

Effects of *Agaricus bisporus* alone or in combination with soybean oil or water as fat substitutes on gel properties, rheology, water distribution, and microstructure of chicken batters

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Abstract

This research aimed to investigate the effect of *Agaricus bisporus* mushrooms alone or in combination with soybean oil or water as fat substitutes on different properties of chicken batter. The cooking yield, water holding capacity, colour, texture, storage modulus (G'), T_2 relaxation time, and microstructure of chicken batters were analysed. Results showed that the above three adding ways of *Agaricus bisporus* mushrooms promoted the transformation of free water into immobilized water and improved the cooking yield, water holding capacity, texture, and rheological properties of the chicken batter. However, the colour of the chicken batter changed significantly ($p < 0.05$). Particularly, the combination of *Agaricus bisporus* and soybean oil demonstrated the optimal effect to improve the gel properties, rheology, microstructure and water-binding ability of the chicken batter. Thus, the results suggest that the concomitant use of *Agaricus bisporus* and soybean oil is a promising strategy for developing healthier chicken products.

Keywords: *Agaricus bisporus*; chicken batters; rheology; water distribution; microstructure

Practical Application: This work is expected to deliver reasonable guidance on the addition of *Agaricus bisporus* powder as fat substitutes into chicken batters for developing healthy chicken products. One can envision that appropriately using *Agaricus bisporus* powder in chicken batters will not only improve the quality and edible safety of chicken products, but also promote the economic value of *Agaricus bisporus*.

1 Introduction

Poultry meat consumption has increased sharply over the past few decades due to growing health concerns and the cost of red meat (Daniel et al., 2011; Samant et al., 2015; Zeng et al., 2019). Emulsified chicken products are one of the most popular emulsified meat products globally (Choe & Kim, 2019). Generally, a chicken sausage contains 20–35% fat, which plays an essential role in various characteristics of emulsified meat products, including emulsion stabilization, cooking yield, flavour, and textural properties (Choi et al., 2009; Varga-Visi & Toxanbayeva, 2017). In recent years, consumers' interest in low-fat meat products has significantly increased because of risks such as hypertension, hyperlipidemia, cardiovascular diseases, and obesity posed by consuming animal fat high in saturated fatty acids and cholesterol (Varga-Visi & Toxanbayeva, 2017). Therefore, several studies have attempted to reduce the fat content of these emulsified chicken products by utilizing fat substitutes or mixtures of functional ingredients and water, and simultaneously minimizing changes in their sensory and textural properties. Some examples of such substitutes are as follows: bacterial nanocellulose (Marchetti et al., 2017), sunflower seed oil and dietary fibre mixture (Choi et al., 2013), dietary fibre extracted from makgeolli lees (Choi et al., 2013), safflower

oil, canola, olive, sunflower, and perilla-canola oil (Kaynakci & Kiliç, 2021; Nieto & Lorenzo, 2021; Shin et al., 2020; Zhang et al., 2018), modified potato and tapioca starches (Hachmeister & Herald, 1998), chicken protein isolate (Silva Moraes et al., 2011), apple pomace fibre (Choi et al., 2016), Hoary basil seed mucilage (Saengphol & Pirak, 2018), and chicken skin and wheat fibre mixture (Choe & Kim, 2019), pineapple dietary fibre and water (Henning et al., 2016).

Agaricus bisporus (white button mushrooms) is widely cultivated in Europe, North America, and China, contributing 35–45% of the world's total mushroom production, and its shelf-life is only a few days (Tarlak et al., 2020). It is rich in protein, carbohydrates, and dietary fibre, and has a low content of fat (Ramos et al., 2019). The protein and polysaccharides contribute to the formation of the meat gel structure, while dietary fibre has water absorption properties and can improve water retention (Kurt & Genççelep, 2018). Moreover, the unique flavour of *A. bisporus* enhances the umami quality and taste of meat products (Dermiki et al., 2013; Wang et al., 2018; Wong et al., 2019). Therefore, *A. bisporus* can play a role similar to fat in emulsified meat products. Additionally, some studies

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have already demonstrated that *A. bisporus* is a promising fat substitute. For instance, the use of *A. bisporus* mushroom as a partial fat replacer improved the sensory quality and maintained the instrumental characteristics of beef burgers (Patinho et al., 2021). The parameters of colour, elasticity, cohesiveness, and cooking yield were significantly enhanced in refrigerated beef patties containing *A. bisporus* and *Pleurotus ostreatus* powder as partial substitutes for fat and salt compared to beef patties without mushroom powder (Cerón-Guevara et al., 2019). The model beef emulsion with 2% *A. bisporus* powder exhibited better gel and texture properties (Kurt & Gençcelep, 2018). However, to our knowledge, there are no studies comparing *A. bisporus* powder alone or in combination with water or soybean oil as a fat substitute in emulsified chicken meat products.

The emulsification process is a crucial step in the production of emulsified meat products. Therefore, to improve the quality of chicken products and minimize its production cost, this study compounded *A. bisporus* with the commonly used soybean oil and water. Our work aims to compare the effects of three different methods of *A. bisporus* addition on the cooking yield, water holding capacity, colour, texture, and dynamic rheological properties of low-fat chicken batter. Moreover, we also determined its microstructure, and water distribution and mobility to understand the role of these three fat replacement methods in the formation of chicken matrix structure and macro-quality of chicken products.

2 Materials and methods

2.1 Materials

Chicken breast meat (Arbor Acres, females, 49-day-old; 72.58% moisture content, 2.34% fat, 22.60% crude protein, and pH 5.89) and pork back fat (moisture content, 12.74%; fat content, 85.78%) were obtained from a local supermarket (Xinxiang, China). All visible connective tissue and fat were trimmed from the chicken breast meat. Furthermore, all visible connective tissue and the remaining lean meat were cut from pork back fat. The meat and pork back fat were ground separately using a grinder (MM-12, Guangdong, China) with a 6 mm perforated plate. Every 500 g of minced meat and 200 g of pork back fat were packed in nylon/polyethylene bags, sealed, stored (-20°C), and used up within 4 weeks. Fresh *A. bisporus* mushrooms (Xinxiang, China) were washed, cut (thickness = 5 mm), and dried (45°C , 8 h) until the moisture content was 7%, crushed using a high-speed grinder, and then passed through a sieve of mesh size 120 mm. The mushroom powder was obtained, which was packed in a polyethylene plastic bag and placed in a desiccator for subsequent experiments. Sodium chloride and sodium tripolyphosphate (JSC Chemical Technology CO., Ltd., China) were analytically pure. Soybean oil, white pepper, sugar, and ice water were of food-grade quality.

2.2 Preparation of chicken batters

Chicken batters were prepared in a cutter bowl (Joyoung S2-A808, Jinan, China) at different occasions in triplicates according to the previously reported method (Zhu et al., 2018).

Chicken meat and pork back fat were thawed overnight at 4°C in advance. The thawed chicken was ground along with salt, tripolyphosphate, and 1/3 ice water at 1500 rpm for 60 s, followed by a 3 min pause. Pork back fat, mushroom powder, the premixture of mushroom powder and soybean oil or water (solid-liquid ratio = 1:2), sugar, ground white pepper, and 1/3 ice water were added according to the formula (Table 1) and then ground at 1500 rpm for 120 s, followed by a 3 min pause. Finally, the remaining 1/3 ice water was added to the batters. The chicken batters were homogenised at 3000 rpm for 60 s. The emulsion was maintained below 10°C throughout the batter preparation. One part of raw meat batter was stored in plastic bags at 4°C for the analysis of rheological properties within 8 h. The other part was placed in 50 mL polypropylene tubes and weighed. Next, the batters were heated to 80°C for 30 min in a water bath, followed by cooling in ice water for 20 min to obtain chicken gel (cooked batters). The prepared chicken gels were stored at 4°C until further analyses.

2.3 Cooking Yield (CY)

The exudate separated from the chicken gel was removed after storage at 4°C overnight. Next, the cooked batters were weighed, and CY was estimated as % weight yield during cooking, expressed as the ratio of cooked batters (g) to raw batter weight (g).

2.4 Water Holding Capacity (WHC)

W_1 grams of chicken gel was wrapped in filter paper and placed in a 50 mL polypropylene tube. The chicken gel was then centrifuged at 4°C for 10 min at 8000 rpm. Next, the sample was taken out of the centrifuge tube, the filter paper was carefully removed, and the weight of the chicken gel after centrifugation (W_2) was obtained. WHC (%) was the percentage of the gel weight after centrifugation (W_2) relative to the original gel weight (W_1).

Table 1. The formulations of chicken batters (units: g/100 g).

Raw material/ ingredients	CK	Ab	Ab+O	Ab+W
Meat batters (100 g)				
Chicken meat	60	60	60	60
Pork back fat	20	16	8	8
Ice water	20	20	20	20
<i>A. bisporus</i> powder		4		
<i>A. bisporus</i> powder +Soybean oil			4+8	
<i>A. bisporus</i> powder+ Ice water				4+8
Total	100	100	100	100
Others (% of meat batters)				
Refined salt	1.4	1.4	1.4	1.4
Sugar	0.65	0.65	0.65	0.65
Sodium tripolyphosphate	0.3	0.3	0.3	0.3
Ground white pepper	0.15	0.15	0.15	0.15

CK: control (100% pork back fat); Ab: CB+80% pork back fat +20% *A. bisporus* powder; Ab+O: CB + 40% pork back fat + 20% *A. bisporus* powder+ 40% soybean oil; Ab+W: CB +40% pork back fat + 20% *A. bisporus* powder + 40% water.

2.5 Colour

A colourimeter (CR-400, Minolta camera Co., Japan) was employed to measure the colour of the chicken gel. The probe of the chromameter was placed close to the surface of the chicken gel (height = 20 mm, diameter = 25 mm) to avoid light leakage. The Hunter's colour values (L^* (lightness), a^* (redness), and b^* (yellowness)) of three chicken gel from each treatment were determined, and the averages were reported. The whiteness value was calculated according to the previously reported method (Wang et al., 2019b).

$$\text{Whiteness} = 100 - \left[(100 - L^*)^2 + a^{*2} + b^{*2} \right]^{1/2} \quad (1)$$

2.6 Texture analysis

A TA-XT Plus texture analyser (Stable Micro Systems, London, UK) was used to measure the texture profile analysis of chicken gel (diameter = 25 mm, height = 20 mm) by compressing chicken gel to 50% with a double compression cycle test according to a previously reported method (Cerón-Guevara et al., 2021) with some modifications. The calibration probe consisted of an aluminium cylinder, P36R, equipped with a 1 kg load cell, and the calibration distance was 30 mm. The settings used for texture analysis were as follows: pre-test speed, 2 mm/s; test speed, 2 mm/s; post-test speed, 5 mm/s; and interval time, 5 s. The measured parameters were hardness (N), springiness (mm), adhesiveness (mNs), and chewiness (N/mm). Each treatment was tested three times.

2.7 Rheological properties

The rheological characteristics of chicken batters were measured using a rheometer (HAAKE MARS III, Thermo Scientific, Massachusetts, USA) according to a previously reported method (Zhou et al., 2018) with some modifications. The chicken batters were placed between two parallel plates (diameter = 40 mm; gap = 1 mm), and the edge of the sample was sealed with methyl silicone oil to avoid contact with air. A temperature ramp sweep was conducted by heating the sample from 20 °C to 80 °C at the rate of 2 °C/min after holding for 2 min. The batters were continuously sheared in an oscillatory mode at a shear frequency of 0.1 Hz. The storage modulus (G') at different temperatures was recorded to characterise the rheological properties of the chicken batters. Each treatment was tested three times.

2.8 Low-Field Nuclear Magnetic Resonance (LF-NMR)

For each treatment, approximately 3 g chicken gel was placed in a 25 mm diameter NMR probe to measure the spin-spin relaxation time (T_2) using an NMI20-040V-I Series NMR analyzer (Suzhou Niumag Co., LTD, Suzhou, China). During the data collection, the transverse relaxation curve was collected with CARR Purcell Meiboom Gill (CPMG) pulse sequence. The test parameters were set as follows: pulse interval, 0.5 ms; main frequency, 20 MHz; offset frequency 625998.37 Hz; TD, 600008; NS, 8; TW, 5500 ms; $\tau = 6.52 \mu\text{s}$; and temperature, 32 °C. The attenuation curve of CPMG was inverted by T_2 inversion fitting software, and the

relaxation spectrum and T_2 were acquired. To increase the reliability of measures, each treatment was tested three times.

2.9 Microstructure

The chicken gel microstructure was observed using a field emission scanning electron microscope (SEM) (Quanta 200, FEI CO., US) (Wang et al., 2019a). First, the chicken gels ($3 \times 3 \times 3 \text{ mm}^3$) were fixed with glutaraldehyde (2.5%) in phosphate buffer saline (pH 6.8) at 4 °C for 24 h. The fixed samples were washed thrice with 0.1 M phosphate buffer (pH 6.8) for 15 min. The washed samples were dehydrated sequentially in 50%, 60%, 70%, 80%, and 90% ethanol for 15 min and then in 100% ethanol for 10 min (thrice). The dried samples were then degreased with chloroform for 1 h. The degreased samples were replaced with a mixture of absolute ethanol and tert-butyl alcohol (volume ratio = 1:1) and then with tert-butyl alcohol for 15 min. Next, the samples were vacuum dried at 45 °C for 5 h. Finally, the vacuum-dried sample was mounted on a bronze stub and sprayed with gold particles. The gold-plated samples were imaged under a microscope at 5 kV. Many micrographs were taken to select the most representative ones.

2.10 Statistical analysis

Data were analysed using analysis of variance (ANOVA) and expressed as mean \pm standard deviation. The differences between means were considered significant at $p < 0.05$. Duncan's multiple range test was used to compare the means and identify significant differences between samples using SPSS Version 20.0 for Windows (IBM, Armonk, NY, USA)

3 Result and discussion

3.1 Cooking Yield (CY), Water Holding Capacity (WHC) and colour

Generally, both CY and WHC can indicate the quality and moisture-binding capacity of meat batter. Table 2 shows the CY, WHC, and colour of the meat batters with or without fat substitutes. The CY and WHC of the samples containing fat substitutes were significantly increased ($p < 0.05$) compared to CK, indicating that the three fat substitutes significantly improved the moisture-binding capacity of meat batter. The increase in CY and WHC might be due to several components such as cellulose, hemicellulose, and lignin present in *A. bisporus* mushrooms (Kurt & Genççelep, 2018), which show good oil-absorbing and water-binding properties (Chaplin, 2003; Han & Bertram, 2017). However, there were no significant ($p < 0.05$) differences between the samples of Ab and Ab+O in CY and WHC in the present study. A possible explanation for this result in our study could be due to the presence of less pork back fat in the sample of Ab+O than Ab.

The color characteristics of meat products are essential for the consumers' acceptance of the product (Vidal et al., 2020). Compared to CK, the values of L^* and whiteness of the samples containing fat substitutes decreased significantly ($p < 0.05$), while the a^* and b^* value were increased significantly ($p < 0.05$). This outcomes may be related to the colour of A.

Table 2. CY, WHC and colour of chicken batters without or with fat substitutes.

Sample	CY	WHC	Whiteness	L^* -value	a^* -value	b^* -value
CK	85.01 ± 1.19 ^c	85.12 ± 0.82 ^c	78.70 ± 0.17 ^a	83.35 ± 0.12 ^a	1.50 ± 0.06 ^c	13.21 ± 0.07 ^c
Ab	90.60 ± 0.94 ^a	88.07 ± 1.31 ^a	59.62 ± 0.09 ^c	63.34 ± 0.09 ^c	1.97 ± 0.11 ^a	16.54 ± 0.08 ^a
Ab+O	89.62 ± 0.47 ^a	87.79 ± 1.13 ^a	63.87 ± 0.12 ^b	67.57 ± 0.15 ^b	1.78 ± 0.08 ^b	15.87 ± 0.10 ^b
Ab+W	87.55 ± 0.81 ^b	86.88 ± 0.30 ^b	60.07 ± 0.29 ^c	63.71 ± 0.21 ^c	1.97 ± 0.14 ^a	16.56 ± 0.12 ^a

CB: chicken batters; CK: control (100% pork back fat); Ab: CB+80% pork back fat +20% *A. bisporus* powder; Ab+O: CB + 40% pork back fat + 20% *A. bisporus* powder+ 40% soybean oil; Ab+W: CB +40% pork back fat + 20% *A. bisporus* powder + 40% water; CY: cooking yield; WHC: water holding capacity. Different letters (a-c) in the same column indicate significant differences ($p < 0.05$) between samples.

bisporus powder, which is low in L^* and whiteness values, and high in a^* and b^* values, due to its characteristic yellowish pigmentation (Cerón-Guevara et al., 2019). These results of L^* and b^* values were consistent with the report that the addition of *A. bisporus* powder reduced the L^* value and increased the b^* value of beef paste (Qing et al., 2021). Furthermore, it has reported that the colour index of low-fat chicken nuggets containing apple pulp increased with the level of apple pulp, which exhibited high redness and yellowness (Verma et al., 2010). In addition, the whiteness, L^* , a^* , and b^* values of cooked batters treated with Ab+O was significantly ($p < 0.05$) different from the treatment of Ab and Ab+W, which might be attributed to the colour of soybean oil and the difference in solubility of the mushroom pigments in water and oil (Jurić et al., 2020; Steglich, 1987).

3.2 Texture properties

Figure 1 shows the texture profile of chicken gel with or without *A. bisporus* powder. Compared with the CK, the addition of *A. bisporus* powder, significantly ($p < 0.05$) improved the hardness, springiness, and chewiness, and reduced the cohesiveness of the batters. The addition of *A. bisporus* powder and soybean oil significantly ($p < 0.05$) enhanced the hardness, springiness, and cohesiveness, and exhibited no significant effect on the chewiness of the chicken gel. The addition of *A. bisporus* powder and water significantly ($p < 0.05$) increased the hardness, springiness, and chewiness, and exhibited no significant effect on the cohesiveness of the batters. Thus, it could be concluded that the combination of *A. bisporus* powder and soybean oil or water significantly ($p < 0.05$) improved the texture characteristics of the batters. These results are supported by a previous study, wherein it was observed that the rich dietary fibre from *A. bisporus* was beneficial for the textural properties of cooked beef emulsion (Kurt & Gençlepe, 2018). The structural characteristics of cooked meat products were closely related to the gelation of myofibrillar proteins (Kang et al., 2020; Westphalen et al., 2006). During the preparation of meat batters, high ionic strength is helpful for the extraction of myofibrillar protein from meat (Gordon & Barbut, 1992). *A. bisporus* powder is rich in Ca, K, Mg, Na, and other elements, which increases the ionic strength of chicken batters and makes it conducive to extract salt-soluble proteins from chicken (Mleczek et al., 2020), proving it beneficial for the formation of the three-dimensional structure of heat-induced gel (Zhang et al., 2018). The Ab+O sample had the highest springiness and cohesiveness, suitable hardness, and similar chewiness to the control group, thus showing the best texture attributes among all the samples. This could be due to the fact

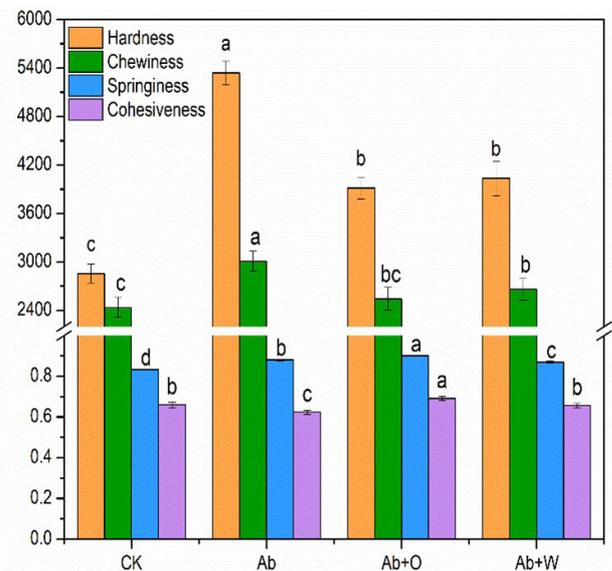


Figure 1. The textural attributes of cooked chicken batters without or with fat substitutes. Note: CB: chicken batters; CK: control (100% pork back fat); Ab: CB+80% pork back fat +20% *A. bisporus* powder; Ab+O: CB + 40% pork back fat + 20% *A. bisporus* powder+ 40% soybean oil; Ab+W: CB +40% pork back fat + 20% *A. bisporus* powder + 40% water. Values bearing different superscripts above the bar with the same colour indicate significant differences ($p < 0.05$) between samples.

that the premixing of *A. bisporus* powder and oil disperses oil droplets in Ab particles, forming smaller oil droplets than fat globules, reducing the accumulation of oil droplets and the interfacial tension between water and oil phases, and accelerating the emulsification process of meat. This result agrees with the results of Choi et al. (2009) who reported the effects of vegetable oils and rice bran fiber on the textural properties of low-fat meat emulsion systems.

3.3 Dynamic rheological properties

Dynamic rheological properties reflect the continuous changes in myofibrillar protein and protein-protein interactions (especially the myosin protein), which are closely related to the intramolecular and intermolecular binding in protein molecules (Sha et al., 2020). The storage modulus (G') indicates the changes in the elastic properties of the gel network structure and matrix strength (Cando et al., 2015). Figure 2 shows the

elasticity of chicken batter during heating by measuring the storage modulus (G').

The curve of G' of all samples showed similar characteristics with increasing temperature (Figure 2). There was a typical initial increase until the peak was reached at about 54.52 °C, which suggested that the myosin head had started aggregating and initiated the protein denaturation, resulting in the formation of weak gel network structures (Zhuang et al., 2018). Subsequently, G' markedly decreased and reached its peak value at 62.5 °C, which might be related to the uncoiling of the myosin tail leading to an increase in protein mobility and destruction of the protein network that had previously formed at a lower temperature (Câmara et al., 2020). The G' value then sharply increased because of the formation of new chemical bonds and increased cross-linking between the proteins, causing the viscous sol to simultaneously transform into an elastic gel network. The rheological results of our study were in accordance with the typical characteristics of meat emulsion (Sha et al., 2020).

The final G' values of the three samples with fat substitutes were higher than that of the control samples, indicating that these substitutes could improve the elastic properties of the gel network structure and matrix strength of the chicken gelling system. This could be attributed to the rich dietary fibre from *A. bisporus* powder, which might act as a filler and dehydrator,

or change the protein structure, thereby enhancing the gel structure. It has reported that dietary wheat could function as a dehydrating agent in surimi and change the tertiary structure of the protein in the sol phase, as reflected in the alterations of the hydrophobic side chain environment, making them more solvent-exposed. After heating, it resulted in hydrophobic contact between protein and fibre and an effortless nonspecific solidification, thereby forming a continuous, compact, and dense uniform structure in batter (Sánchez-González et al., 2009).

Among all the treatments, the sample of Ab+O showed the highest G' value. In contrast, the sample of Ab+W displayed the lowest G' value, indicating that Ab+O showed the best ability in improving the meat batter elasticity than Ab and Ab+W. This was in agreement with the findings of the texture profile in this study. One possible explanation for the improved elasticity of chicken batter is that the premixing of *A. bisporus* powder with soybean oil is similar to oil drop pre-emulsification. Many studies have confirmed that the addition of pre-emulsified vegetable oil could improve the G' level of meat batters (Choi et al., 2009; Zhuang et al., 2016).

3.4 LF-NMR spin-spin relaxation (T_2)

The low-field pulse NMR technique measures the T_2 relaxation time and estimates the fluidity and structural properties of the components of the water molecules partially fixed by the proteins in the gel system.

Figure 3 and Table 3 show the continuous distribution of NMR T_2 relaxation time and three T_2 relaxation times of water protons and corresponding peak area fractions of water in cooked chicken batters. Three separate peaks were identified as T_{2b} , a fast minor component (0–3% of total water), T_{21} , a major component (91–98% of total water), and T_{22} , a slow component (1–6% of total water) with the relaxation time of 0–1 ms, 40–60 ms, and 300–900 ms, respectively. In general, T_{2b} reflects water molecules tightly bound or closely connected to myofibrillar proteins through strong hydrogen bonds (Pearce et al., 2011). T_{21} corresponds to immobilized water trapped within the myofibrillar protein matrix and tightly bound to monolayer water molecules. T_{22} is related to free water that is weakly bound to the gel system and is the most fluid water component in the gel (Luo et al., 2020). There were no significant ($p < 0.05$) differences in T_{2b} of all samples, while the PT_{2b} of Ab+O was significantly ($p < 0.05$) smaller than that of the other three samples, suggesting that the addition of *A. bisporus* powder and soybean oil changed the bonding state of water and protein in chicken gel. It has reported that bamboo shoot dietary fibre did not influence the proportion of

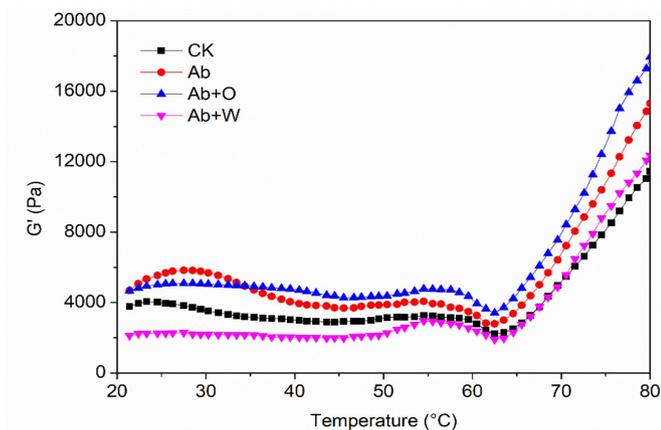


Figure 2. The storage modulus (G') of chicken batters without or with fat substitutes. Note: CB: chicken batters; CK: control (100% pork back fat); Ab: CB+80% pork back fat +20% *A. bisporus* powder; Ab+O: CB + 40% pork back fat + 20% *A. bisporus* powder+ 40% soybean oil; Ab+W: CB +40% pork back fat + 20% *A. bisporus* powder + 40% water. Values bearing different superscripts above the bar with the same colour indicate significant differences ($p < 0.05$) between samples.

Table 3. T_2 relaxation times and corresponding peak areas percentages of water in cooked chicken batters.

	T_{2b} (ms)	T_{21} (ms)	T_{22} (ms)	PT_{2b} (%)	PT_{21} (%)	PT_{22} (%)
CK	0.43 ± 0.18 ^a	57.22 ± 3.04 ^a	811.13 ± 25.71 ^a	2.94 ± 0.56 ^a	91.61 ± 1.41 ^d	5.46 ± 0.17 ^a
Ab	0.43 ± 0.12 ^a	49.77 ± 2.15 ^b	351.12 ± 23.41 ^c	2.38 ± 0.45 ^a	95.82 ± 1.24 ^b	1.80 ± 0.43 ^c
Ab+O	0.57 ± 0.10 ^a	49.77 ± 3.23 ^b	351.12 ± 20.62 ^c	0.42 ± 0.12 ^b	97.92 ± 1.03 ^a	1.66 ± 0.25 ^c
Ab+W	0.65 ± 0.08 ^a	57.22 ± 2.01 ^a	513.59 ± 20.48 ^b	2.66 ± 0.36 ^a	93.24 ± 1.36 ^c	3.52 ± 0.36 ^b

CB: chicken batters; CK: control (100% pork back fat); Ab: CB+80% pork back fat +20% *A. bisporus* powder; Ab+O: CB + 40% pork back fat + 20% *A. bisporus* powder+ 40% soybean oil; Ab+W: CB +40% pork back fat + 20% *A. bisporus* powder + 40% water. Different letters (a–d) in the same column indicate significant differences ($p < 0.05$) between samples.

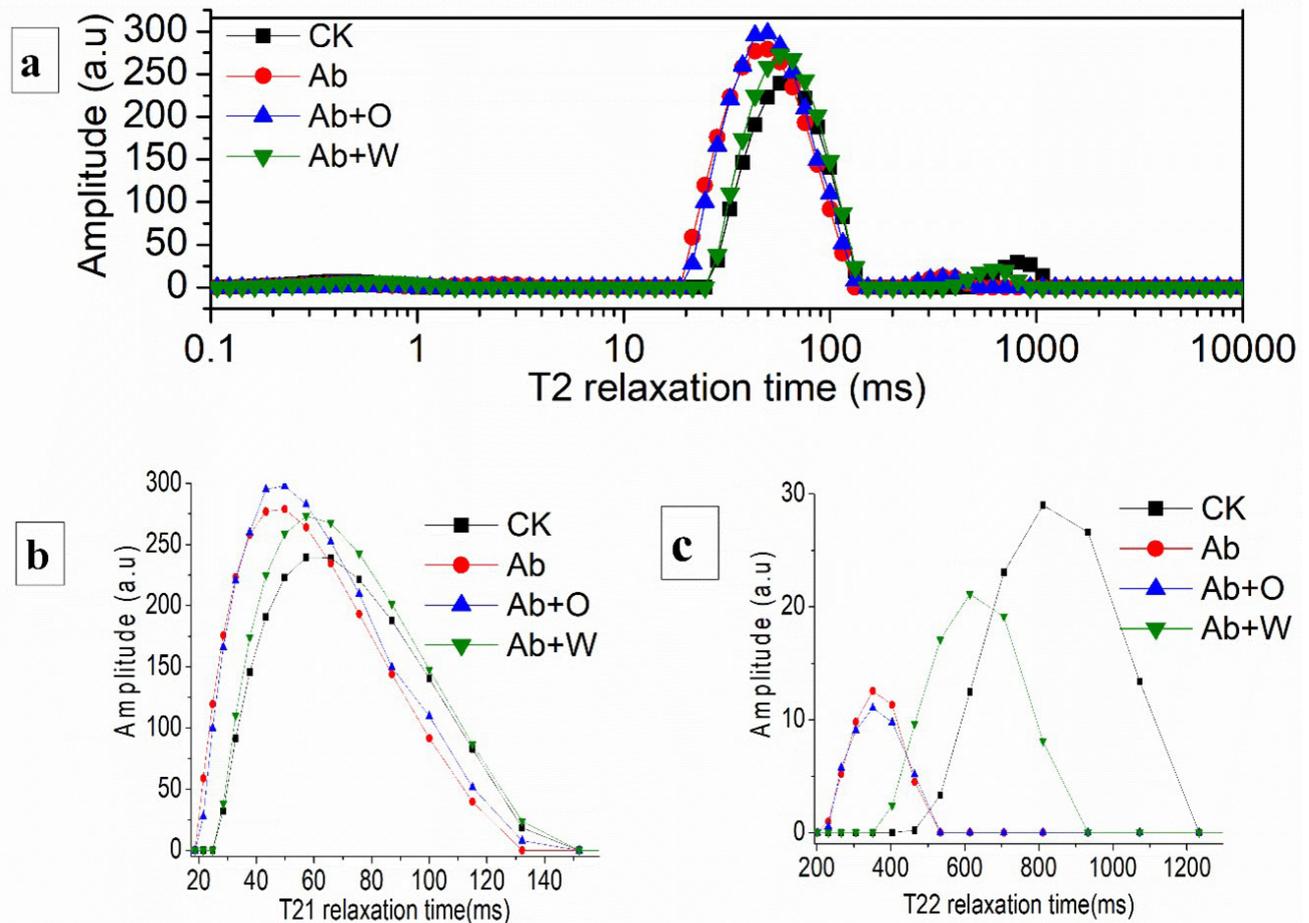


Figure 3. The continuous distribution of NMR T_2 relaxation time of chicken gel without or with fat substitutes. Note: T_2 relaxation time of chicken gel (a). T_{21} relaxation time of chicken gel (b). T_{22} relaxation time of chicken gel (c). CB: chicken batters; CK: control (100% pork back fat); Ab: CB+80% pork back fat +20% *A. bisporus* powder; Ab+O: CB + 40% pork back fat + 20% *A. bisporus* powder+ 40% soybean oil; Ab+W: CB +40% pork back fat + 20% *A. bisporus* powder + 40% water. Values bearing different superscripts above the bar with the same colour indicate significant differences ($p < 0.05$) between samples.

bound water in pork meat batter, which was consistent with the results of the samples of Ab and Ab+W in present experiment (Li et al., 2020). Compared to CK, the T_{21} values significantly decreased in the samples of Ab and Ab+O ($p < 0.05$), indicating that *A. bisporus* powder or the mixture of *A. bisporus* powder and oil could significantly ($p < 0.05$) enhance the gel's ability to bind to water and weaken the freedom degree of moisture. A significant ($p < 0.05$) decrease in T_{22} was observed in the gel system of chicken batter of Ab and Ab+O, suggesting that the addition of *A. bisporus* powder or mixture of *A. bisporus* powder and soybean oil decreased the water mobility of chicken meat gel and increased CY and WHC of chicken batters.

The amount of immobilized water in the Ab and Ab+O samples was significantly ($p < 0.05$) higher, while the amount of free water was significantly lower than those of the other two groups. The variation trend of T_{21} and PT_{21} between Ab and Ab+O was inconsistent. However, it has been reported that specific changes or trends between T_{21} and PT_{21} might be incompatible (McDonnell et al., 2013; Zhuang et al., 2018).

The incompatibility between T_{21} and PT_{21} might be ascribed to the fact that the chicken batters with *A. bisporus* powder and soybean oil formed a denser gel structure. The smaller the gel pore, the smaller the degree of freedom of the water wrapped by the myofibrillar protein matrix. A part of the free water in cooked chicken batters could have transformed to immobilized water, which may be related to the gel network structure.

3.5 Microstructure

Figure 4 shows the SEM images of cooked chicken batters without or with various fat substitutes. The SEM images indicated that the addition of different fat substitutes affected the microstructure of cooked chicken batters. A smooth microstructure can effectively bind water, while a coarse microstructure is fragile and has a low water holding capacity (Li et al., 2020). As shown in Figure 4, the SEM micrograph of the CK exhibited a granular, rough aspect and large, irregular cavity structure. The cavity in the microstructure of the gel was the water channel (Zhuang et al., 2018). The water channels in

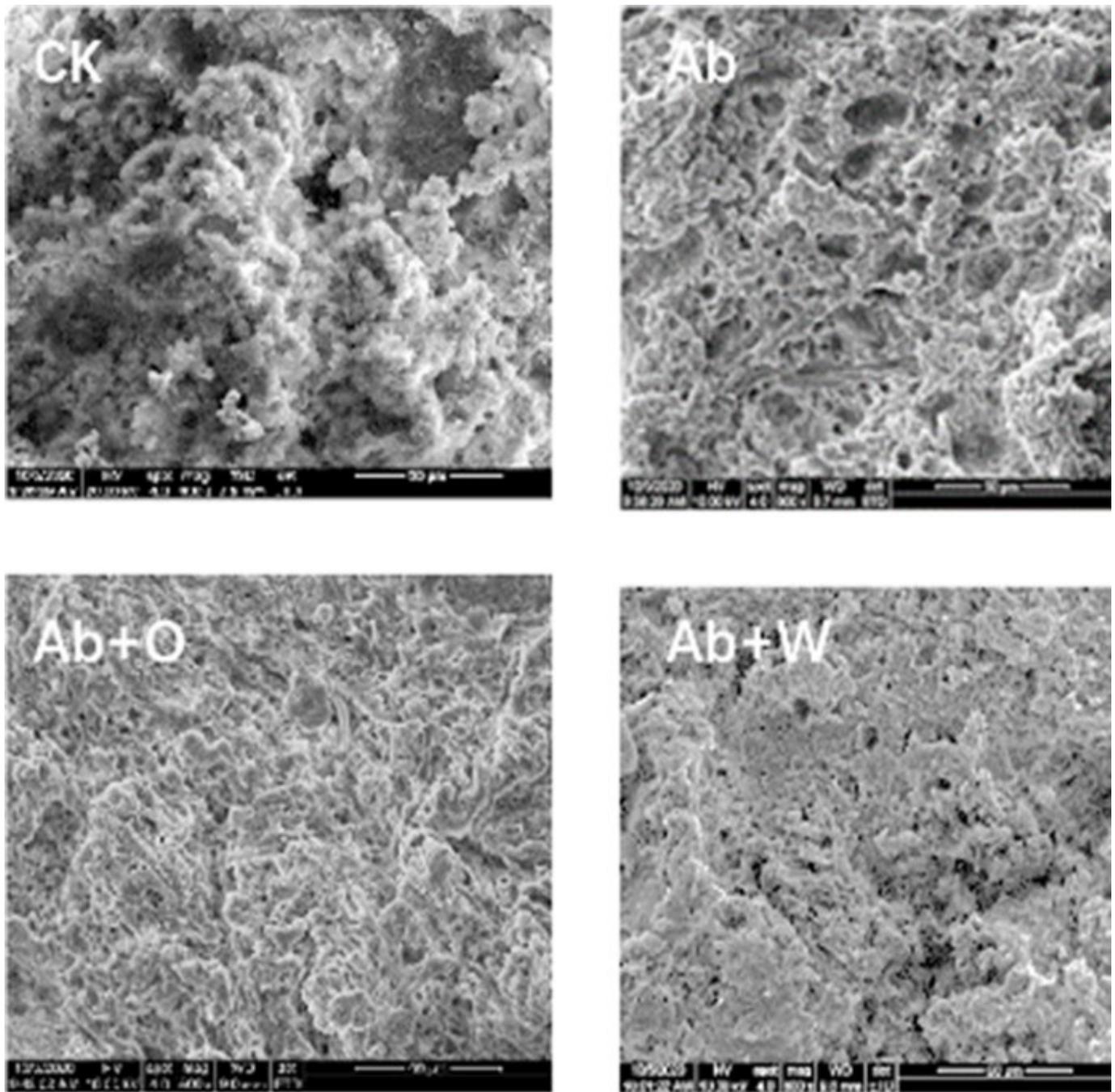


Figure 4. SEM images of chicken gel without or with fat substitutes at 1000 × magnification. Note: CB: chicken batters; CK: control (100% pork back fat); Ab: CB+80% pork back fat +20% *A. bisporus* powder; Ab+O: CB + 40% pork back fat + 20% *A. bisporus* powder+ 40% soybean oil; Ab+W: CB +40% pork back fat + 20% *A. bisporus* powder + 40% water.

the SEM micrograph of the CK had cross throughout the gel network, and it was easy for water to escape from the protein network in macropores, increasing the water loss. In contrast, the water channels in the samples with fat substitutes became thinner or even disappeared; the SEM micrograph exhibited a relatively uniform, compact, homogeneous, and ordered network structure. A possible explanation could be that the cellulose in *A. bisporus* powder expanded after absorbing water and shrunk the water channel, making the gel network structure

more compact. Therefore, it could be inferred that the CY of the samples with various fat substitutes should be greater than that of the CK, which was consistent with the result of CY in our study. In particular, the bridge between adjacent macromolecules was more obvious in the SEM micrograph of the Ab+O sample, followed by Ab and Ab+W samples. A more apparent bridge could prevent the formation of interconnected water channels and assist in generating further independent water cavities, thereby obtaining better gel quality and excellent WHC. This

was in agreement with the results of WHC in this study. Previous studies have demonstrated that WHC of meat products is linked to the microstructure (Han et al., 2009; Trout, 1988). Generally, the diameter of the pores within the protein lattice range from 0.1 to 1.0 μm , which is inversely proportional to the magnitude of the force that immobilizes water (Siegel & Schmidt, 1979). When water is immobilized in pores, the distance of water diffusion to the water/protein interface is shortened than unfixed water, resulting in a more significant proportion of immobilized water molecules to diffuse to the protein/water interface. Therefore, the T_2 value of the immobilized water in the pores is lower than that of free water, and as the pore size decreases, the T_2 value gradually decreases, approaching that of bound water (Trout, 1988). Consequently, the addition of three fat substitutes formed more and smaller porous microstructure, which caused a decrease in T_2 value, thus resulting in a higher WHC.

4 Conclusion

Based on this study, it can be concluded that the addition of *A. bisporus* powder or the combination of *A. bisporus* powder and soybean oil or water transforms part of free water into immobilized water and significantly ($p < 0.05$) improves CY, WHC, texture, and rheological properties of the chicken batter. However, the colour of the chicken batter changed significantly ($p < 0.05$). Particularly, the chicken batter with *A. bisporus* powder and soybean oil showed a higher CY, WHC and texture parameters, a more uniform and dense microstructure, and a relatively minor color change. Therefore, the results suggested that a combination of *A. bisporus* powder and soybean oil could be utilized to replace pork back fat for the production of reduced-fat and healthy chicken products. This study only investigated the use of *A. bisporus* mushrooms with soybean oil or water as a fat substitute in chicken batter. Future studies are needed using *A. bisporus* with other vegetable oils or mushrooms for the production of healthy meat products.

Ethical approval

Ethics approval was not required for this research.

Conflict of interest

The authors declare that they have no conflict of interest.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

Haijuan Nan, investigation, methodology and writing-original draft. Haoyu Zhou, methodology, writing-review and editing. Bo Li, conceptualization, project administration, writing-review and

editing. Tetiana M. Stepanova, formal analysis, writing-original draft, methodology, supervision. Natalia V. Kondratiuk, formal analysis, writing-original draft and methodology.

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