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Effects of vine pepper oil on the deterioration of texture quality in boiled rabbit meatballs: Inhibition of hydrophobic interactions and formation of covalent aggregates

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ABSTRACT

This study explored the influence of Sichuan characteristic vine pepper oil (VPO) on the quality of boiled rabbit meatballs. Sensory analysis revealed that VPO addition alleviated the strong undesirable odor of the meatballs. However, increasing VPO content significantly reduced the texture quality and water-holding capacity (WHC), particularly when VPO exceeded 3 % (*v/w*). SEM and BET-specific surface area analysis indicated that higher VPO levels promoted globular aggregate formation on the gel surface, increased the number of large pores, and resulted in uneven pore volume distribution. Elevated VPO content reduced the storage modulus and hindered the gel-strengthening phase, and intermolecular force analysis demonstrated significant inhibition of hydrophobic interactions between protein molecules during heating. SDS-PAGE further showed that increased VPO induced covalent bonding between meat proteins and VPO components, forming excessive macromolecular aggregates that disrupted the integrity of the gel structure, ultimately leading to reduced product texture and WHC.

1. Introduction

Rabbit meat is considered a functional food due to its low levels of fat (approximately 1.8 % in loin muscles), cholesterol (47.0 mg/100 g in loin muscles), and sodium (37.0 mg/100 g in loin muscles), as well as its high protein content (approximately 22.4 % in loin muscles), polyunsaturated fatty acids (32.5 % of total fatty acids), essential amino acids (10.34 % in loin muscles), and B vitamins (e.g. 8.7–11.9 μg of vitamin B12 in 100 g lean edible portion) (Dalle Zotte & Szendro, 2011). Additionally, its relatively high energy value (899 kJ/100 g in the forelegs and 603 kJ/100 g in the loin) meets the nutritional needs of modern humans, particularly for populations requiring a balanced diet, such as pregnant women, adolescents, and the elderly (Buitrago-Vera et al., 2016; Wang et al., 2022). China is the world's largest producer and consumer of rabbit meat, with a long history of consumption. According to FAOSTAT (2020), China produced 488,000 tons of rabbit meat in 2020, accounting for 57.07 % of global production. Rabbit meat consumption in China primarily involves frozen and fresh products, while deep-processed products are typically prepared through methods such as deep-frying, sauce-braising, and curing (Li et al., 2018). However, the industry faces challenges in developing these processed products: excessive saltiness and spiciness, along with severe product homogenization, hinder the healthy growth of the Chinese rabbit industry. Additionally, rabbit meat accounts for less than 1 % of total national meat consumption (Zhang et al., 2023). Therefore, the development of innovative and healthier deep-processed products is essential to ensure the sustainable growth of this industry.

Emulsified meat products such as kung-wan and surimi paste enjoy substantial consumer popularity (Hu et al., 2024). However, analogous rabbit meat products remain underrepresented in the market, primarily due to thermally induced lipid oxidation of high unsaturated fatty acid content, which generates undesirable odor compounds during processing (Abdel-Naeem et al., 2021). While the exceptionally low intramuscular fat content of rabbit meat (approximately 1.8 % in loin muscles)

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confers nutritional advantages (Dalle Zotte & Szendro, 2011), research demonstrates that intramuscular lipid content critically governs essential quality parameters including juiciness, tenderness, and flavor profile development (Petracci & Cavani, 2013). This paradox highlights the need for innovative processing strategies that address both thermal oxidation mitigation and optimal lipid supplementation in rabbit meat systems. Prior studies in low-fat meat matrices, particularly washed surimi, demonstrate that strategic incorporation of exogenous lipids can simultaneously enhance product quality and nutritional value (Fang et al., 2021). Unlike other fat replacement approaches employing polysaccharides, flours, hydrocolloids or specific animal proteins in low-fat meat products (Fadillah & Dirpan, 2024), rabbit meat's inherent high protein-to-fat ratio makes it particularly amenable to polyunsaturated fatty acid (PUFAs)-rich lipid fortification. Emerging evidence suggests that oils containing bioactive compounds not only improve sensory characteristics but also impart health benefits. For instance, virgin coconut oil incorporation reduces fishy odorants in surimi gels during freeze-thaw cycles while enhancing flavor complexity (Shen et al., 2024). Similarly, sturgeon oil emulsions enriched with ω-3 PUFAs have been shown to optimize both nutritional profiles and characteristic flavors in surimi products compared to traditional plant derived oils (Yuan et al., 2024). These findings position exogenous oils with distinctive flavor profiles as promising candidates for developing rabbit meat emulsions that counteract lipid oxidation while compensating for inherent fat deficiencies.

The textural properties and WHC of emulsified meat products constitute critical quality determinants, influencing sensory attributes such as chewiness and juiciness. However, lipid supplementation exerts complex multiphase effects mediated through three primary mechanisms: interfacial protein-lipid interactions, gel matrix structural modulation, and physical embedding effects. First, lipid source and emulsification methodology significantly influence protein cross-linking patterns and emulsion stability. Unsaturated oil droplets demonstrate superior emulsification efficiency due to enhanced protein adsorption at oil-water interfaces, forming stable interfacial films that prevent droplet coalescence and ensure homogeneous lipid distribution (Zheng et al., 2021; Zhou, Chen, et al., 2019). Second, lipid content modulates gel network formation through conformational protein transitions. Optimal oil concentrations promote α-helix to β-sheet transitions in myofibrillar proteins (MPs), strengthening hydrophobic interactions and yielding denser gel matrices with improved WHC and textural integrity. Excessive lipid incorporation (>50 mg/mL) conversely disrupts protein rearrangement, weakening intermolecular interactions and compromising gel strength (Han et al., 2021). Third, lipid globules function as physical fillers within the protein matrix, reducing interstitial porosity through direct space occupation. This action restricts water and lipid mobility via pore size reduction and physical entrapment, thereby improving product cohesiveness (Akgun et al., 2016; Song et al., 2022). Thus, elucidating how exogenous lipids affect protein behavior and gel matrix microarchitecture is essential for optimizing meat paste gel quality.

Vine pepper (*Zanthoxylum armatum* DC), a member of the Rutaceae family, is a characteristic medicinal and edible resource renowned for its unique "fragrant and numbing" flavor. Vine pepper oil (VPO), the primary processed derivative of this plant, is widely utilized as a condiment in the catering industry and in developing foods with "spicy-numbing" sensory profiles. Its distinct aroma effectively masks undesirable odors in food products (Liu et al., 2023). However, research on the development of "spicy-numbing" meat paste products remains limited, and the impact of VPO dosage on sensory quality and gel formation mechanisms in such products is poorly understood. Therefore, we hypothesize that the unique flavor profile of VPO may mitigate the odor generated by the oxidation of unsaturated fatty acids in rabbit meat during heating. Furthermore, the addition of this oil could address textural deficiencies resulting from the meat's intrinsically low-fat content, particularly in attributes such as juiciness and mouthfeel. This study aims to investigate the effects of VPO on the sensory attributes of heat-induced rabbit meatballs and elucidate its mechanism of action on gel properties through protein behavior analysis and microstructural characterization.

2. Materials and methods

2.1. Materials

Fresh Ira rabbit carcasses (75 days old, average weight about 2.3–2.5 kg/live rabbit, male) were purchased from Pixian Wal Mart Supermarket. The carcasses were quickly transported to the laboratory and hind legs and *M. longissimus dorsi* muscles were segmented and stored at −20 °C.

2.2. Preparation of spicy-numbing rabbit meatballs

The thawed rabbit meat was cut into small pieces and crushed into paste using a meat grinder, and various additives are added to the minced meat, including salt 1.5 %, monosodium glutamate 0.5 %, ice water 5 %, white sugar 1.5 %, white pepper 0.15 %, potato starch 1 %. Different levels (1 %,3 %,5 %,7 %) of VPO were added to meat paste respectively and mixed evenly (*v/w*), meat paste without VPO was used as the control group. The meat paste was made into a ball shape, with each meatball weighing 25 g. Each of carcasses was randomly selected for the preparation of spicy-numbing rabbit meatballs, the process was repeated three times.

2.3. Sensory evaluation

The rabbit meatballs were boiled at 80 °C for 10 min and subsequently cooled to room temperature prior to sensory evaluation. The sensory evaluation protocol was adapted from the methodology described in the previous study by Ruan et al. (2023) with minor modifications. A trained sensory panel consisting of 10 members (5 males and 5 females), all experienced in meat product evaluation, conducted the assessment. Six meatballs were prepared for each experimental formulation and evaluated across five sensory attributes using a 7-point hedonic scale (Table S1), where higher scores indicated greater acceptability of the specific quality parameter. Evaluations were conducted under standardized laboratory conditions with controlled ambient lighting and temperature maintained at 25 °C. This study protocol received ethical approval from the Institutional Review Board of the College of Food and Bioengineering at Xihua University. Prior to participation, all panel members were fully informed about the experimental procedures and voluntarily agreed to participate. The research team ensured the protection of participants' rights and privacy throughout the study, with explicit consent obtained for the collection and utilization of both personal information and experimental data.

2.4. Quality characteristics of rabbit meatballs

The quality characteristics of cooked rabbit meatballs were analyzed by texture properties, WHC, and yield. The texture determination method is as follows: meatballs were cut into a cube with a side length of 1.5 cm, and measured by P/36 R probe of a texture analyzer (TA-XTPLUS, Stable Micro Systems, London, UK) at 25 °C. The pre-test speed is 1.0 mm/s, testing speed is 0.5 mm/s, measured velocity is 0.5 mm/s, the number of presses is 2 times with target deformation of 30 %. Each group of samples was measured 6 times.

Yield was characterized by determining the weight of meatballs before and after heating. Briefly, meatballs were weighed before heat treatment and recorded as "M_1"; After boiling, drained the water then cooled it down, and recorded it as "M_2". The yield (%) was calculated according to the formula:

$$\text{Yield}\ (\%) = M_2/M_1 \times 100\%$$

WHC was characterized by determining the weight of meatballs before and after centrifugation. Meatballs were cut into a cube with a side length of 1.5 cm and wrapped by three layers of filter paper. The cube was centrifuged at 3000 r/min at 4 °C for 15 min. The ratio of weight of cube after centrifugation to that before centrifugation is WHC.

2.5. Observation on microstructure of meatball

2.5.1. Scanning electron microscope (SEM)

The method of observing the microstructure of meatball with SEM was referred to Jiang et al. (2021) with slight modification. Cooked meatball was cut into a 0.5 cm cube and fixed in phosphate buffer (0.1 M, pH 7.2, containing 2.5 % glutaraldehyde) at 4 °C for 24 h, then the sample was washed by the 0.1 M phosphate buffer (pH 7.2) for twice and dehydrated for 30 min at different gradient ethanol (30, 50, 70, 90, 100 %), respectively. The dehydrated sample was rapidly frozen in liquid nitrogen and then freeze-dried in a vacuum freeze dryer (FDU-1200, lingyi Biological Technology Co.,Ltd., Shanghai, China). The dried gel was sprayed with gold, and the microstructure of paste gel was observed and photographed by SEM.

2.5.2. Determination of BET specific surface area and pore size

The method of observing the BET specific surface area and pore size distribution of meatball was referred to Zhang et al. (2024) with slight modification. The BET specific surface area and pore size distribution of dried gel was characterized by using an automatic rapid surface area and microporous analyzer (ASAP 2020, Micromeritics, USA). The measurement procedure for this equipment is consistent with the study by Zhang et al. (2024).

2.6. Rheological behaviours

The rheological properties of meatball during the heating process were determined according to Wei et al. (2023) with minor modification. The 40 mm parallel plates with a gap distance of 0.5 mm was chosen to measure the variation trends of storage modulus (G ′), loss modulus (G ″), and phase angle (tan δ, G ″/G ′) at different temperatures (20, 30, 40, 50, 60, 70, and 80 °C). The paste was added between the two plates and sealed with silicone oil to prevent protein hydration during heating.

2.7. Surface hydrophobicity

The cooked meatball was dissolved phosphate buffer (20 mM, pH 6.0) and homogenized fully at 7000 r/min for 1 min (HR-25D, Shanghai Huxi Industrial Co., Ltd., Shanghai, China). Then, the homogenate was mixed with bromophenol blue (BPB) reagent (0.1 % *w/v*) in a volume ratio of 10:1, the mixture was place in a rotating shaker and oscillated at 400 r/min for 20 min to allow it to fully react. Subsequently, the mixture was centrifuged at 6000 r/min and 4 °C for 15 min, the obtained supernatant was diluted with 9-fold phosphate buffer (20 mM, pH 6.0). The surface hydrophobicity value was calculated following the method of Jiang et al. (2021).

2.8. Molecular forces

The analysis method of intermolecular forces in meatball followed the method of Wei et al. (2023). Firstly, four different reagents were prepared to determine the intermolecular interaction in meatball. 8 M urea dispersed in phosphate buffer (50 mM, pH 7.0) to determine the intermolecular hydrogen bonds; 0.5 % (*w/v*) sodium dodecyl sulfate (SDS) dispersed in phosphate buffer (50 mM, pH 7.0) to determine the hydrophobic interactions between molecules; 0.25 % (*v*/v) β-mercaptoethanol (β-ME) dispersed in phosphate buffer (50 mM, pH 7.0) to determine the intermolecular disulfide bonds; 0.5 M Sodium thiocyanate dispersed in phosphate buffer (50 mM, pH 7.0) to determine the electrostatic interactions between molecules. Rabbit meatball was added into above reagent at a mass to volume ratio of 1:9, the mixture was homogenized fully at 7000 r/min for 1 min (HR-25D, Shanghai Huxi Industrial Co., Ltd., Shanghai, China). The homogenate was transferred to test tube and heated in a water bath (80 °C) for 1 h, cooled it to room temperature and centrifuged at 10000 r/min for 15 min. The protein content in obtained supernatant was analyzed by Kjeldahl method. The relevant measuring instruments are automatic kieldahl apparatus (K1160, HaiNeng Future Technology Group Co., Ltd., Jinan, China).

2.9. SDS-PAGE

Protein properties in cooked meatball was analyzed by SDS-PAGE. The minced gel was mixed with electrophoretic buffer at a mass to volume ratio of 1:3 (m/v), the mixture was reacted in a boiling water for 5 min and centrifuged at 8000 ×*g* for 10 min at 4 °C, then 7 μL of supernatant was added to a 5 % for subsequent electrophoresis analysis. The proteins are separated on a separation gel based on their molecular weight. Subsequently, the electrophoresis gel was stained and decolorized and this process is based on the study by Zhang et al. (2024).

2.10. Statistical analyses

The average value of the relevant measurement data is calculated from triplicates and expressed as mean ± standard error. All experiments were repeated three times (each repeat used 5 kg of rabbit meat). Principal component analysis (PCA) was processed on SPSS (version 22) and drawn on Origin 8.1 software. ANOVA analysis of variance was used to evaluate the difference significance of the results. A significant difference in all statistical analyses was determined using Tukey's significant difference (HSD) test at 5 % significance level ($P < 0.05$).

3. Results and discussion

3.1. Sensory evaluation

The sensory evaluation of boiled rabbit meatballs treated with varying levels of VPO is shown in Fig. 1. The texture scores of the meatballs decreased while flavor scores increased with higher VPO concentrations. VPO contains unique volatile flavor compounds derived from bioactive constituents such as alkaloids, volatile oils, amides, and phenols (Liu et al., 2023). These compounds effectively mask unpleasant odors generated by unsaturated fatty acid oxidation, thereby enhancing the overall flavor profile. However, VPO addition was found to negatively impact meatball texture. Studies indicate that incorporating external lipids into meat products can alter the rheological properties and microstructure of meat gels, subsequently affecting sensory

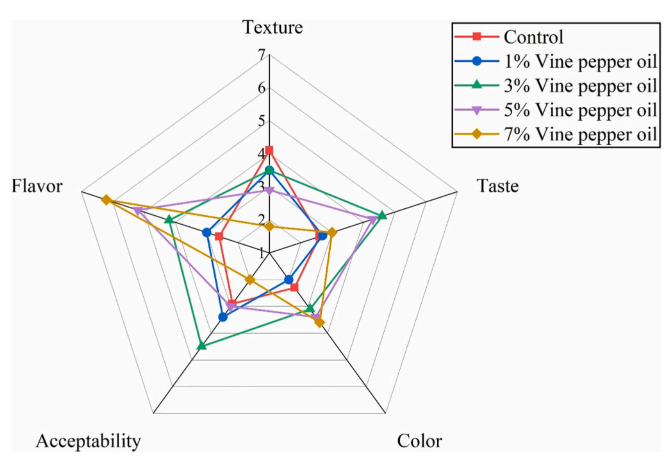

Fig. 1. Effect of VPO content on the sensory properties of rabbit meatballs.

characteristics including texture and WHC (Song et al., 2022). Color differences among samples were less pronounced compared to texture and flavor variations, primarily manifesting as brightness changes induced by VPO. Overall taste scores, determined by the balance between flavor and texture, reached optimal levels with moderate VPO concentrations. Insufficient VPO failed to adequately counteract undesirable odors, whereas excessive VPO introduction of polyphenolic compounds (e.g., phenolic acids, isoflavones, saponins) led to bitter/astringent tastes that reduced consumer acceptance (Zeeb et al., 2018). Overall, it appeared that 3 % VPO exhibited good sensory properties.

3.2. Texture properties, yield and WHC

Texture properties including hardness, springiness, cohesiveness, and chewiness serve as critical indicators for evaluating the chewing quality of meat products. As shown in Table 1, no significant differences ($P > 0.05$) were observed in textural parameters between control and 1 % VPO-treated meatballs. However, a concentration-dependent decline in textural characteristics became evident with increasing VPO levels ($P < 0.05$), consistent with sensory evaluation results (Fig. 1). Sensory panelists notably perceived textural degradation corresponding to elevated oil concentrations. Hardness reflects the force required for food deformation, while cohesiveness indicates the internal binding strength maintaining structural integrity. This phenomenon aligns with established relationships between meat paste gel texture and protein content in gel systems (Zhou, Chen, et al., 2019). Increased lipid incorporation reduces system protein content, disrupting protein network formation and compromising structural compactness (Han et al., 2021; Liang et al., 2020). Our findings demonstrate significant reductions in hardness and cohesiveness ($P < 0.05$) even at low VPO concentrations (3 %), potentially attributable to VPO's high UFA content (Liu et al., 2023; Rashed et al., 2021). Chen, Zhang, et al., 2022reported that UFA supplementation (e.g., linoleic acid C18:2) induces greater texture softening compared to saturated fatty acids like stearic acid. Notably, springiness and resilience - parameters reflecting gel's elastic recovery post-compression - showed no significant variation between control and 3 % VPO samples ($P > 0.05$). The 3 % VPO formulation exhibited softer yet more elastic characteristics, potentially contributing to improved sensory acceptance (Fig. 1).

The structural integrity of gel networks in minced meat systems critically determines product yield and WHC, as well-organized matrices effectively immobilize water and lipids to preserve meat juiciness. As shown in Fig. 2, VPO concentrations exceeding 3 % induced significant reductions in both yield and WHC ($P < 0.05$). This suggests that excessive lipid incorporation may either: (1) fail to function as effective fillers within the protein gel matrix for uniform network formation, or (2) exceed the system's emulsification capacity, resulting in compromised structural integrity and subsequent moisture migration (ZZhou, Yang, et al., 2019; He et al., 2022). Notably, the 3 % VPO formulation maintained comparable yield and WHC to controls ($P > 0.05$), likely due to preserved gel network uniformity and continuity.

3.3. SEM and BET pore volume

To better understand the mechanism of quality deterioration of meatball gel, the microstructure of the gel profile was analyzed. As shown in Fig. 3, meatball gels with low contents of VPO showed a network structure with fewer holes and smaller pore sizes, especially in the control group, where the gel profile was generally smooth and flat. However, as the VPO content increased from 3 % to 7 %, clear cavities and cracks became larger in the gel profile, and an increasing number of spherical particles were observed. The macropore formations could be attributed to VPO droplets coalescing throughout the gel matrix to form larger aggregates, ultimately disrupting the protein network during slow conductive heating (He et al., 2022). The increasing number of spherical particles was considered to result from the emulsification-driven combination of fat particles and protein molecules, and their formation was closely related to the cross-linking of lipids and proteins through hydrophobic or covalent interactions during heating (Han et al., 2021). Large oil droplets could also generate an interfering effect on protein-protein interactions in the gel matrix by increasing intermolecular distances between protein chains (as indicated by the larger voids), thereby affecting the cross-linking mode of MHC and subsequent MPs aggregation behavior (Fang et al., 2021). Meatball gels with 1 % and 3 % VPO exhibited fewer cavities and cracks, which depended on more appropriate cross-linking and interactions between protein-lipid or protein-protein molecules to form a denser and more uniform gel network. These observations align with the WHC and yield results shown in Fig. 2.

To comprehensively understand the effect of VPO on the network structure of meatball gel, the volume and distribution of internal pores in the gel profile were analyzed. As shown in Fig. 3, the pore size distribution was predominantly concentrated within 0–20 nm, and both the volume and number of pores in the control and low VPO doses (<3 %) were relatively low. Interestingly, the 1 % VPO group exhibited a lower number of internal pores than the control, which was contrary to the higher number of external pores observed by SEM (Fig. 3), which may be attributed to the low oil content acting as a filler within the gel matrix, thereby promoting internal molecular cross-linking (Han et al., 2021; Song et al., 2022). However, as the VPO content increased (particularly in the 5 % and 7 % VPO groups), the volume and number of internal pores significantly increased, with pore sizes appearing non-uniform. These observations align with the SEM microstructure of the gel profile (Fig. 3). As mentioned earlier, the elevated oil content correspondingly reduced the protein concentration in the minced meat system and hindered protein cross-linking, ultimately inhibiting the formation of an ordered internal gel network. Furthermore, components in vegetable oil may interfere with MPs cross-linking during gel network formation, leading to increased pore volume and quantity (Han et al., 2021; Liang et al., 2020) and decreased product texture and WHC.

3.4. Dynamic rheological properties of rabbit meatball incorporated with VPO

Changes in dynamic rheological properties during temperature ramp tests can be used to evaluate the cross-linking behavior of proteins during gel network formation. Fig. 4 illustrates the effects of VPO on the rheological properties of rabbit meatballs during heating from 25 °C to 80 °C. The storage modulus (G′) of all groups exhibited a distinct upward trend starting at 40 °C, reaching a prominent peak around 50 °C. This phenomenon indicates the initiation of myosin head cross-linking and the formation of a weak gel network (Wei et al., 2023). As temperatures continued to rise, G′ experienced a secondary increase and approached

Table 1
Textural properties of rabbit meatball with different incorporation levels of vine pepper oil.

Treatment	Hardness(g)	Springiness(%)	Cohesiveness (%)	Gumminess (g)	Chewiness (g)	Resilience (%)
Control	3079.13 ± 85.22^{b}	83.00 ± 1.85^{ab}	67.93 ± 1.02^{c}	2091.47 ± 30.01^{c}	1736.43 ± 62.73^{c}	29.57 ± 0.60^{bc}
1 %	3046.90 ± 347.45^{ab}	84.30 ± 0.61^{ab}	67.77 ± 0.85^{c}	2065.34 ± 249.25^{bc}	1742.60 ± 223.41^{bc}	29.87 ± 0.31^{c}
3 %	2409.78 ± 405.91^{a}	85.20 ± 0.44^{b}	65.13 ± 0.06^{b}	1569.62 ± 264.27^{ab}	1338.45 ± 232.45^{ab}	28.13 ± 0.81^{b}
5 %	2463.94 ± 192.48^{a}	83.70 ± 6.68^{ab}	62.17 ± 5.20^{abc}	1537.17 ± 235.26^{a}	1296.83 ± 307.13^{ab}	25.20 ± 3.31^{abc}
7 %	2410.87 ± 334.19^{a}	82.67 ± 1.42^{a}	62.47 ± 1.08^{a}	1507.18 ± 214.35^{a}	1246.37 ± 182.76^{a}	25.67 ± 1.01^{a}

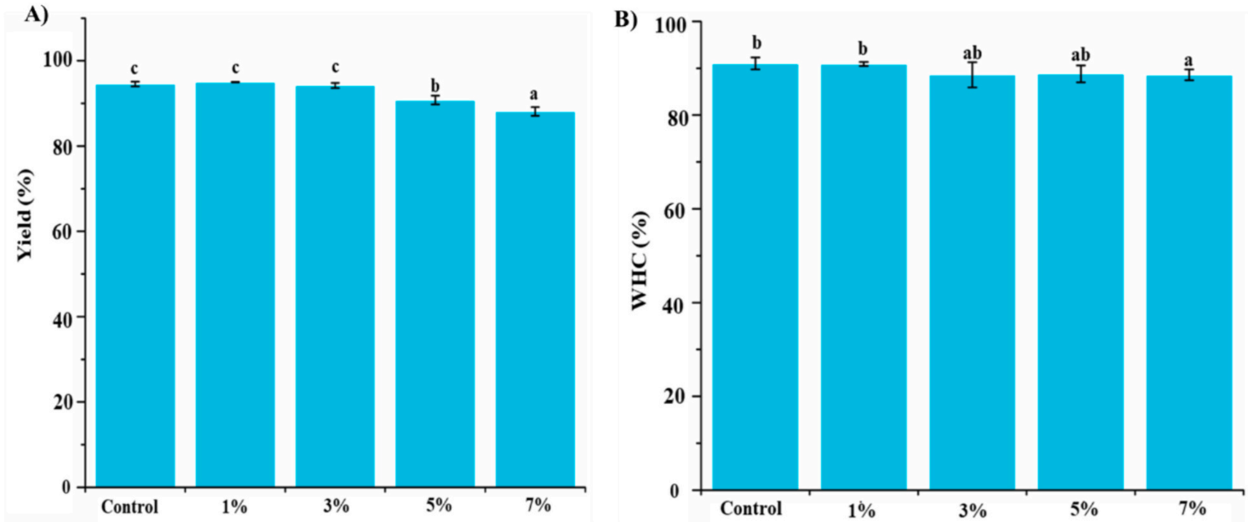

Fig. 2. Effect of VPO content on the yield (A) and WHC (B) of rabbit meatballs. Different letters (a-c) indicate significant differences ($P < 0.05$).

its maximum at approximately 70 °C before stabilizing. This "gel-strengthening phase" suggests complete disruption of MPs spatial structure, enabling protein unfolding and the formation of thermally irreversible gel networks through disulfide bonds (S—S) or hydrophobic interactions (Yuan et al., 2024). Ultimately, all samples showed moderate G′ reduction at higher temperatures, likely attributable to structural degradation caused by myosin tail denaturation (Kong et al., 2016).

Both G′ and loss modulus (G″) demonstrated consistent temperature-dependent trends across all VPO concentrations (Fig. 4A and B). Notably, VPO-modified meatball paste exhibited delayed G′ elevation around 60 °C compared to the control (Fig. 4A). However, no significant differences in G′ values were observed between experimental groups within the 40–60 °C range, suggesting that VPO addition minimally affects myosin head cross-linking while significantly influencing subsequent hydrophobic interactions and disulfide bond formation during MPs-mediated gelation. This observation aligns with previous findings that excessive oil content may create spatial hindrance through protein-oil hydrophobic interactions, thereby restricting protein structural rearrangement and unfolding (Han et al., 2021; Yan et al., 2020). From 60 °C to 80 °C, G′ values of 1–5 % VPO formulations remained consistently lower than the control group, showing an inverse correlation with VPO concentration. This reduction may stem from VPO-induced disaggregation of the actin-myosin network, which enhances protein mobility and increases intermolecular spacing (Li et al., 2024). Such structural modifications are further exacerbated by incomplete protein emulsification of oil droplets, leading to coalescence of lipid phases that physically separate matrix proteins and impede cross-linking (Yuan et al., 2024).

The loss modulus (G″) characterizes the viscous behavior of gel systems. As shown in Fig. 4B, the temperature-dependent viscosity trends mirrored those of the storage modulus. Consistent with the G' pattern, the 7 % VPO formulation exhibited the highest G" values between 70 and 80 °C, demonstrating that elevated VPO content significantly enhances system viscosity during thermal processing. This observation aligns with Chen, Lin, et al. (2022) findings where high soybean oil concentrations promoted larger emulsion microgel particles, consequently increasing flow resistance and apparent viscosity. The tangent delta (tanδ) ratio serves as an elasticity indicator, with lower values reflecting greater elastic dominance. Fig. 4C reveals distinct phase behavior patterns: compared to the control, 1–5 % VPO formulations showed significant tanδ reduction at 50–60 °C but marked elevation at 65–80 °C, while the 7 % VPO group maintained the highest values throughout. This dual-phase response suggests that low VPO concentrations promote myosin head cross-linking to enhance initial elasticity, yet subsequently inhibit high-temperature gel maturation - a phenomenon consistent with the earlier discussed G' behavior. Notably, although the 7 % VPO group displayed G' elevation at 70–80 °C (Fig. 4A), its sustained high tanδ values indicate impaired sol-gel transition, reflecting suppressed myosin cross-linking efficiency.

3.5. *Surface hydrophobicity of rabbit paste*

Hydrophobic interactions in rabbit meat paste play a crucial role in determining emulsion gel quality, with surface hydrophobicity serving as a key indicator of these interactions (Han et al., 2021). As shown in Fig. 5, surface hydrophobicity exhibited a statistically significant increase ($P < 0.05$) with rising oil content, reflecting enhanced protein-oil hydrophobic interactions. Notably, when VPO concentration exceeded 1 %, no further significant differences in surface hydrophobicity were observed ($P > 0.05$), suggesting protein emulsification capacity reached saturation. This saturation phenomenon aligns with Han et al.'s (2021) findings in chicken MPs gels supplemented with CO. The observed hydrophobicity enhancement in paste systems may originate from two mechanisms: (1) Oil-induced protein denaturation and structural unfolding, exposing buried hydrophobic residues (Han et al., 2021); (2) Thermodynamic instability arising from the positive free energy between hydrophobic oil phases and aqueous environments, which promotes protein-oil hydrophobic interactions (Han et al., 2021; McClements, 2011). However, excessive oil-water interfacial interactions may disrupt protein network formation. Alzagtat and Alli (2002) demonstrated that enhanced protein-oil hydrophobic interactions can reduce intramolecular hydrophobic associations, thereby altering protein aggregation patterns during thermal denaturation. This dual effect explains the non-linear relationship between oil content and gel quality parameters observed in the current study.

3.6. *Intermolecular forces between protein molecules*

The contribution of molecular interactions to gel formation was assessed through protein solubility measurements following selective disruption of intermolecular forces using chemical reagents. As shown in Fig. 6, hydrogen bond content within protein molecules remained statistically unchanged across treatments ($P > 0.05$). This observation aligns with previous reports identifying hydrogen bonds and hydrophobic interactions as primary stabilizing forces in MPs-based emulsion gels (Yao et al., 2025). Hydrogen bonding plays dual critical roles in maintaining water structure stabilization and preserving protein

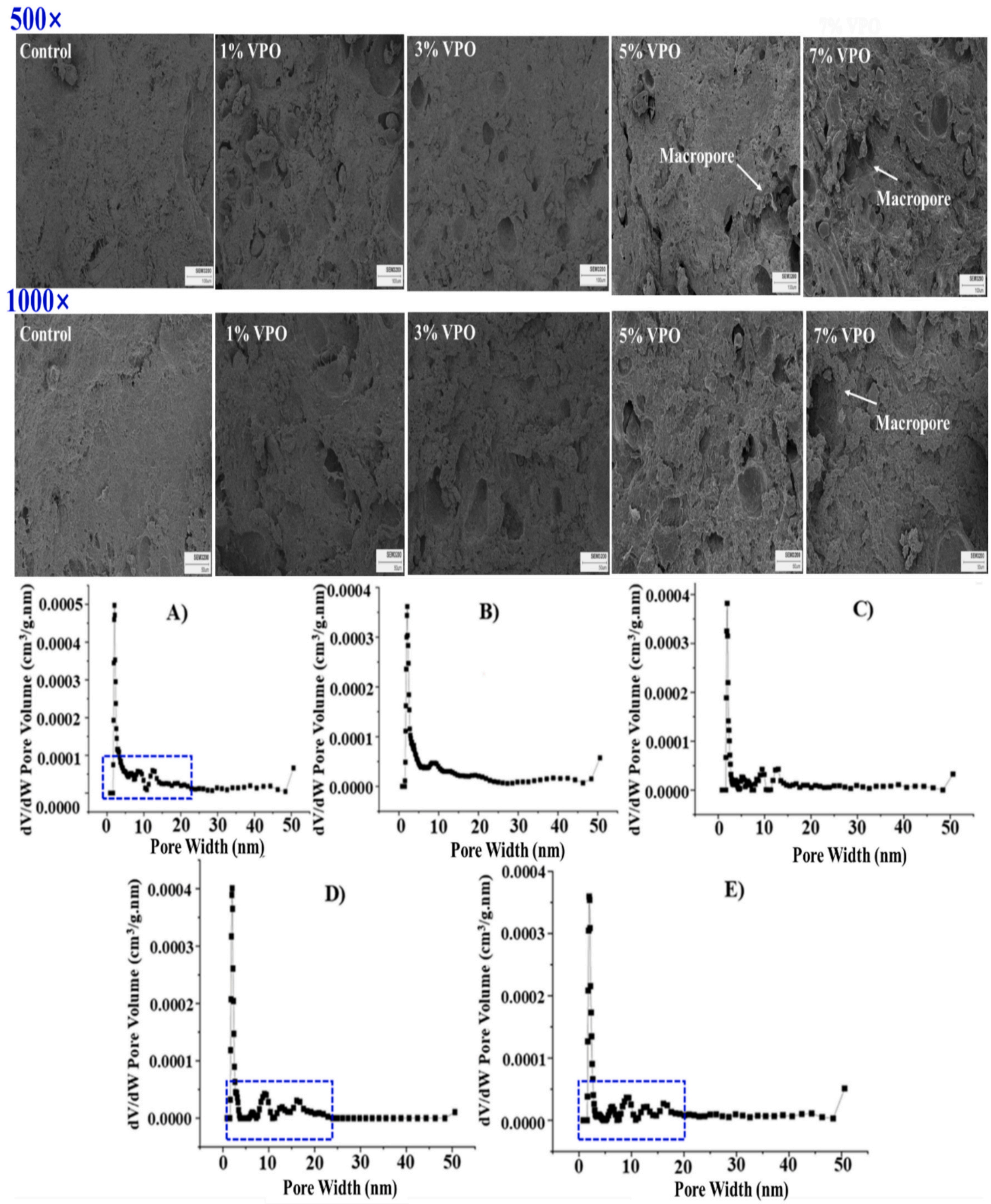

Fig. 3. The SEM images and pore volume of rabbit meatball gels with different incorporation levels of VPO.

secondary conformation within the gel matrix, both essential for WHC (Cao et al., 2018). While oil composition - particularly polar group content - can theoretically influence hydrogen bond formation (Yan et al., 2020), our data demonstrate VPO incorporation induced no significant hydrogen bond alteration ($P > 0.05$), potentially attributable to structural compensation through VPO's unique polar constituents. Contrastingly, hydrophobic interactions - another key stabilization mechanism - showed significant reduction in all VPO groups except 3 % addition ($P < 0.05$). This finding corroborates our earlier rheological analyses, suggesting elevated VPO content disrupts hydrophobic cross-linking, facilitates actin-myosin network disaggregation, and promotes macromolecular particle formation - collectively impairing gel matrix integrity.

Disulfide bonds and electrostatic interactions exhibited VPO concentration-dependent responses. While 3 % VPO showed unique behavior, other concentrations caused no significant changes in these forces ($P > 0.05$). This biphasic response aligns with existing models where moderate oil addition enhances charge-mediated cross-linking through amino acid exposure, whereas excessive lipid content sterically hinders such interactions (Yao et al., 2025; Zhou et al., 2017). Our results suggest optimal VPO incorporation (≤3 %) promotes three-dimensional network formation through balanced disulfide bonding, electrostatic attraction, and hydrophobic interactions. Although this formulation slightly reduced meatball firmness (Table 1), it significantly

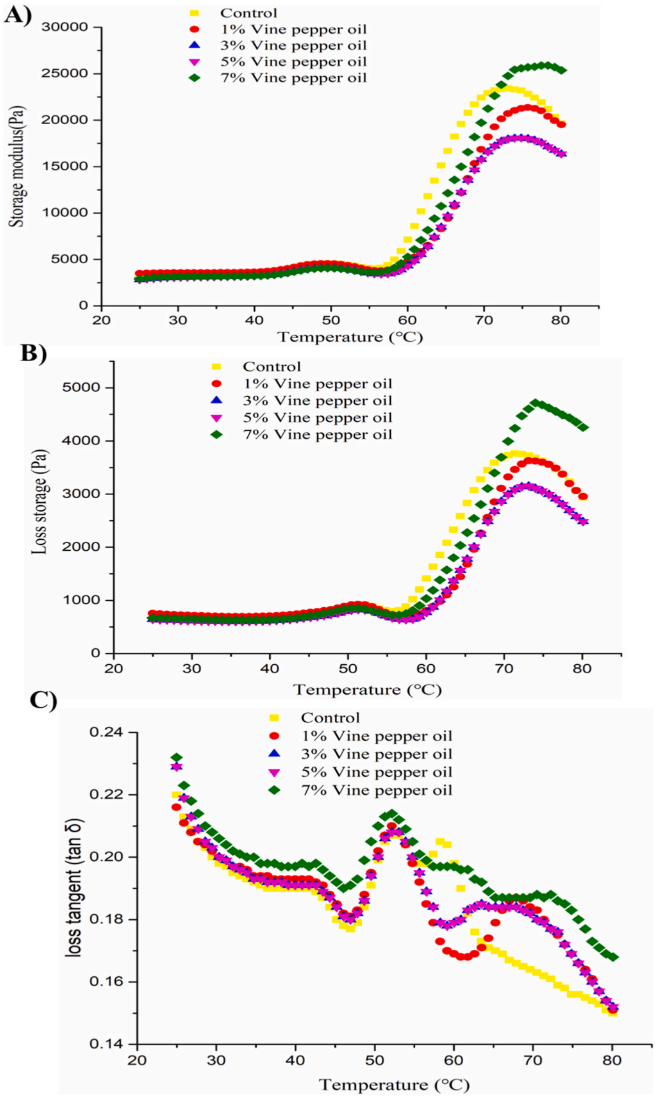

Fig. 4. Rheological behavior analysis (storage modulus (G′) (A), loss modulus (G″) (B), and loss tangent (tan δ) (C)) of rabbit meatballs gel with different incorporation levels of vine pepper oil under the heating treatment.

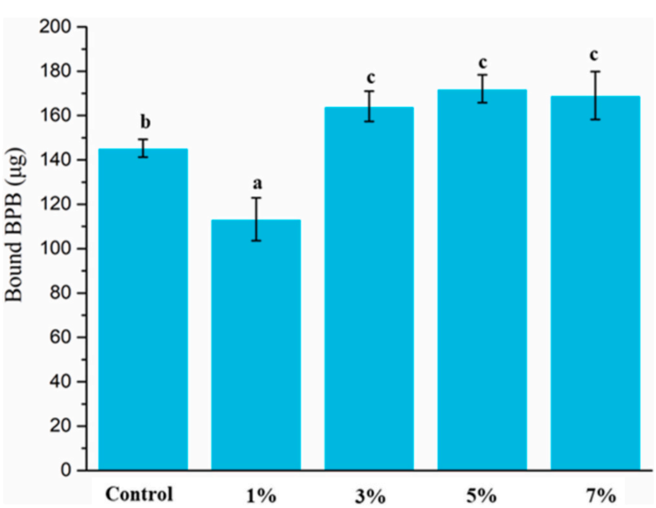

Fig. 5. Effect of VPO content on the surface hydrophobicity of rabbit meatballs gel. Different letters (a-c) indicate significant differences ($P < 0.05$).

improved sensory acceptance, juiciness, and product yield (Fig. 1 and 2), demonstrating the practical benefits of controlled molecular interaction modulation.

3.7. Protein profile analysis

To investigate the molecular mechanisms underlying VPO's effects on thermally induced protein gelation, SDS-PAGE analysis was conducted to characterize protein aggregation patterns. As shown in Fig. 7, under non-reducing conditions (β-ME absent), increasing VPO concentrations (particularly 5 % and 7 %) promoted the formation of high molecular weight (HMW) aggregates at the gel top. This demonstrates that elevated VPO levels induce covalent macromolecular aggregation, disrupting three-dimensional network formation - consistent with our rheological and microstructural observations (Fig. 3 and 4). The reducing condition (β-ME present) revealed distinct aggregation mechanisms: control and 1 % VPO groups showed complete dissolution of HMW species, confirming disulfide bonding as their primary aggregation pathway. However, residual aggregates persisted in higher VPO formulations (≥3 %), suggesting covalent cross-linking between meat proteins and VPO components. This irreversible aggregation correlates with the observed gel quality deterioration, likely caused by bioactive substances (e.g., unsaturated fatty acids [UFAs]) in VPO forming covalent adducts with MPs. Huang et al. (2022) reported similar phytochemical-mediated aggregation phenomena, where plant polyphenols formed covalent complexes with muscle proteins, impairing their functional properties. These findings highlight the dual role of VPO components: while moderate concentrations (≤3 %) facilitate desirable disulfide-mediated cross-linking, excessive VPO introduces competing covalent interactions that compromise gel integrity. Future studies should characterize the chemical nature of these protein-VPO adducts to optimize formulation strategies.

3.8. Principal component analysis of quality characteristics

To comprehensively assess the relationship between quality parameters and VPO's impact on rabbit meatball characteristics, principal component analysis (PCA) was performed and the distance among indicators or groups can to some extent reflect whether their correlation is strong (Yang et al., 2016). As demonstrated in Fig. 8A, hydrogen bonding and hydrophobic interactions showed strong positive correlations with key textural properties (sensory texture, resilience, cohesiveness), product yield, and WHC, while maintaining moderate associations with chewiness and hardness. Conversely, surface hydrophobicity, tan δ values at 80 °C, and sensory flavor intensity exhibited negative correlations with these quality indicators. This pattern suggests that VPO concentrations exceeding 3 % impair gel network formation by disrupting hydrophobic interactions between meat proteins, ultimately compromising product texture and functional properties. Notably, while VPO incorporation enhanced flavor profiles and surface hydrophobicity (Fig. 1 and 5), the observed textural deterioration in rabbit meat proteins likely stems from two mechanisms: (1) limited emulsification capacity at elevated VPO levels, and (2) formation of covalent macromolecular aggregates through interactions with VPO components (Fig. 7B). These structural modifications hinder the gel-strengthening phase (Fig. 4) and promote pore formation within the gel matrix (Fig. 3). This dual effect highlights the potential for developing VPO encapsulation strategies to optimize its distribution as active fillers, thereby improving textural and juiciness parameters without compromising flavor enhancement. The PCA biplot (Fig. 8B) clearly differentiates sample groups, identifying 3 % VPO as the critical threshold for quality deterioration. Beyond this concentration, significant reductions in texture and juiciness were observed. These findings establish a formulation optimization framework: maintaining VPO below 3 % preserves essential textural properties while achieving flavor improvement, whereas higher concentrations require advanced delivery systems

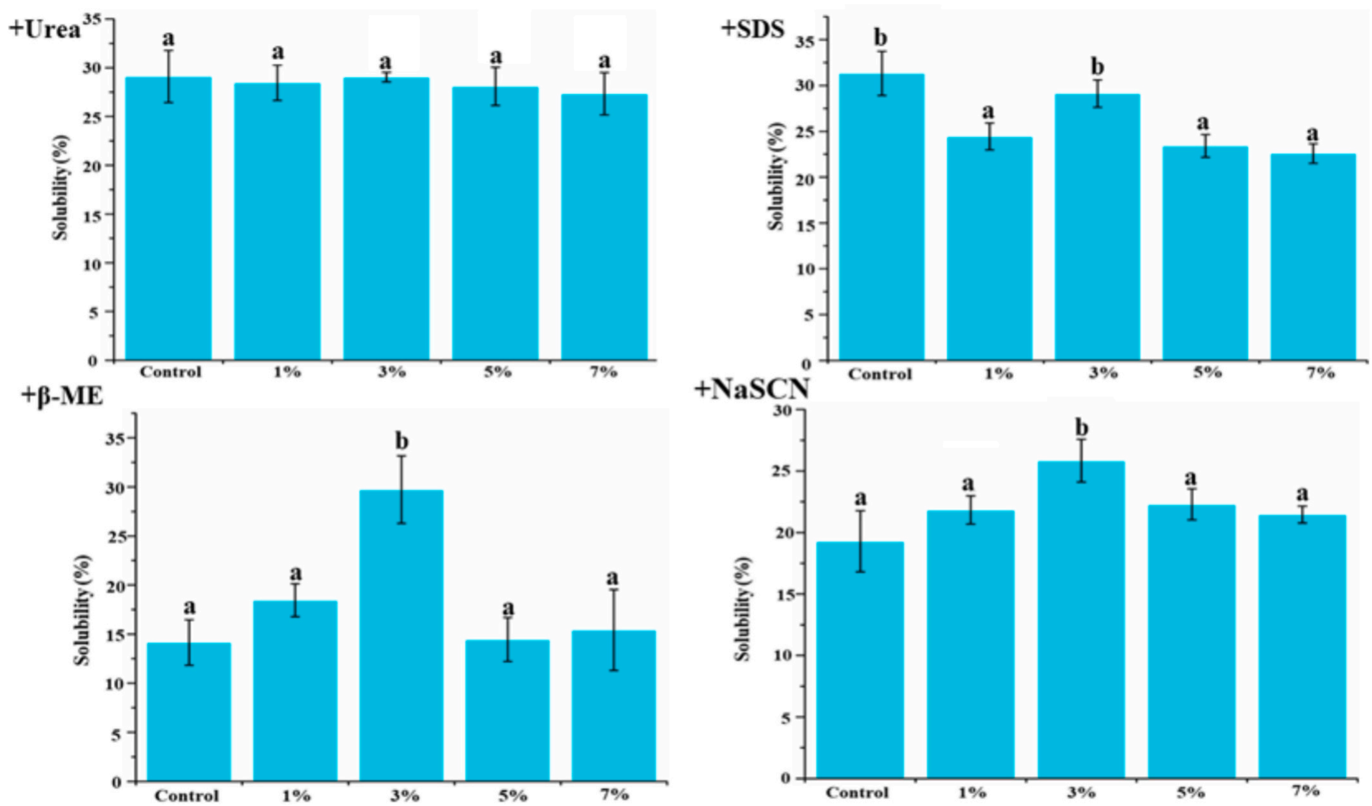

Fig. 6. Solubility (%) of rabbit meatballs gel with different incorporation levels VPO in different force-disruption solutions. (A) 8 mol·L^{-1} urea to disrupt the intermolecular hydrogen bonds. (B) 0.5 % sodium dodecyl sulfate (SDS) to disrupt the hydrophobic interactions. (C) 0.25 % β-mercaptoethanol (β-ME) to disrupt the disulfide bonds. (D) 0.5 mol·L^{-1} sodium thiocyanate (NaSCN) to disrupt electrostatic interactions. Different letters (a-b) indicate significant differences ($P < 0.05$).

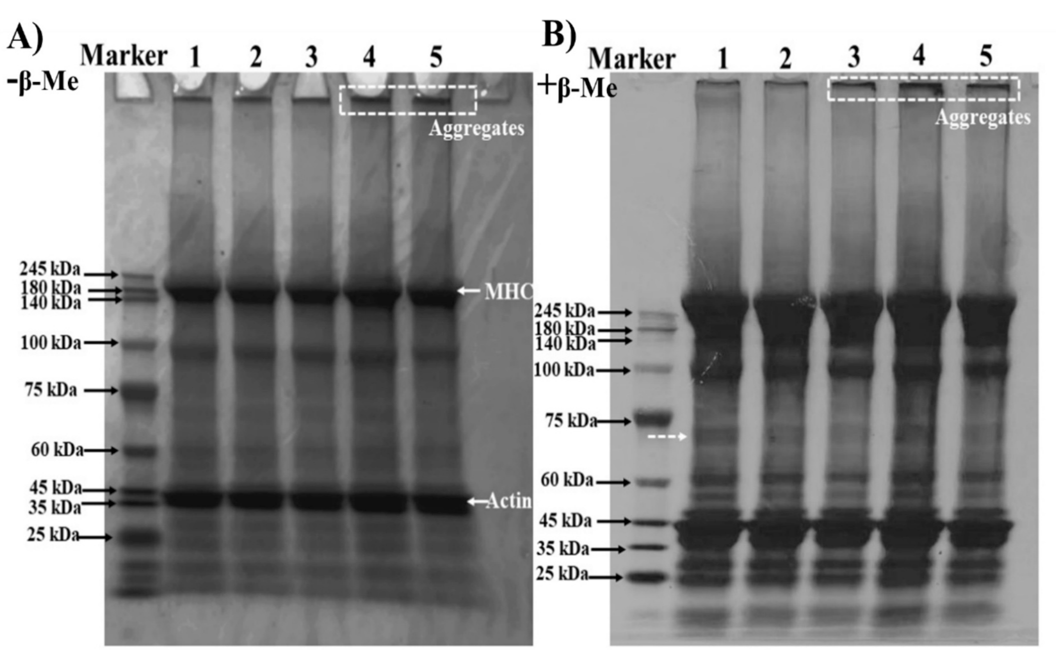

Fig. 7. SDS-PAGE patterns in non-reducing mode (A) and reducing mode (B) of rabbit-meatball proteins subjected to different VPO treatments.

to mitigate adverse effects on protein interactions.

4. Conclusion

The VPO could enhance the sensory characteristics of boiled rabbit meatballs. However, an excessive concentration would lead to deterioration of the product's texture and juiciness, especially when its concentration exceeded 3 % (*v*/*w*). The increase in VPO led to more cavities, cracks, and spherical particles in the gel profile. Additionally, the pore volume increased, and the pore volume distribution became uneven.

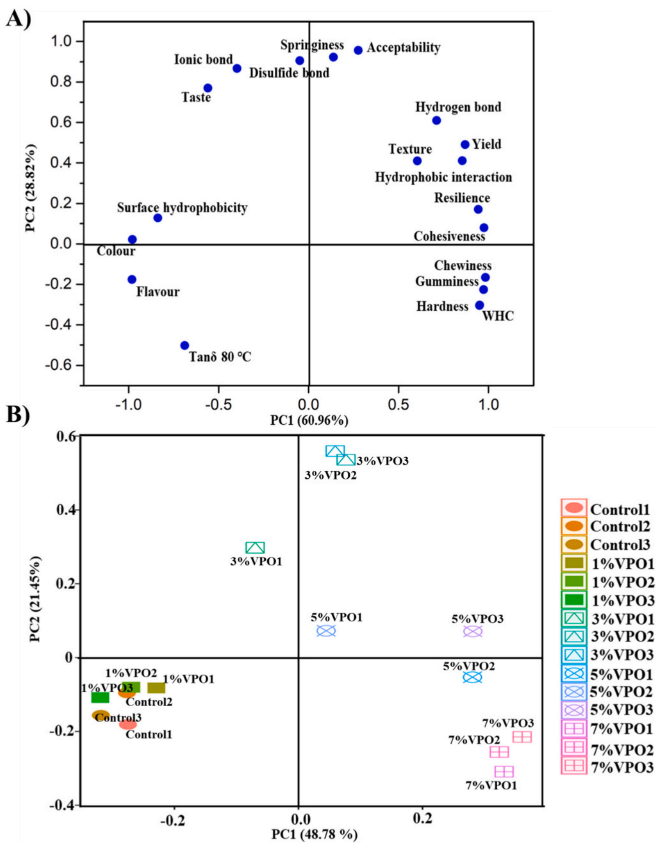

Fig. 8. Principal component analysis for indicators (A) and groups (B) of rabbit meat-ball.

The deterioration of gel quality caused by the addition of VPO was closely related to the weakened intermolecular forces, particularly hydrophobic interactions. Moreover, the components in VPO could form covalent aggregates with the proteins in rabbit meat. These two factors hindered the gel-strengthening phase during heating, thereby disrupting the gel network structure and resulting in the deterioration of the product's texture and juiciness. While VPO exerted a positive effect on the sensory flavor of boiled rabbit meatballs, future studies should focus on improving their texture and juiciness while ensuring better sensory flavor at higher VPO doses.

CRediT authorship contribution statement

Chang Su: Writing – original draft, Investigation, Data curation, Conceptualization. **Dong Zhang:** Supervision, Data curation, Conceptualization. **Yuxin Huang:** Software, Methodology, Investigation. **Jiaxin Chen:** Visualization, Validation, Formal analysis, Conceptualization. **Hongjun Li:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Yong Tang:** Writing – review & editing, Resources, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102420.

Data availability

Data will be made available on request.

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