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Pea protein–*Artemisia sphaerocephala Krasch* gum interpenetrating-network emulsion gel as a fat replacer in pork meatballs: Water retention, texture, oxidation, and freeze–thaw stability

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Abstract

Emulsion gels can replace pork backfat in comminuted meat products, but their performance depends on replacement level and processing process. This study evaluated a vitamin E-loaded pea protein isolate–*Artemisia sphaerocephala Krasch* gum (PPI–ASKG) interpenetrating-network emulsion gel in pork meatballs. Pork backfat was replaced by the emulsion gel at mass ratios of 0%, 25%, 50%, 75% and 100%, and quality attributes of the meatballs were measured before and after freeze–thaw cycles (FTCs). The water-holding capacity increased initially and then decreased, peaking at the 25% replacement level (E25). E25 also exhibited the lowest cooking loss (4.27% vs. 5.98% for the full-fat control) and thawing loss (3.2% vs. 6.5% for the full-fat control), while hardness, gumminess and chewiness remained comparable to those of the fresh-control. The 50% replacement level (E50) maintained low cooking and thawing losses, whereas higher replacement levels increased liquid losses and reduced hardness by up to 49.1%. After FTCs, the gel-containing formulations retained higher lightness and lower thiobarbituric acid reactive substances (TBARS) values than the control. A moderate replacement level (25–50%) mitigated freeze–thaw damage to the myofibrillar protein network and retained immobilized water, thereby stabilizing the WHC of meatballs. Electronic-nose analysis further revealed that sulfur-containing volatile responses increased progressively with the replacement level, reflecting the beany off-flavor of pea protein. These findings provide a practical approach for reducing the saturated fat content of meatballs while improving water retention and freeze–thaw stability without compromising texture.

Introduction

Animal fat provides the tenderness, juiciness and mouthfeel of comminuted meat products, but its saturated fatty acids are associated with cardiovascular disease. Meatballs are among the most widely consumed comminuted products worldwide and are commonly distributed frozen, which makes both their fat content and their freeze–thaw behavior commercially relevant. Replacing animal fat while preserving the technological and sensory functions of the native fat phase is therefore an active goal in meat processing [1] .

Substituting animal fat with liquid vegetable oils improves the fatty acid profile but softens texture and accelerates oxidation, so structured alternatives are needed. Emulsion gels address this by immobilizing the oil within a viscoelastic protein, polysaccharide or composite matrix, thereby mimicking the solid-like behavior of native fat while keeping the oil in a healthier liquid form [2]. In comminuted products such as sausages and meatballs, their effect on water retention, texture and oxidation depends on both gel formulation and replacement level [3, 4].

Repeated freeze–thaw exposure presents a stringent test because ice formation and recrystallization can redistribute water, disrupt muscle structure and promote protein and lipid oxidation [5, 6]. These changes are associated with increased thawing or cooking losses and altered textural properties in meat products. Protein- and polysaccharide-based ingredients can moderate some of these changes, but their

effects depend on material identity, concentration and interactions with the meat matrix [7, 8]. Freeze-thaw performance should consequently be evaluated as a coupled response of the dispersed lipid phase, interfacial layer, hydrocolloid matrix and myofibrillar protein network [9, 10].

Protein-polysaccharide-based emulsion gels have shown that matrix formation and interfacial organization can be associated with rheology and freeze–thaw stability in model systems [11]. At the application level, plant-protein emulsions and protein–polysaccharide emulsion gels have been used as fat replacers in minced meat products such as meatballs, patties and sausages. For example, Zhang et al. [8] reported that an egg white protein–sodium alginate double-network emulsion gel improved the freeze–thaw stability of minced meat gel, and Zou et al. [12] found that a cassia bean gum/soy protein isolate composite emulsion gel kept the quality of meat sausage comparable to that of the full-fat control at 50% fat replacement. Despite this progress, protein–polysaccharide interpenetrating-network emulsion gels have rarely been examined as fat replacers in meat products, and the optimal replacement level for such gels remains undefined. We previously prepared a PPI–ASKG interpenetrating-network gel with good freeze–thaw stability [13, 14], but its performance as a fat replacer in a real comminuted product has not been evaluated.

Here, we examined the formulation response of pork meatballs containing a vitamin E-loaded PPI–ASKG emulsion gel [14]. Five formulations replaced 0%, 25%, 50%, 75% or 100% of pork backfat mass with the emulsion gel. Water-holding capacity, cooking loss, thawing loss, color, low-field nuclear magnetic resonance (LF-NMR), magnetic resonance imaging (MRI), texture profile, lipid oxidation and electronic-nose responses were assessed before and after FTCs. This work is expected to provide a practical basis for reduced-fat meatballs with desirable quality attributes.

Materials and methods

Materials

Pork tenderloin, pork backfat and olive oil were purchased from a local supermarket. Pea protein isolate (PPI; 85 wt% protein) was obtained from Shandong Huatai Food Co., Ltd. (Shandong, China). Vitamin E (VE) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Thiobarbituric acid (TBA) and butylated hydroxytoluene (BHT) were purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). *Artemisia sphaerocephala Krasch* gum (ASKG; molecular weight 142 kDa) was supplied by Zhejiang Yiming Bio-Technology Co., Ltd. (Zhejiang, China), glucono-δ-lactone (GDL) was obtained from Shandong Keyuan Biochemical Co., Ltd. (Shandong, China). All other reagents were of analytical grade.

Preparation of VE-loaded emulsion gels

VE powder (0.15 wt%) was dispersed in olive oil (16 g) and sonicated for 30 min in the dark. PPI (20 wt%) and ASKG (1.5 wt%) stock solutions were prepared separately in phosphate-buffered saline (10 mM, pH

7.0). PPI solution (7.5 g) and VE-loaded olive oil (3.0 g) were homogenized at 18,000 rpm for 2 min. ASKG stock solution (3.0 g) and buffer (1.5 g) were added to the resulting emulsion (10.5 g), stirred at 500 rpm for 15 min, re-homogenized at 18,000 rpm for 2 min, and sonicated in an ice-water bath at 240 W for 2 min using 3 s on/3 s off pulses. After adjustment to pH 7.0, GDL (2 wt%) was added and the mixture was heated at 85°C for 1 h. The final PPI and ASKG concentrations were 10 wt% and 0.3 wt%, respectively [14].

Preparation of pork meatballs

Pork tenderloin and pork backfat were minced separately for 1 min. The remaining ingredients were added according to Table 1, and the mixture was minced for a further 1 min. Batter temperature was controlled during mincing to limit heat accumulation. The batter was transferred to spherical molds (2.5 cm diameter) and cooked in boiling water at 100°C for 15 min. Cooked meatballs were stored at 4°C for further investigated. Pork backfat was replaced by the emulsion gel at levels of 0%, 25%, 50%, 75% and 100%, corresponding to formulations designated E0, E25, E50, E75 and E100, respectively.

Table 1
Formulation of pork meatballs (g/100g)

Ingredient	E0	E25	E50	E75	E100
Pork tenderloin	60	60	60	60	60
Pork backfat	30	22.5	15	7.5	0
Emulsion gel	0	7.5	15	22.5	30
Ice water	4.8	4.8	4.8	4.8	4.8
Salt	2	2	2	2	2
Sugar	1	1	1	1	1
Starch	1.5	1.5	1.5	1.5	1.5
Sodium tripolyphosphate	0.2	0.2	0.2	0.2	0.2
Chicken essence	0.3	0.3	0.3	0.3	0.3
White pepper powder	0.1	0.1	0.1	0.1	0.1
Ginger powder	0.1	0.1	0.1	0.1	0.1

Freeze-thaw treatment

Meatballs were subjected to five freeze–thaw cycles (5FTC). Each cycle comprised freezing at − 20°C for 22 h followed by thawing in a 30°C water bath for 2 h. Measurements were conducted on fresh samples and on samples after the fifth cycle.

Water-holding capacity (WHC) and cooking properties

Water-holding capacity (WHC) was measured using 5 g fresh samples centrifuged at 8,000 × g for 30 min [11]. The tubes were inverted to drain released liquid, and surface moisture was removed with filter paper. WHC was calculated as:

$$WHC\,(\%) = \frac{W_0 - W_1}{W_0} \times\ 100\%$$

1

where W_0 and W_1 are the sample masses before and after centrifugation, respectively.

Cooking loss was determined using the method adapted from previous study [15]. Fresh samples portions (5 g) were placed in 10 mL glass beakers and heated at 100°C for 15 min. Surface liquid was removed before the samples were reweighed. Cooking loss was calculated as the following equation:

$$Cooking\ loss\,(\%) = \frac{W_0 - W_1}{W_0} \times\ 100\%$$

2

where W_0 and W_1 are the sample masses before and after heating, respectively.

Thawing loss and reheating loss

Thawing loss was calculated from the sample mass before (W_0) and after (W_1) thawing. For reheating loss, thawed meatballs were heated in a 75°C water bath for 25 min and weighed before (W_2) and after reheating (W_3). The properties were calculated as the following equations [16]:

$$Thawing\ loss\,(\%) = \frac{W_0 - W_1}{W_0} \times\ 100\%$$

3

$$Reheating\ loss\,(\%) = \frac{W_2 - W_3}{W_2} \times\ 100\%$$

4

Appearance and color measurement

Surface and cross-sectional images were acquired using a Canon EOS 6D camera. Instrumental color was measured with a CR-400 chroma meter after calibration against a white standard plate and was

reported as L* (lightness), a* (redness/greenness), and b* (yellowness/blueness). Three readings were collected at different positions on each sample and averaged for analysis [17].

Low-field nuclear magnetic resonance (LF-NMR) measurements

To perform LF-NMR measurements, 5 g of each sample was placed into glass tubes with a cylindrical shape (internal diameter of 16.5 mm) and subsequently analyzed using a nuclear magnetic resonance instrument (NMI20–040 V-I, Niumag Electric Corporation, Shanghai, China). The following acquisition parameters were employed: a frequency of 200 kHz, an interscan delay equal to 3000 ms, 8 signal accumulations, and 15,000 echoes. Distributions of transverse relaxation time (T_2) were obtained by applying an inverse Laplace transformation with the software provided by the instrument.

For magnetic resonance imaging (MRI) analysis, images with proton density weighting were collected using the following settings: a slice thickness of 3 mm, repetition time set to 1500 ms, echo time set to 20 ms, and 200 signal averages. The 20 ms value corresponds to the effective echo time.

Texture properties

Texture profile analysis (TPA) was conducted with a TA.XT.Plus texture analyzer (Stable Micro Systems, UK) and a cylindrical P/0.5 probe. The reported settings were a pre-test speed of 2.0 mm/s, a post-test speed of 1.0 mm/s, a compression distance of 10 mm, and a trigger force of 5.0 g. Hardness, springiness, cohesiveness, gumminess and chewiness were calculated using the instrument software [18].

Thiobarbituric acid reactive substances (TBARS)

Solution A was prepared by combining TBA (1.88 g), distilled water (414 g), 12 M hydrochloric acid (8.8 mL) and Trichloroacetic Acid (TCA) (75 g). Solution B comprised BHT (0.5 g) in absolute ethanol (25 mL; 2% w/v). Solutions A and B were mixed at 100:3 (v/v), stirred for 2 h, and sonicated for 10 min. Meatball sample (1 g) was mixed with 2 mL reagent, vortexed for 5 s, heated at 75°C for 1 h, cooled for 10 min, and centrifuged at 3,800 rpm for 15 min. Supernatant absorbance was measured at 532 and 580 nm. TBARS was calculated as the following equation [19]:

$$TBARS(mg/kg) = \frac{A_{532} - A_{580}}{W} \times 9.48$$

5

where A_{532} and A_{580} are the absorbances at 532 and 580 nm, respectively, and W is the sample mass (g).

Electronic nose analysis

Electronic-nose measurements were performed with a PEN 3.5 system (Airsense Analytics, Germany) using a procedure adapted from Deng et al. [20]. Minced sample (2.0 g) was sealed in a 50 mL

headspace vial and equilibrated at room temperature for 60 min. Acquisition and cleaning times were 60 and 180 s, with sample and cleaning gas flow rates of 300 and 600 mL/min, respectively. Responses from 56–58 s were retained. Fifteen 10-dimensional response datasets were reported. The nominal sensitivity classes of the ten sensors are listed in Table 2.

Table 2
The performance of the electronic nose sensors

Sensor	Performance
W1C	Sensitive to aromatic compounds
W5S	Broad range response, sensitive to nitrogen oxides
W3C	Sensitive to aromatic compounds
W6S	Sensitive to hydrogen
W5C	Sensitive to alkenes and aromatic compounds
W1S	Sensitive to methane
W1W	Sensitive to organic sulfides
W2S	Sensitive to alcohols
W2W	Sensitive to aromatic compounds, sulfur- and chlorine-containing organic compounds
W3S	Sensitive to alkanes

Statistical analysis

Experiments were reported as having been performed at least in triplicate, and values are presented as mean ± standard deviation. The reported analysis used one-way analysis of variance in SPSS v27.0.1, with $p < 0.05$ as the significance threshold. Origin 2021 was used for plotting.

Results and discussion

Appearance and color

The appearance of meatballs before and after 5FTC is shown in Fig. 1, and the corresponding color parameters (L*, a*, b*) are summarized in Table 3. Before 5FTC, the E0 meatball (full-fat control) exhibited a relatively rough surface, a porous cross-section, and a relatively dark appearance (Fig. 1A). Pork color can be influenced by myoglobin chemistry, lipid oxidation, water distribution, light scattering and formulation composition [21]. With the incorporation of emulsion gel, the meatball surface became visibly smoother and the internal structure more compact (E25–E50). When the substitution ratio was further increased to E75 and E100, the cross-section again became looser and more porous, indicating that excessive replacement of pork backfat disrupted the integrity of the meat protein gel network [17]. In addition, as the emulsion gel content increased, the color of the meatballs gradually became

lighter/brighter. After 5FTC, the E0 meatball showed pronounced surface and cross-sectional browning (Fig. 1B), whereas the meatballs containing the emulsion gel largely retained their original color and structural appearance. The protective effect was particularly evident in the E25–E100 groups, possibly indicating that the PPI–ASKG interpenetrating network emulsion gel effectively scavenges the free radicals generated during frozen storage [22].

The quantitative color data (Table 3) corroborated these visual observations. Before 5FTC, the L* value increased significantly from E0 (76.92) to E25 (78.35) and then plateaued for E50–E100 (78.35–78.49). This indicates that incorporating the lipid phase into meat batter in a finely dispersed and stable form leads to small, uniformly distributed fat particles in the cooked product. These particles enhanced visible light scattering from both cut and exterior surfaces [23]. Consequently, L* increases and overall visual brightness is improved. Wang et al. [24] also reported that emulsion droplets have smaller particles than fat, which enlarges the relative surface area, enhancing light reflection and thus improving the L* of meatballs. The a* value increased with the substitution ratio, indicating a more reddish appearance. This is consistent with the suppression of myoglobin oxidation to metmyoglobin in the emulsion gel-containing formulations, given that a* is closely associated with the redox state of myoglobin [21]. The b* value decreased from E0 (17.42) to E100 (15.17, $p < 0.05$), which is consistent with the reduced extent of both lipid oxidation and the browning reaction in the lower-fat emulsion gel systems [25].

After 5FTC, the L* value of the E0 group decreased sharply, whereas those of the emulsion gel-containing samples remained relatively stable. This trend is consistent with a previous report, showing that the L* value of pork meatballs decreased after 30-days of freezing storage [16]. The higher L* values observed in E25–E100 compared with E0 after 5FTC confirm that the emulsion gel inhibited pigment oxidation during frozen storage [21, 25]. All samples showed a decline in a* values after 5FTC, relative to their fresh counterparts. This loss of redness may be attributed to cold-induced denaturation of myoglobin, the primary meat pigment, as reported previously [26]. The b* values were also markedly influenced by the emulsion gel content, increasing in all samples after FTC. These results agree with previous study, who attributed the increased yellowness to myoglobin denaturation, metmyoglobin accumulation and lipid oxidation [16]. Collectively, these findings indicate that the incorporation of emulsion gel can help mitigate quality deterioration after FTC.

Table 3
Color parameters (L*, a*, b*) of meatballs with various fat replacers

	Before 5FTC			After 5FTC		
	L*	**a***	**b***	**L***	**a***	**b***
E0	76.92 ± 0.27^{b}	0.75 ± 0.13^{c}	17.42 ± 0.12^{a}	66.71 ± 0.69^{b}	0.33 ± 0.02^{b}	18.27 ± 0.08^{a}
E25	78.35 ± 0.52^{a}	0.93 ± 0.29^{bc}	15.98 ± 0.60^{b}	78.17 ± 0.46^{a}	0.45 ± 0.15^{a}	16.03 ± 0.07^{b}
E50	78.35 ± 0.11^{a}	0.94 ± 0.11^{bc}	15.83 ± 0.15^{bc}	78.26 ± 0.21^{a}	0.44 ± 0.04^{a}	16.11 ± 0.08^{b}
E75	78.46 ± 0.38^{a}	1.22 ± 0.14^{ab}	15.79 ± 0.22^{bc}	78.38 ± 0.32^{a}	0.43 ± 0.05^{a}	16.09 ± 0.09^{b}
E100	78.49 ± 0.98^{a}	1.38 ± 0.17^{a}	15.17 ± 0.50^{c}	78.48 ± 0.22^{a}	0.44 ± 0.04^{a}	15.44 ± 0.51^{c}

Values are means ± SD (n = 3 independent replicates). Different superscript lowercase letters within the same column indicate significant differences at $p < 0.05$.

Water-holding capacity (WHC) and cooking properties

The WHC and cooking loss of pork meatballs are shown in Fig. 2. WHC is a key quality attribute of comminuted meat products, because it directly governs the perceived juiciness and visual appearance of the final product. As the substitution ratio increased from 0% to 100%, the WHC first increased and then decreased (Fig. 2A), with the highest value observed in E25 and the lowest in E100 ($p < 0.05$). This result suggested that a certain proportion of fat replacement by emulsion can improve WHC, which is consistent with the results reported by previous study [27]. The initial increase for E25 may be attributed to the fact that olive oil is rich in unsaturated fatty acids (predominantly oleic acid), which can interact with pork myofibrillar proteins during chopping to form a viscoelastic interfacial film around the dispersed oil droplets. This interfacial structure promotes a more homogeneous distribution of oil droplets within the meat batter and contributes to the formation of a denser and more cohesive gel network, thereby reducing centrifugal water loss [10]. When the substitution ratio exceeded 50%, however, the WHC of E75 and E100 decreased significantly and the two groups were not significantly different from each other (Fig. 2A), suggesting that excessive replacement of pork backfat by the emulsion gel compromises the integrity of the protein gel network. At these high substitution levels, the proportion of meat-derived structural components is reduced, and the resulting looser microstructure provides less resistance to centrifugal force, leading to greater expressible juice and lower WHC. This observation is consistent with previous studies, reporting that partial fat replacement by emulsion gels alters the properties and water distribution of comminuted meat systems in a substitution-ratio-dependent manner [28].

Cooking loss reflects the ability of meat products to retain moisture during thermal processing and is largely governed by heat-induced changes in the connective tissue network and the conformation of myofibrillar proteins [29]. Cooking loss followed a U-shaped pattern (Fig. 2B). The values for E0, E25, E50, E75 and E100 were 5.98%, 4.27%, 5.60%, 7.88% and 8.37%, respectively. E25 had the lowest cooking loss and was significantly lower than E0 ($p < 0.05$), corresponding to a 28.6% reduction. These results suggest that replacing 25–50% of pork backfat with the PPI–ASKG interpenetrating-network emulsion gel can compensate for its structural and water-binding ability, with the gel network providing further water retention during heating. Previous studies have shown that protein- or polysaccharide-based fat replacers can effectively preserve, or even improve, the moisture retention and gel network of reduced-fat meat products [4, 30]. In contrast, cooking loss increased markedly at higher substitution levels, with E100 showing the highest value (8.37%), approximately 40% higher than that of E0 (Fig. 2B). This is in agreement with the WHC results and indicates that excessive replacement of pork backfat by the emulsion gel leads to a loose and less compact meat batter structure, which leads to greater release of water and soluble matter during cooking [12]. Jiang et al. [25] similarly reported that excessive incorporation of emulsion-type fat replacers in beefballs increased juice loss and cooking loss, attributed to the weakened lubricating and binding action of animal fat toward meat particulate fractions upon high-level substitution.

Thawing loss and reheating loss

Both the thawing loss and reheating loss (Fig. 3) exhibited a clear U-shaped dependence on the fat substitution ratio. It decreased from 6.5% in E0 to 3.2% in E25 and 3.5% in E50, then increased to 8.5% in E75 and 11.2% in E100. The lower thawing losses of E25 and E50 indicate that, at these moderate substitution levels, the PPI–ASKG interpenetrating network of the emulsion gel effectively stabilizes the oil droplets against ice-crystal-induced coalescence and integrates well with the meat protein matrix, thereby preserving the integrity of the meat batter network during freeze-thaw process [8]. Furthermore, the higher water-holding ability of E25 and E50 likely enhanced water entrapment within the myofibrillar protein network, leading to lower cooking loss [31]. In contrast, E100 exhibited highest thawing loss values. This can be attributed to the fact that the dispersed oil droplets were highly susceptible to ice-crystal damage. During freeze-thaw treatment, ice crystals grew within and around the oil droplets and penetrated the oil–water interfacial layer, leading to coalescence and flocculation of the dispersed phase upon thawing [32]. This destabilization of the emulsion released bound water and increased drip loss [33]. Consequently, the combined effects of a weakened meat matrix and emulsion destabilization resulted in the highest thawing loss in E100.

Reheating loss reflects the amount of juice and soluble matter released from the meat matrix during thermal treatment, with water being the major component of such loss [10]. The PPI–ASKG complex can adsorb at the oil–water interface and form elastic, high-density fat droplets with a continuous interfacial layer. During reheating, this elastic interfacial structure restricts the expansion and coalescence of oil droplets and retains the water originally bound within the gel network [10], which accounts for the significantly lower reheating losses of E25 and E50 compared with E0. When the substitution ratio

exceeded 50%, the system contained a higher fraction of free water, which expands upon reheating and induces structural collapse upon cooling to ambient temperature, thereby weakening the integrity of the meat batter network. Consequently, the E75 and E100 groups could not retain the juice released during reheating, leading to the highest reheating losses in these groups [34]. Within each substitution level, cooked meatballs consistently exhibited higher reheating loss than their raw counterparts (Fig. 3B). This is consistent with partial denaturation and shrinkage of the myofibrillar protein gel during pre-cooking. These changes further reduce the WHC upon subsequent reheating [29].

LF-NMR and MRI analysis

The LF-NMR distributions were presented as three assigned water populations, T_{21}, T_{22}, and T_{23} interpreted as bound, immobilized and free water, respectively (Fig. 4). According to low-field NMR transverse relaxation (T_2) analysis, water in all meatball samples existed mainly as immobilized water, with bound and free water representing only minor fractions. For samples before cycling, incorporation of the emulsion gel markedly altered the water distribution of meatballs: the T_{22} peak shifted to shorter relaxation times, and its peak area increased as the substitution ratio increased from E0 to E50 (Fig. 4A). These results further indicate reduced water mobility and an increased proportion of immobilized water. The PPI–ASKG interpenetrating-network emulsion gel formed a denser and more cross-linked network, which restricted the movement of water molecules and converted part of the free water into immobilized water [7, 35]. When the substitution ratio was further increased to E75 and E100, however, the T_{22} peak shifted toward longer relaxation times and its peak area decreased (Fig. 4A), indicating increased water mobility and a lower proportion of immobilized water. This phenomenon can be attributed to the inability to sustain the stability of immobilized water and fat molecules under excessive fat-substitution levels, resulting in the diffusion of water into the meat-gel network [34]. These observations are consistent with the WHC results in Section 3.2.

After 5FTC, all samples showed a increase in the relative area of the T_{23} peak (Fig. 4B), indicating a shift of water from the immobilized to the free population. This is consistent with the mechanical damage to the protein gel network caused by the growth and recrystallization of ice crystals during frozen storage, which expelled immobilized water from the gel matrix and released it as free water [6, 36, 37]. E25 and E50 showed only a small increase in the T_{23} peak area and largely retained the T_{22} profile, whereas E0 exhibited a shift from T_{22} to T_{23} (Fig. 4B). A moderate level of emulsion gel (25–50%) therefore suppressed the freeze-thaw-induced damage to the myofibrillar protein gel network and preserved the immobilized water population, thereby maintaining the WHC of the meatball [5, 36]. The E100 group was the most susceptible to freeze-thaw-induced water redistribution, consistent with its higher thawing loss and reheating loss.

MRI was used to visualize the spatial distribution of water in the meatball, and the pseudo-color proton density images of meatballs with different substitution ratios are shown in Fig. 5. In the proton density-weighted images, the bright (red/yellow) regions indicate areas with high water content, whereas the

dark (blue/green) regions indicate areas with low water content [13]. For fresh samples, the red/yellow signal first increased and then gradually decreased with the substitution ratio from E0 to E100, indicating that the retained water content followed the same trend. The gel network structure strongly influences water distribution, because a denser and more ordered structure retains water more effectively [18]. The chemical composition, muscle structure and water content also affect it. After 5FTC, E0 showed almost no red/yellow signal, indicating severe water loss. The red/yellow signal first increased and then gradually decreased with the substitution ratio, in agreement with the T_{22} results. Meatballs with a moderate emulsion gel content formed a denser gel network, which improved their freeze-thaw stability and water-binding capacity.

Texture properties

The texture profile analysis (TPA) parameters of pork meatballs before and after 5FTC are summarized in Table 4. Before 5FTC, the hardness, cohesiveness, gumminess and chewiness decreased as replacement level increased. The textural attributes declined significantly, likely because the added emulsion gel partially damaged the protein network [38]. Hardness decreased by 49.1% from E0 (2582 g) to E100 (1313 g), whereas E25 did not differ from E0 for the reported fresh-sample TPA parameters. This finding is consistent with Zhou et al. [39], who reported that replacing up to 50% of chicken fat with a soy protein fibril–red palm oil Pickering emulsion did not significantly alter the hardness or chewiness of meat emulsion gels. The softer texture caused by the emulsion gel (E50-E100) is probably related to the physical state of the fat phase. Pork backfat is solid at room temperature and its solid-fat crystals reinforce the meat batter network, whereas vegetable oils remain liquid and provide less structural support [3]. Kerdlapee et al. [15] also reported that replacing animal fat with a soy protein-based emulsion reduced the texture parameters of meat emulsion gels.

After 5FTC, the hardness, gumminess and chewiness of all samples decreased. Springiness remained largely unchanged, whereas cohesiveness increased. The general decrease in hardness, gumminess and chewiness can be attributed to the damage of the myofibrillar protein gel network by ice crystals. During frozen storage, ice crystals form and recrystallize, mechanically disrupting the protein network and lowering the force required for the first compression [6]. In parallel, the oxidative denaturation and aggregation of myofibrillar proteins further weaken the network structure [16]. For the emulsion gel-containing groups, the coalescence of oil droplets during freezing and thawing reduced the reinforcing effect of the oil droplets on the gel matrix, which explains the larger hardness loss in samples [8, 16]. The result was agreement with previous study reported by Pan et al. [6].

Table 4
Texture profile parameters of pork meatballs before and after 5FTC

	Samples	Hardness (g)	Springiness	Cohesiveness	Gumminess (g)	Chewiness
Before-5FTC	E0	2582 ± 115^{a}	0.79 ± 0.00^{a}	0.55 ± 0.01^{a}	1415 ± 90^{a}	1116 ± 69^{a}
	E25	2558 ± 70^{a}	0.79 ± 0.01^{a}	0.53 ± 0.02^{a}	1407 ± 152^{a}	1099 ± 135^{a}
	E50	2010 ± 71^{b}	0.78 ± 0.00^{a}	0.45 ± 0.01^{b}	897 ± 40^{b}	700 ± 32^{b}
	E75	1603 ± 36^{b}	0.76 ± 0.01^{ab}	0.42 ± 0.02^{b}	726 ± 98^{bc}	530 ± 88^{bc}
	E100	1313 ± 34^{c}	0.73 ± 0.03^{b}	0.40 ± 0.01^{b}	531 ± 31^{c}	402 ± 23^{c}
After-5FTC	E0	2492 ± 27^{a}	0.79 ± 0.00^{a}	0.58 ± 0.02^{a}	1414 ± 76^{a}	1111 ± 58^{a}
	E25	2330 ± 72^{b}	0.76 ± 0.01^{a}	0.57 ± 0.03^{a}	1354 ± 134^{a}	1020 ± 57^{a}
	E50	1667 ± 41^{c}	0.75 ± 0.01^{a}	0.48 ± 0.02^{b}	805 ± 63^{b}	610 ± 42^{b}
	E75	1336 ± 33^{d}	0.72 ± 0.01^{a}	0.42 ± 0.01^{b}	630 ± 9^{bc}	458 ± 53^{c}
	E100	1245 ± 39^{d}	0.72 ± 0.06^{a}	0.42 ± 0.02^{b}	506 ± 9^{c}	377 ± 9^{c}

Values are mean ± standard deviation (n = 3, as originally reported).

Different lowercase letters denote significant differences among samples within the same freeze–thaw treatment ($p < 0.05$).

Lipid oxidation

TBARS values, expressed per kilogram of product, differed strongly among formulations. For pork meatballs, lipid oxidation mainly arises from the high susceptibility of their fatty acids to oxidation [40]. The TBARS values of the pork meatballs before and after 5FTC are shown in Fig. 6. Before 5 FTCs, the TBARS value of the full-fat control E0 (4.3 mg/kg) was significantly higher than those of the emulsion gel groups (1.3–1.5 mg/kg). Higher TBARS values indicate more pronounced lipid oxidation and degradation in the meat system [41]. The PPI–ASKG interpenetrating-network emulsion gel replacement samples showed lower TBARS values, suggesting that the emulsion effectively reduced lipid oxidation in the meat system during storage. This behavior are most likely attributable to the combined reduction in total fat content and the antioxidant components of the formulation (the added vitamin E together with the native tocopherols in olive oil) [39]. Previous study also reported that the PPI-based Pickering emulsion may also lower TBARS content in the replacement samples by limiting free radical attack on fatty acids [42]. The similar TBARS values among E25, E50, E75 and E100, despite their increasing VE content, can be

explained by the dose-plateau behavior of vitamin E as a chain-breaking antioxidant: once the VE concentration is sufficient to quench the radicals generated in the system, further VE addition does not further reduce TBARS [43].

After 5FTC, the TBARS value of E0 increased sharply to 12.0 mg/kg ($p < 0.05$), indicating substantial lipid oxidation in the full-fat control during frozen storage. In contrast, the TBARS values of E25-E100 showed only minimal changes and were not significantly different from their fresh samples. The pronounced suppression of TBARS accumulation in the VE-loaded groups is consistent with the antioxidant role of VE in inhibiting lipid peroxidation, as previously reported in previous study [44].

Volatiles analysis of meatballs

Electronic nose (E-nose) systems utilize a sensor array to generate characteristic response patterns for headspace volatile compounds; when combined with chemometric data analysis, these systems allow rapid differentiation among samples. This approach has been widely applied to evaluate flavor differences in meat products [45]. The volatile profiles of the pork meatballs before and after 5FTC were measured with a PEN 3.5 electronic nose equipped with ten metal-oxide sensors (W1C, W5S, W3C, W6S, W5C, W1S, W1W, W2S, W2W and W3S). Among the ten sensors, only W1W (sensitive to organic sulphides) and W5S (sensitive to nitrogen oxides) showed significant differences ($p < 0.05$) among the five formulations (Fig. 7). The result suggested that the PPI–ASKG interpenetrating-network emulsion gel replacement samples altered the headspace pattern of organic sulfide- and nitrogen-oxide-related volatiles, the majority of which are heterocyclic compounds containing nitrogen, oxygen and sulfur [46].

Before 5FTC, the W1W response increased progressively with the substitution ratio, consistent with the accumulation of sulphur-containing volatiles. PPI contains appreciable amounts of sulfur-containing amino acids, mainly methionine and cysteine. During processing, these amino acids undergo oxidative and thermal degradation, releasing volatile compounds such as H_2S and methanethiol. These compounds are perceived as the characteristic “beany” off-flavor of legume-based proteins [47]. The response signal of the W5S sensor shows a trend of initial decrease followed by subsequent increase with the increase of emulsion gel content. This phenomenon is likely driven by reactive oxygen species produced via protein hydrolysis in meat, which trigger lipid oxidation [46]. The elevated W5S responses observed for E75 and E100 are consistent with their higher PPI contents, which provide more free amino groups and reactive amino acid side chains [47]. Consistent with this observation, Guan et al. [46] also reported that increasing fat replacer content leads to markedly divergent response values of the W1W and W5S sensors to sample odors.

After 5FTC, the overall sensor pattern was similar to that before 5FTC, with W1W and W5S remaining the most discriminating sensors. The W5C response (sensitive to alkenes and aromatic compounds) decreased slightly after 5FTC. This behavior may be attributed to degradation or volatilization loss induced by freeze-thaw cycles, leading to the reduced sensor response [48]. Lou et al. [49] also observed a reduction in aromatic flavor compounds in duck meat after freeze-thaw treatment.

Conclusions

Replacing pork backfat with the VE-loaded PPI–ASKG interpenetrating-network emulsion gel resulted in level-dependent changes in the properties of pork meatballs. At 25% replacement, the emulsion gel was incorporated into the myofibrillar protein matrix, yielding a denser and more continuous network that converted free water into immobilized water. Consequently, water-holding capacity reached its maximum, cooking and thawing losses were minimized, and textural attributes remained comparable to those of the full-fat control. At replacement levels of 50% and above, however, the emulsion gel progressively disrupted the continuity of the protein network, leading to decreased hardness, gumminess, and chewiness as well as increased liquid losses. After 5TCs, all emulsion gel-containing formulations exhibited higher lightness and markedly lower TBARS values than the control, which can be attributed to the combined capacity of the denser network and antioxidant components to mitigate ice-crystal damage and lipid oxidation. Future studies employing composition-matched controls and direct interfacial and structural characterization are needed to distinguish the individual contributions of the PPI–ASKG network and vitamin E.

Declarations

CRediT authorship contribution statement

Wenru Ma: Investigation, Data curation, Formal analysis, Writing - original draft, Validation. Yuehan Shan: Data curation, Writing-review & editing. Lingqi Si: Writing - review & editing. Lei Dai: Writing - review & editing. Qingjie Sun: Resources, Funding acquisition. Man Li: Writing - review & editing. Taotao Dai: Writing - review & editing. Xingfeng Xu: Conceptualization, Supervision, Project administration.

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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Figures

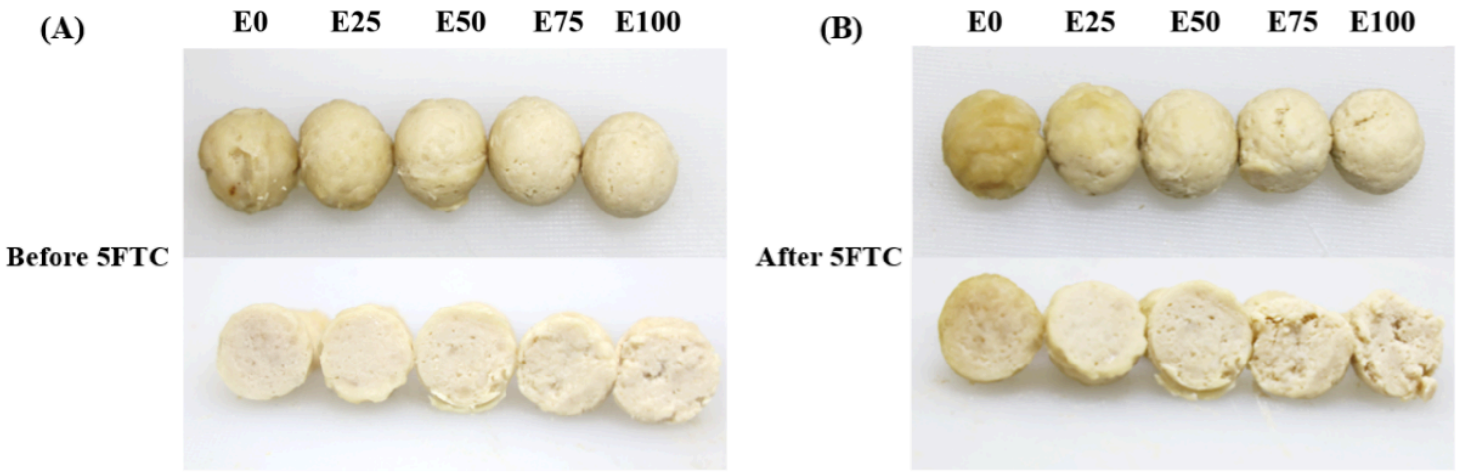


Figure 1

Surface and cross-sectional appearance of pork meatballs before (A) and after 5FTC (B)

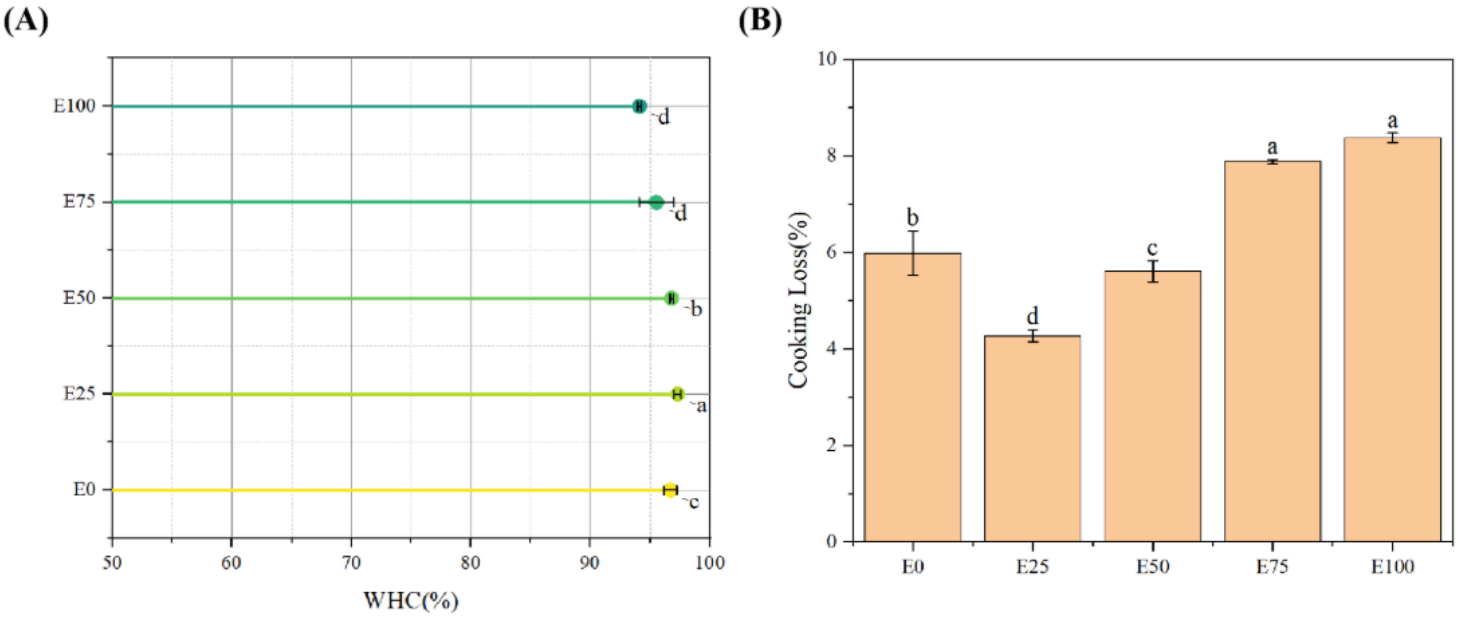


Figure 2

Water-holding capacity (A) and cooking loss (B) of pork meatballs

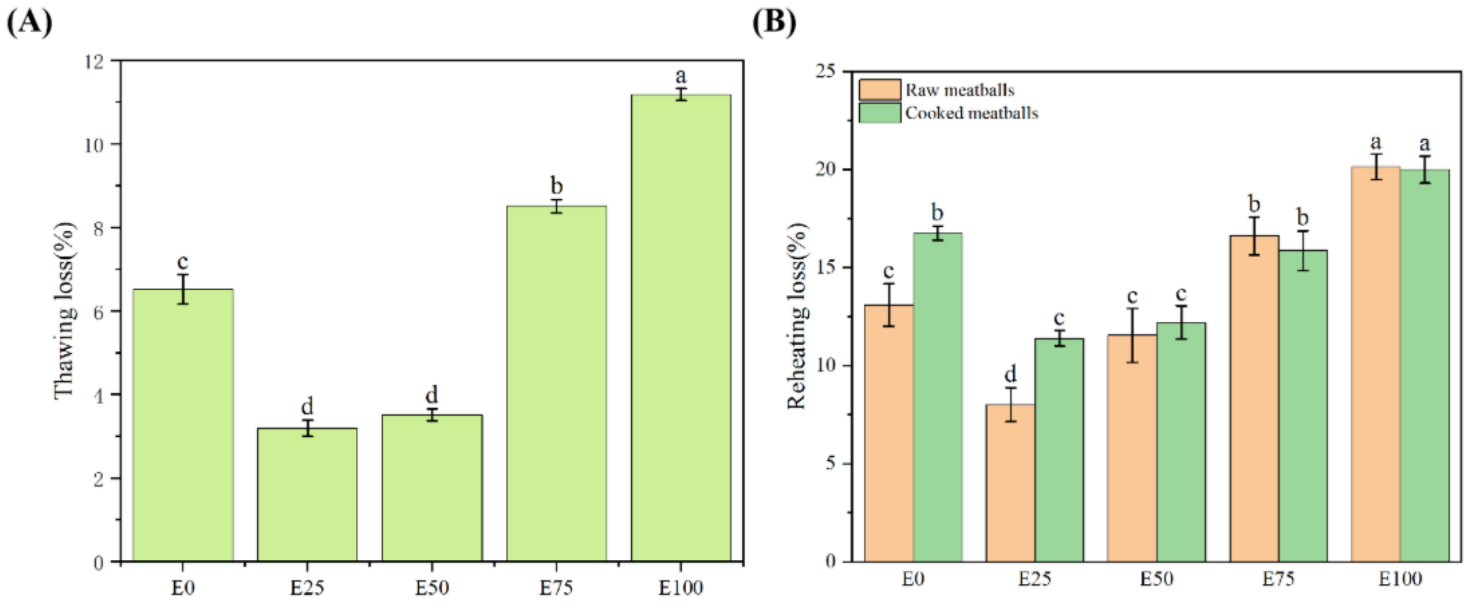


Figure 3

Thawing loss (A) and reheating loss (B) of pork meatballs

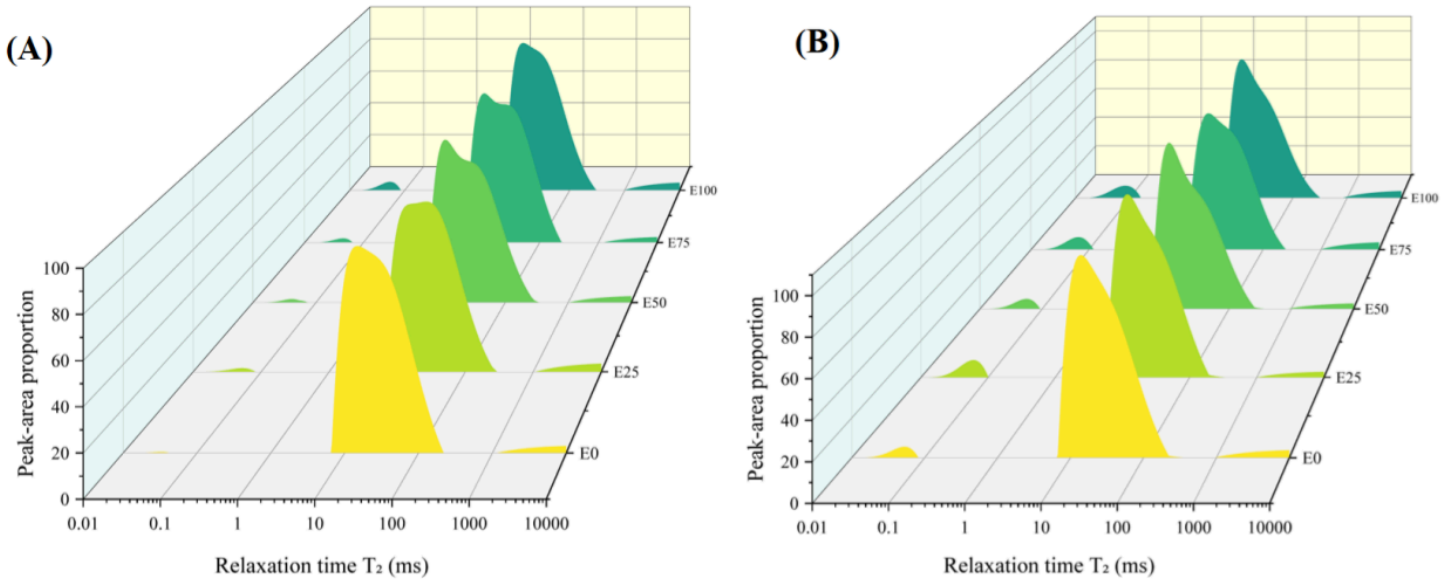


Figure 4

LF-NMR transverse-relaxation-time distributions of pork meatballs before (A) and after (B) 5FTC

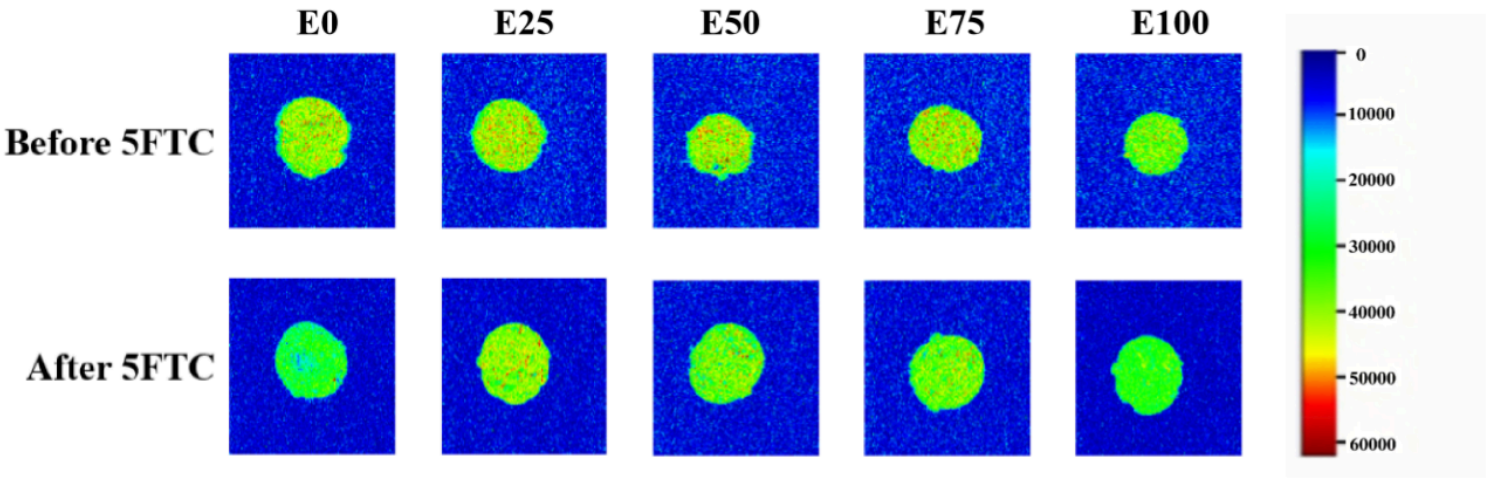


Figure 5

Magnetic resonance images of pork meatballs before and after freeze-thaw treatment

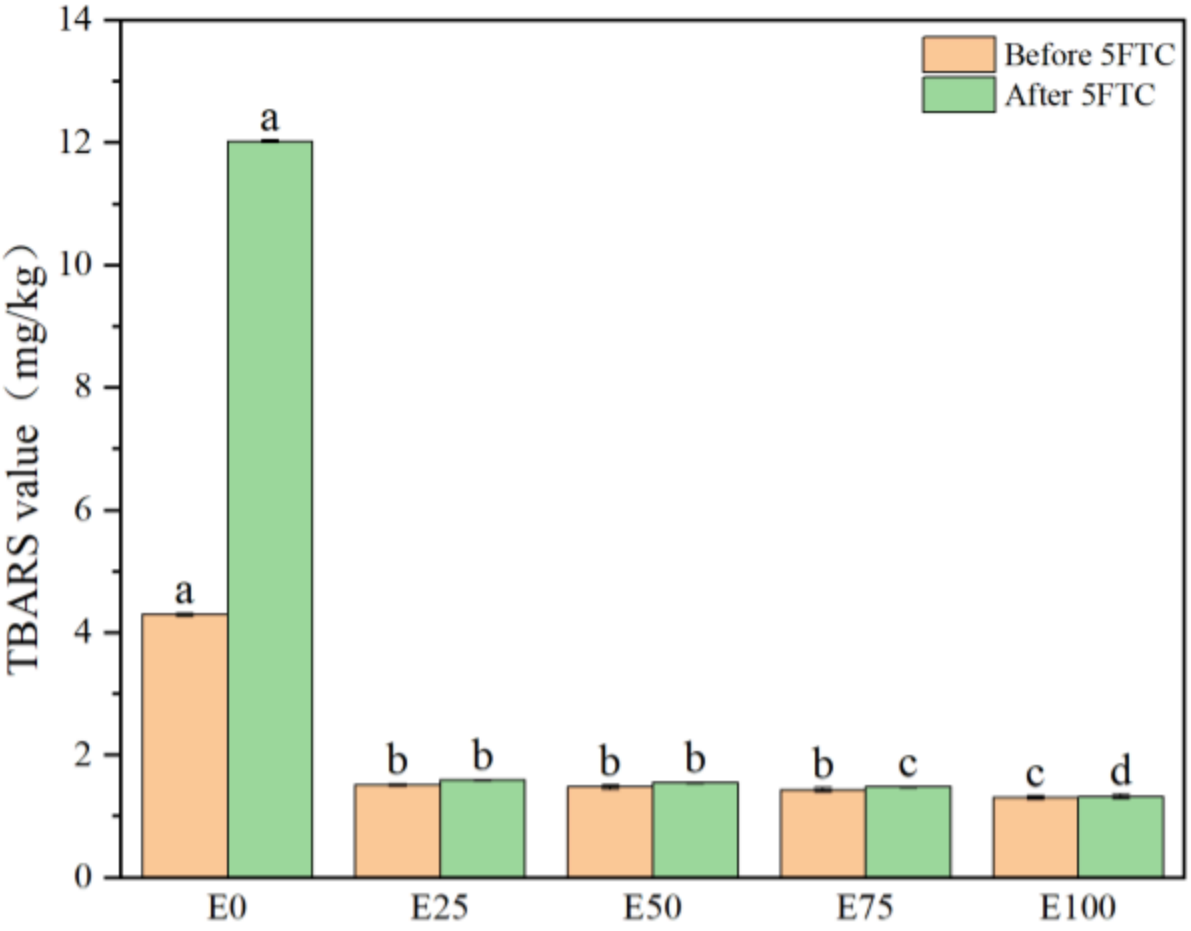


Figure 6

Thiobarbituric acid reactive substances (TBARS) in pork meatballs before and after 5FTC. Different lowercase letters denote significant differences among different samples within the same freeze–thaw

treatment ($p < 0.05$)

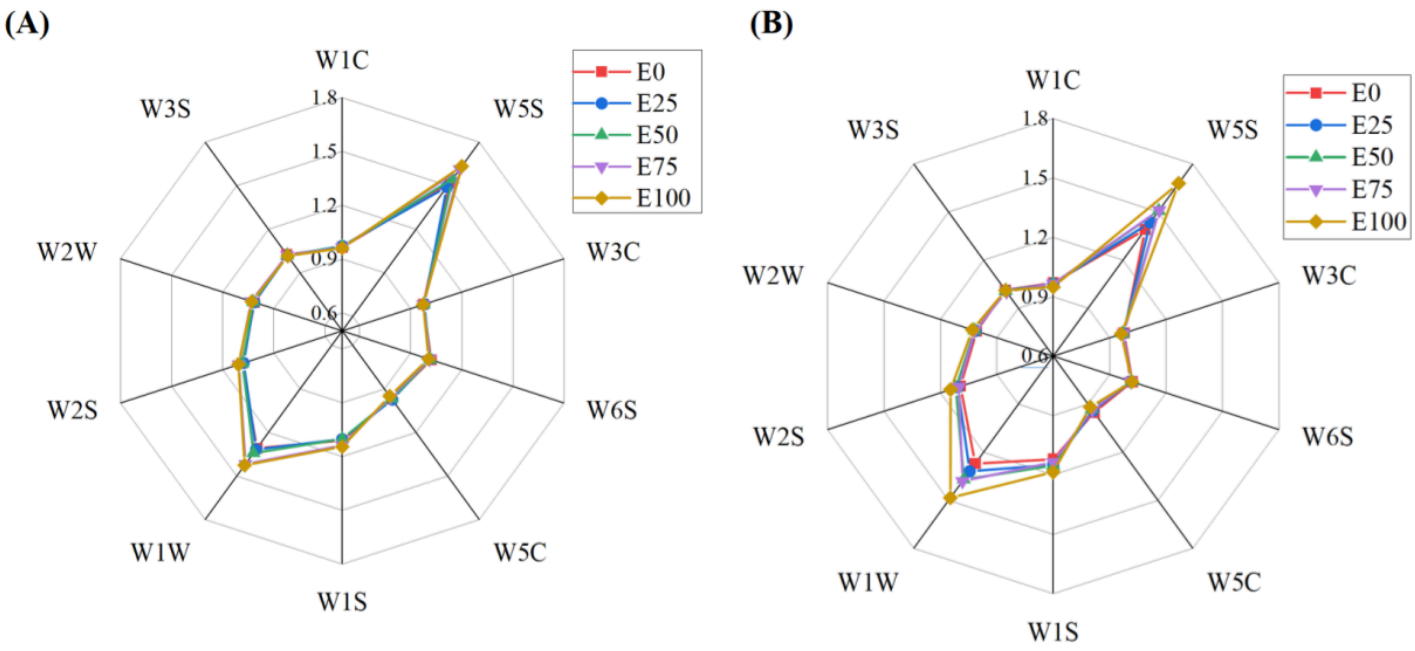


Figure 7

Electronic-nose response fingerprints of pork meatballs before and after 5FTCs