenes were formed because of CP incorporation. Cis-verbenol, caryophyllene, and trans-α-bergamotene were the dominant compounds, which were responsible for the woody, sweet, and cinnamon aroma. Isoborneol, humulene, and β-farnesene were famous for their therapeutic properties (Petrović et al., 2022). Similar to terpenes, the total number and concentration of alcohols were greatly increased by the incorporation of CP. These alcohols contributed to the faint floral and grassy scent of the bread owing to their high odor threshold. 1-Heptyn-3-ol, (z)-4-decen-1-ol and phenylethyl alcohol were detected in all samples, while 2,6-Dimethyl-3,7-octadiene-2,6-diol, 2-furanmethanol and 3-methyl-3-cyclohexen-1-ol were absent in the control. Among alcohols, 1-heptyn-3-ol was a vital component in bread, formed during the oxidative decomposition of linoleic acid, which gave the bread an aroma profile of mushroom (Nissen, Samaei, Babini, & Gianotti, 2020). Esters were mainly derived from the esterase-catalyzed reaction of alcohols and acids during microbial metabolism. Methyl 2-ethylhexanoate and benzyl decanoate were absent in the control, and ethyl caprylate and acetic acid, bornyl ester were found in all samples. Ethyl octoate was the most important ester owing to its highest content, providing fruity and mushroom notes of bread. All ketones identified were absent from the control, with the highest concentration being chrysanthenone. Aldehydes were produced by the thermal oxidation of lipids and were the main contributors to the aroma of bread due to their low odor threshold. Furfural, benzaldehyde, and 2-hexenal were detected in all samples. Furfural is a typical Maillard-type aroma composition that held an odor with almond characteristics and a bread-like flavor. However, the presence and accumulation of furfural were detrimental to the safety and quality of food. Surprisingly, the inclusion of CP effectively reduced its concentration in bread. This result was supported by the report of Mildner-Szkudlarz, Rozanska, Piechowska, Waskiewicz, and Zawirska-Wojtasiak (2019), who found that the polyphenols, especially ferulic acid, showed superior inhibition in chemical hazards caused by the Maillard reaction. Regarding other volatile groups, santolina triene (0.96–1.79 μg/g, hydrocarbons), butylated hydroxytoluene (0.09–1.03 μg/g, phenols) and acetic acid (0.12–0.65 μg/g, phenols) were the dominant compounds in bread.

3.8 Sensory evaluation

Table S2 shows sensory evaluation scores for bread prepared with different formulations. No significant ($p < 0.05$) differences were observed between control, CP3, and CP6 in terms of appearance, texture, and taste, while the scores of CP3 and CP6 were higher than these of CP9, CP12 and CP15. Notably, CP6 exhibited the highest scores for appearance, texture, and overall acceptability, which implied it was more attractive to sensory evaluators than the control. These findings indicated that the addition of appropriate amounts of CP brings the desired flavor and texture to wheat bread. To further identify the underlying causes of flavor and texture formation and improved the sensory properties of bread, mantel's test correlation was performed. The thickness of the line indicates the strength of the correlation, while the change in its color indicates the significance of the difference. As shown in Fig. 4C, overall acceptability was observed to be positively correlated ($p < 0.01$, $r > 0.80$) with lightness, total phenolic content, antioxidant capacity, and the number of cells. For appearance, greenness, specific volume, and the number of cells were found to be highly correlated ($p < 0.01$, $r > 0.80$) with it. For texture, the effect of SR, 1047/1022 value, cell wall thickness, and coarse/fine clustering ($p < 0.05$) were significant for it. The number of cells, coarse/fine clustering, hardness, and ketones showed a significant ($p < 0.01$, $r > 0.80$) effect on the taste. In addition, the major contribution of aroma volatiles contained in CP to the overall acceptability of the bread was reflected in the content of terpenes, ketones, and phenols. These results would help us to understand the barriers to plant-rich bread consumption, and provide guidance to produce wheat-chrysanthemum composite flour-based products available with comparable sensory properties to refine wheat bread.

Conclusion

In this study, the rheological behavior, structural and baking properties of doughs with different substitution levels of chrysanthemum powder (CP) were investigated. Results indicated that the incorporation of CP reduced relative crystallinity and the orderliness of the starch structure. Flow and SAOS tests showed that replacing wheat flour with CP increased consistency index, and viscoelastic modulus of doughs, compared to the control, implying weakened plasticity of the dough system, which was related to the poor baking performance of the bread. LAOS tests showed that doughs exhibited typical *I* (strain thinning) behavior. The SEM observation revealed that a denser and more coherent gluten network than the control at 3% and 6% CP substitution levels, whereas at substitution levels above 9% a looser gluten network and an uneven gluten film were observed. For bread, no obvious difference was observed in the textural attributes of the control, CP3, and CP6, while CP9, CP12, and CP15 exhibited coarser cells and thicker cell walls, which is undesirable for consumers. Furthermore, GC-MS results indicated that the flavor of the bread was dramatically enriched by CP. Notably, the inclusion of CP at the 6% level not only improved the rheological and structural properties of doughs, but also increased the phenolics, antioxidant capacity and volatiles in breads. This further was confirmed by sensory evaluation, which showed that CP6 scored highest in terms of appearance, texture, and overall acceptability. These results provided insights into the relationship between the rheological behavior and microstructure of chrysanthemum-rich doughs, which would be a valuable guide to developing more chrysanthemum-based products in the food industry.

Declarations

Author Contribution

Huihuang Xu: Data curation, Formal analysis, Writing - Original Draft. Min Wu: Funding acquisition, Project administration, Writing - Reviewing and Editing. Nan Shang: Writing - Reviewing and Editing. Wenguang Wei: Conceptualization, Methodology. Fei Gao: Methodology.

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Conflict of Interest Statement

The authors declare no competing interests.

Data Availability

All data generated or analyzed during this study are included in this published article.

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Figures

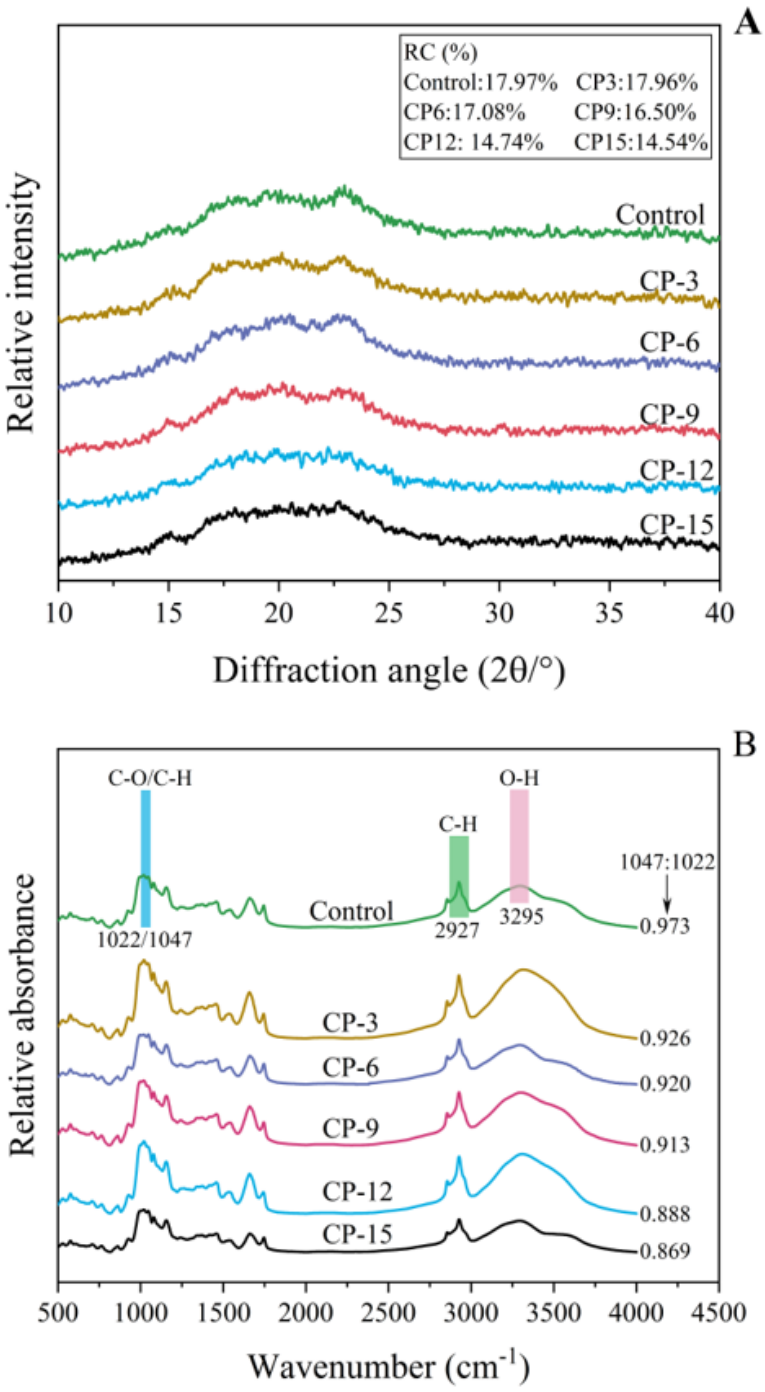


Figure 1

X-ray diffractograms (A) and FTIR spectra (B) of doughs with different substitution levels of chrysanthemum powder (Control: Dough without chrysanthemum powder).

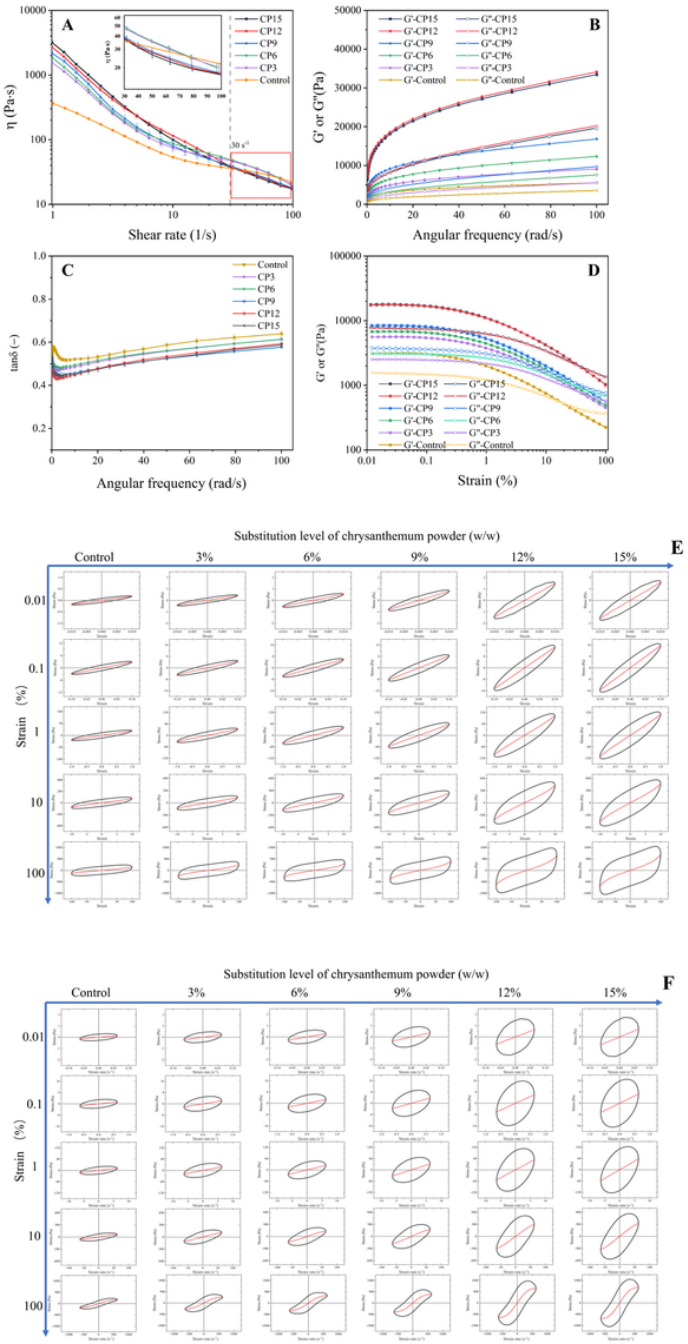


Figure 2

Rheological behavior curves of dough with different substitution levels of chrysanthemum powder: (A) Apparent viscosity; (B) Elastic and viscous moduli; (C) Dependence of the phase angle on the oscillatory frequency; (D) Large amplitude oscillatory shear behavior; (E) The elastic Lissajous curves (the black and red lines represented the total stress and the decomposed elastic stress, respectively); (F) The viscous Lissajous curves (the black and red lines represented the total stress and the decomposed viscous stress, respectively).

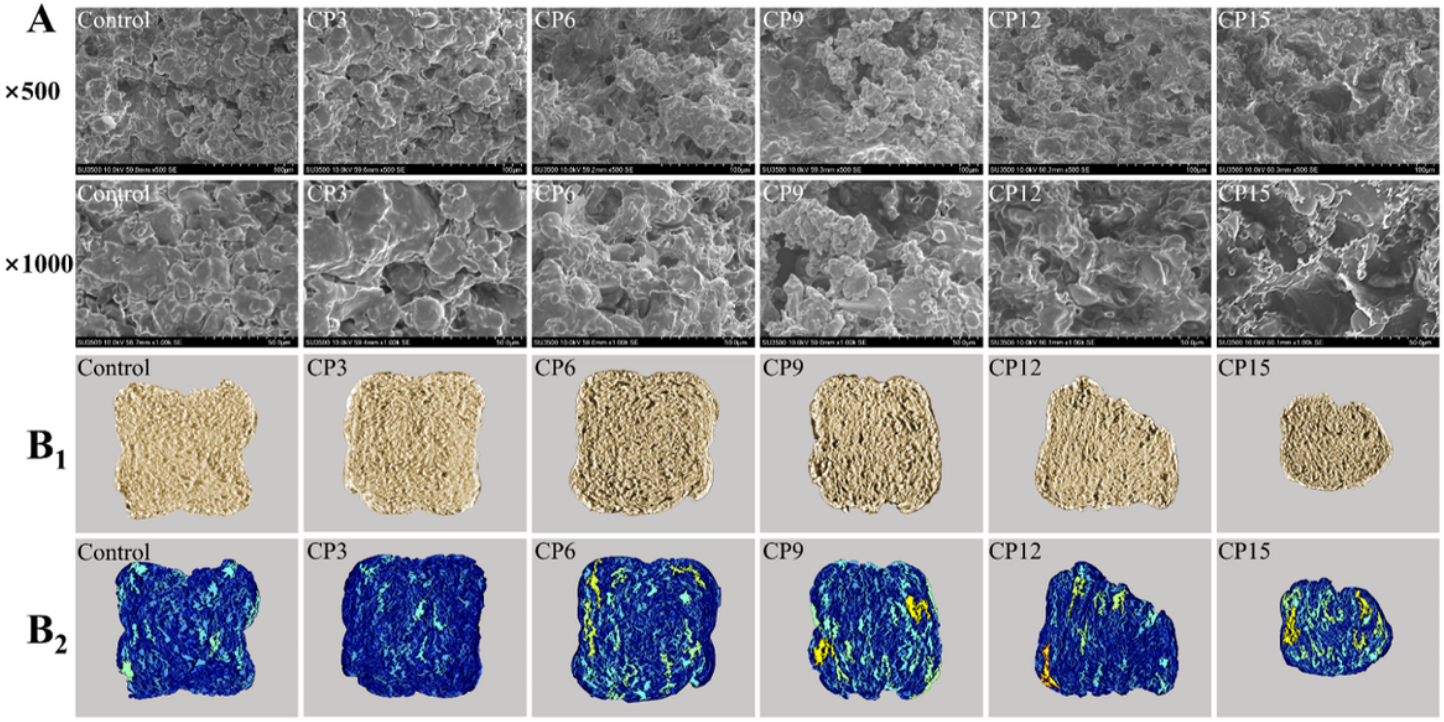


Figure 3

SEM images of dough with different substitution levels of chrysanthemum powder (A) and digital images obtained from C-cell imaging system of bread slices with different substitution levels of chrysanthemum powder. (Control: Bread without chrysanthemum powder; A: brightness correction chart; B: stomata chart).

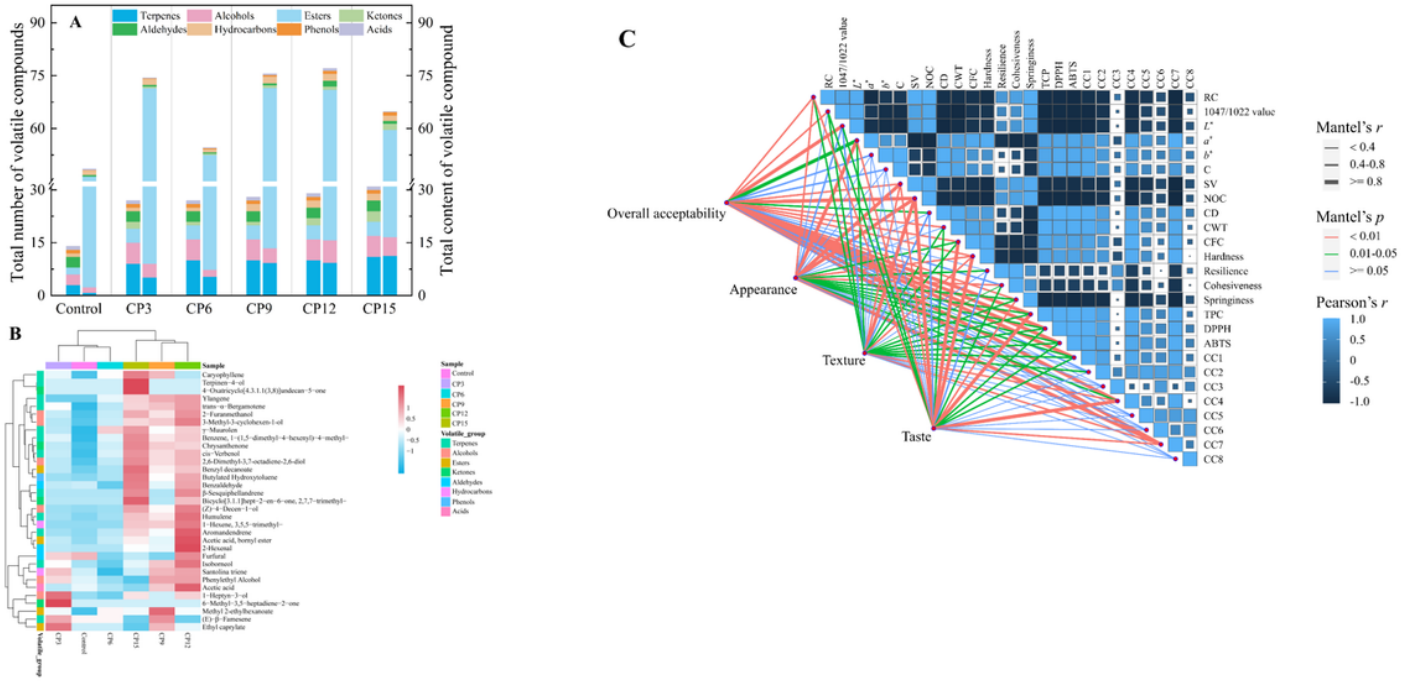


Figure 4

The total number, total content (A) and hierarchical clustering analysis (HCA) and heat map (B) of volatile compounds detected in bread with different substation levels of chrysanthemum powder, and mantel's test correlation between sensory evaluations and physicochemical properties of doughs and bread (C). RC: relative crystallinity SV: special volume; NOC: number of cells; CD: cell diameter; CWT: cell wall thickness; CFC: coarse/fine clustering; TPC: total phenolic content; CC: content of volatile compound; C1: terpenes; C2: alcohols; C3: esters; C4: ketones; C5: aldehydes; C6: Hydrocarbons; C7: phenols; C8: Acids.

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