

Article

The Effects of Fermentation Time and the Addition of Blueberry on the Texture Properties and In Vitro Digestion of Whey Protein Gel

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Abstract: The interaction of blueberry and whey protein has strong antioxidant properties and potential antibacterial and anti-aging functions during the fermentation process. In this study, the properties of fermented gels derived from whey protein mixed with blueberry juice were investigated for the production of probiotic-rich products such as jelly and pudding. The microstructure, water-holding capacity, texture changes, rheological properties, and digestive characteristics of fermented gels were evaluated in vitro. The fermented gels with a mixture of whey protein and blueberry exhibited a honeycomb structure, observed by SEM. The adhesiveness of the gel with a mixture of blueberry and whey protein was the highest at 7.5 h and 8.0 h, respectively. The storage modulus (G') and loss modulus (G'') of the mixed gels were higher than those of whey protein gels before 6 h of fermentation. When the fermentation time was 8 h, the release of polyphenols, flavonoids, and proteins was fastest and greatest during the digestion of gastric and intestinal fluid ether for the whey protein fermented gel and the mixed fermented gel. The water-holding capacity of the mixed gels was lower than that of the whey protein fermented gels during the fermentation period of 8 h. The viable counts of the mixed fermented gels could reach 10^7 CFU/mL, which was higher than those of whey protein gels after 6 days of storage.

Keywords: fermentation; blueberry juice; whey protein; lactic acid bacteria; gelation



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1. Introduction

Protein gel is a three-dimensional (3D) network of protein molecules with a specific spatial structure. Protein molecules interact with each other or combine with polyphenols, water, lipids, and salts in the environment to form large aggregates through non-covalent and covalent bonds during the gelation process. Globular proteins play a crucial role in gelation. Whey protein can create an excellent gel to enhance the rheological qualities of foods [1]. Many techniques have been used to study the formation of whey protein gels, including physical, chemical, and biological modifications. Whey proteins are abundant in branched chains and sulfur-containing amino acids [2]. Microbial fermentation plays an important role in promoting gelation and changing product characteristics. The metabolites produced by lactic acid bacteria (LAB) significantly affect the qualities of the gel. An increased concentration of hydrophobic amino acids improves hydrophobic contact among protein fragments and the structure of the gel network [3]. Exopolysaccharides (EPSs) can enhance gel texture and water retention through the filling effect. Gels produced

with fermentation induced by probiotics are significantly different from those produced with fermentation induced by gluconate- δ -lactone (GDL) when the formation time of fermentation-induced gels is shortened [4]. Yang et al. found that strains of *Lactobacillus delbrueckii*, *Streptococcus thermophilus*, *Lactobacillus plantarum*, and *Lactobacillus paracasei* affected the structure and the interaction of protein molecules in gels [3]. Individual strains of fermentation-induced gel protein exhibited minimal levels of cross-linking. However, the gel network in mixed cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* is dense [5,6]. Fermented gels are more complicated than ordinary acid-induced gels. Whey proteins are degraded by LAB enzymes during gel formation, changing the dispersion of polar peptides and sulfhydryl groups' emergence or concealment [7]. In addition, lowering the pH strengthens the protein-binding areas or fills in the gaps in the network's architecture through hydrogen bonding and electrostatic forces [8]. LAB fermentation does not need additional acidifiers to create acidic conditions [6]. The fermentation-induced gel formation process is gentle. Pang et al. found that slow gelation formed a finer network structure with a porous, homogeneous structure containing similar pore sizes. However, a difference in the acidification rate did not cause a significant difference in the strength or particle size in the internal structure of the gels, as long as the final pH was similar [4]. The advantage of cold gelation is that cold gels can form at lower protein concentrations and temperatures [9]. The secondary structure of proteins is affected by the development of hydrogen bonds or hydrophobic interactions between polyphenols and proteins during the co-fermentation of blueberry and whey proteins.

The addition of nonprotein components could regulate the interaction forces and change the structure and texture of protein gels. Phenolic compounds are natural cross-linkers and gel enhancers of many animal proteins. Polyphenols with polyaromatic rings and polyhydroxyl structures can interact with whey proteins to form gel structures with good properties [10]. It has been found that adding tea polyphenols to animal protein increases gel strength. The presence of polyphenols accelerates protein aggregation. Whey-protein-polyphenol conjugates have been used to improve the thermal stability, solubility, emulsification, antioxidant, film-forming, and gelling properties of whey protein [11]. Whey-protein-polyphenol conjugates could alter biological activities and have nutritional and health-promoting properties; for example, they can reduce the allergenicity of β -LG and BSA against LDL oxidation and atherosclerosis inhibition, and they have higher antioxidant and antiviral activities than pure proteins [12]. Furthermore, the structure of protein gels is related to the digestion process. Though protein gels are initially digested orally by way of physical disintegration, they are digested in the stomach by the biochemical material environment. The initial hardness and softening of half of the gels reduces their digestion time and their mass transport in gastric fluids [13]. The release of protein or other functional molecules in the gels is also related to the structure of the gels during the digestion process. Therefore, the matrix of the gels is very important for the bioutilization of their nutrients in the human body. The particle size and concentration of edible gels also has an impact on in vitro gut fermentation, including on gas production, short-chain fatty acids, and ammonia [14]. In a previous study, we discovered that anthocyanin release rates were reduced in a system made from blueberry and whey protein in low-acid simulated gastric fluid. The LAB in the fermented system were more resistant to in vitro digestion [15]. The interaction between whey protein and polyphenols through hydrogen bonds during fermentation could protect LAB against high temperatures and low temperatures during the drying process [16]. In a previous study, we also found that a fermented product with mixed whey protein and blueberry juice had the ability to inhibit the proliferation of *E. coli* [17]. Currently, studies provide little information on the structure of whey protein in relation to the aggregation or network framework of gels in the presence or absence

of polyphenols. Probiotic gels can replace jelly pudding foods, increasing the nutritional value and variety of the product.

Fermentation-induced gels generated by mixed strains of *Lactobacillus delbrueckii* and *Streptococcus thermophilus* under different fermentation times were evaluated in this study. The microstructure and color changes of whey protein fermented gels and fermented gels with a mixture of whey protein and blueberry juice under different fermentation times were investigated. The rheological properties and texture of gels formed over various fermentation periods were compared. The release of active ingredients in gastric juices and the extent of swelling were determined to evaluate the bioactivity of the gels during two hours of digestion. This will help to provide an understanding of how fermentation times affect gel characteristics and protein arrangement, and even how to control the fermentation process.

2. Materials and Methods

2.1. Materials

Whey protein concentrate (WPC) with a protein content of at least 80% was obtained from Shanghai HowYou Food Technology Co., Ltd (Shanghai, China). Blueberries were collected during the 2022 harvest period in Daxinganling, China. *L. delbrueckii* 134 and *Streptococcus thermophilus* grx 02 were obtained from Yangzhou University, Yangzhou, Jiangsu, China.

2.2. Preparation of Fermentation-Induced Gels

One system consisted of 12 g WPC, 12 g sugar, and 33.34 mL blueberry juice, dissolved in distilled water to obtain a final volume of 200 mL. The other system was obtained by dissolving 12 g of sugar and 12 g of WPC in distilled water to obtain a final volume of 200 mL. The blueberry juice was prepared by using a crusher after weighing blueberries to obtain a blueberry juice content of 17% (*w/v*). The pH of the two systems was adjusted to 7.0. These systems were stirred for 20 min using a magnetic stirrer at 800 rpm, at 25 °C. The mixed solution was then pasteurized at 85 °C for 10 min, before being cooled down to 40 °C for fermentation. The second generation of *L. delbrueckii* 134 and *S. thermophilus* grx02, at a ratio of 1:1, were inoculated into the above two systems to initiate fermentation at 42 °C for 4.5, 5, 5.5, 6, 6.5, 7, 7.5, and 8 h (IW 4.5–8). Scheme 1 shows the preparation and structure of the blueberry and whey protein fermented gels.

2.3. Scanning Electron Microscopy Observation

The gels were formed in a 50 mL glass container. A small piece of gel ($3 \times 3 \times 3 \text{ cm}^3$) was excised using a scalpel and lyophilized. The microstructures of the different samples were observed using a scanning electron microscope (Gemini SEM 300, Carl Zeiss AG, Oberkochen, Germany). Images were obtained at 200× magnification.

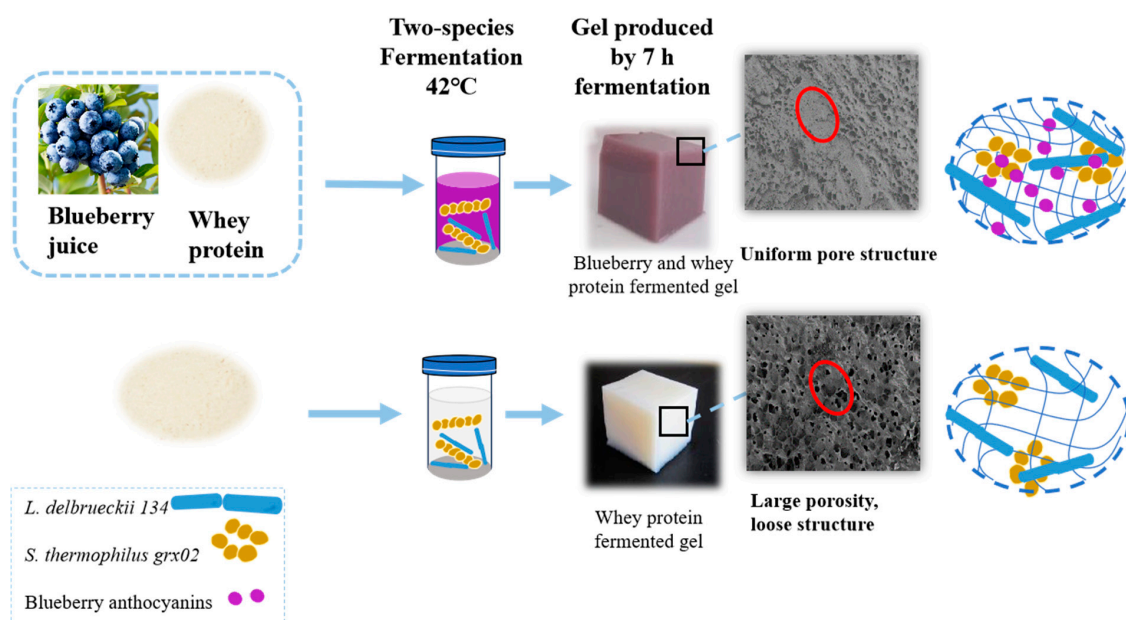
2.4. Color Measurement

The color of the samples was measured using a Minolta Chroma Meter CR-400 colorimeter (Minolta Ltd., Milton Keynes, UK). The measurement results were expressed as L (lightness), a (redness), and b (yellowness) values of the gels, where ΔL^* , Δa^* , and Δb^* are the differences between the color coordinates before fermentation and at a specific fermentation time.

2.5. Texture Measurement

The textural characteristics of the fermentation-induced gels were determined using a TMS-Pro texture analyzer (Food Technology Corporation, Sterling, VA, USA), equipped

with a max load of 2500 N/550 lbf and a flat-surface cylindrical probe with a diameter of 7.5 mm. The fermented gels were stored at 4 °C and equilibrated to room temperature (25 °C) prior to the texture analysis. The test speed was 1 mm/s. The probe was inserted into the gels to a penetration depth of 20 mm. The withdrawal speed of the probe was 1 mm/s. The texture exponent program was used to assess the hardness, elasticity, cohesiveness, adhesiveness, gumminess, and chewiness of the gels. All texture measurements were evaluated in triplicate [18].



Scheme 1. A schematic diagram showing the characteristics of gel produced with whey protein alone and that mixed with blueberry, fermented by *Lactobacillus delbrueckii* 134 and *Streptococcus thermophilus* grx 02.

2.6. Rheological Measurement of Fermented Gels

Coaxial cylinder geometry was conducted with a rotational rheometer (Malvern Instruments Ltd., Worcestershire, UK), with a vane upper geometry of 25 mm and 4 blades (4V25 SR0619SS), to test the rheological attributes of various samples at 25 °C. The shear rate was varied from 0.01 to 100 s⁻¹, and the relationship between the shear rate and the increased viscosity (η) of the gel was analyzed. Once the cooling process was completed, a frequency test was performed in the frequency range of 0.01–100 Hz at a fixed strain of 1% and a temperature of 25 °C. The changes in storage (G') and loss (G'') moduli were recorded automatically.

2.7. Water-Holding Capacity

Water-holding capacity (WHC) was evaluated as reported by Zhang et al. [19], with appropriate modification. The gels were stored at 4 °C for 24 h, before being centrifuged. In tubes, 2 g of the gels were centrifuged at 10,000× g at 4 °C for 10 min. The tubes were then inverted and emptied of water.

2.8. In Vitro Gastric Digestion

2.8.1. Swelling

A 10 g sample of gel was placed into 30 mL of simulated gastric fluid (SGF) without enzyme, and stirred at 100 rpm and at a temperature of 37 °C. The gel was removed from the medium, and its surface was dried using a paper towel. Then, the swelling rate was calculated using the following formula.

SGF: 7.0 mL of 37% HCl and 2.0 g of sodium chloride were dissolved in 1000 mL of distilled water and adjusted to pH 1.2 [20,21].

$$\text{Swelling (\%)} = [(W_t - W_0)/W_0] \times 100\%$$

where W_0 is the initial gel weight (g) and W_t is the gel weight after digestion.

2.8.2. Release of Protein

The gels were incubated in SGF containing pepsin (2000 U/mL) at 37 °C for 2 h in a shaking incubator [16]. The digestion fluid was centrifuged at 1500× *g*, and the absorbance of the whey protein in the supernatant was measured at 280 nm to determine the concentration of the released protein.

The released protein is the amount of protein released into the SGF, and the total protein is the amount of protein within the gel matrix.

2.8.3. Release of Total Phenolic

The total phenolic content in the gels was determined using Folin–Ciocalteu reagent, following the methodology described by Zahid et al. [22].

2.8.4. Release of Anthocyanin Concentration

The classic pH-differential method was conducted according to the procedure described by Li et al. [23], with minor modifications. A 1 mL volume of each sample was diluted with 9 mL of potassium chloride buffer (pH 1.0) or 9 mL of sodium acetate buffer (pH 4.5). The absorbance of the samples was measured at 520 and 700 nm, followed by 20 min of incubation at 25 °C.

2.9. Viable Bacterial Count Calculation

The enumeration of viable bacteria was evaluated as reported by Z. Chen et al. [24]. First, a 1 g sample of gel was taken aseptically and placed in a sterilized 50 mL centrifuge tube containing 10 mL of normal saline (with a preset rotor in the tube). The gel was broken using a sterilized glass tube, and the liquid was evenly diluted after full shaking. Then, 1 mL of the diluent was injected into 9 mL of saline along the tube wall, and shaken to mix evenly. Three gradients of 1 mL of 10^{-6} , 10^{-7} , and 10^{-8} diluent were added to an empty plate, and 20–25 mL of MRS medium was added. After solidifying the agar, the plate was turned over and incubated in a 36 ± 1 °C incubator for 48 ± 2 h. The number of colonies on the plate was counted to calculate the number of colony-forming units. Plates with colonies between 30 and 300 were selected for counting, and then multiplied by the dilution factor to obtain the total number of colonies for the test samples. The number of viable bacteria were determined after storage periods of 0, 2, 4, 6, 8, and 10 days. All experiments were performed in triplicate.

2.10. Statistical Analysis

The experimental data were statistically analyzed using SPSS v.11.5 software (SPSS, Inc., Chicago, IL, USA). One-way ANOVA was used to examine the effects of different treatments. The data are expressed as the mean value $\pm$ standard deviation.

3. Results

3.1. Microstructural Examinations

At the beginning of fermentation, many pores were present on the gel surface, which was due to the fermentation by the lactic acid bacteria. At this moment, the hydrophobicity and flexibility of the protein molecules were higher, which increased the foaming ability

of the protein in the fermentation system. Upon extension of the fermentation time, the pores were reduced in number or became smaller, because the growth of LAB consumes oxygen during fermentation [25]. The gel surface structure was photographed using a camera. As shown in Figure 1, protein gel did not form after fermentation by *L. delbrueckii* and *S. thermophilus* for 4.5 h. The whey protein gel formed at 5.5 h was white, slightly transparent, and easily deformed for juice outflow, as shown in Figure 1. With extension of the fermentation time, the hardness, water retention, and elasticity of the gel were enhanced. The whey protein–blueberry juice hybrid fermented gel had the form of an aerated liquid at 4.5 h and a gel at 5.5 h. Moreover, the states of the whey protein–blueberry mixed blueberry gel at different fermentation times were similar to those of the whey protein fermented gel, according to their surface appearance. Scanning electron microscopy (SEM) was also used to analyze the changes in the gel structure of the fermented gels. The SEM microstructures of the fermentation-induced gels after various incubation times are shown in Figure 1. Fermentation with *L. delbrueckii* and *S. thermophilus* resulted in a thick gel network. As shown in Figure 1, the microstructure of the gel after fermentation for 7.5 h was different in the whey protein fermented system in comparison to the whey protein and blueberry fermented system. The gel with a mixture of blueberry and whey protein, after fermentation for 7.5 h, showed a multifaceted, unbroken fiber network framework and honeycomb-like attributes that differed from the irregular plate and pore structure formed at 4.5 h [6]. According to a previous study, the gel structure usually becomes unstable and loose with an increase in internal pores [26]. Therefore, the surfaces of the gels were relatively rough at first, then gradually became smooth and delicate. After 6 h of fermentation, the whey protein and blueberry juice gel began to form a more porous surface, whereas the whey protein gel took 7.5 h to attain this state. The whey protein gel was more porous than whey protein and blueberry juice gel after fermentation for 8.0 h. The whey protein and blueberry juice fermented system experienced an increase in protein content or increased aggregation of local protein molecules, because of the presence of anthocyanins. Whey proteins are globular structures with sulfhydryl groups and disulfide bonds that are used to construct gel networks. Therefore, the more closely that protein molecules aggregate with each other, the denser the gel network becomes. This could lead to fewer pores and increase interaction among molecules [27].

3.2. Color Analysis

The gels with fermentation induced by *Streptococcus thermophilus* and *Lactobacillus delbrueckii* showed significant variation ($p < 0.05$) in ΔL , Δa , and Δb values, as shown in Table 1. The ΔL value indicates the brightness or whiteness of a product before and after changes [28]. The ΔL values of the whey protein fermented gels changed between 13.09 and 17.72. The brightness of the whey protein fermented gels increased from 4.5 to 6 h. In addition, the brightness change was not significant from 6.5 to 8 h. The brightness of the whey protein–blueberry mixed fermented gels was substantially higher than that of the whey protein gels ($p < 0.05$) at 8.0 h. High values of ΔL and Δb indicate that the whiteness/brightness and yellowness of the whey protein gel fermented for 6 h were greater than those of the other samples, as shown in Table 1. The values of Δa for the whey protein–blueberry juice mixed fermented gel varied more than those for the whey protein gel ($p < 0.05$). Significant color changes in the whey protein gel and the gel with a mixture of whey protein and blueberry are shown in Figure 1. The increased values of Δa indicate that the whey protein–mixed blueberry fermented gels became red with the change in fermentation time. The increased values of Δb indicate the dominance of blue over yellow. Δa and Δb reached their maximum values at 7 h, and there was no significant change in the fermentation process thereafter ($p > 0.05$). As the fermentation time increased,

acid production increased gradually. Therefore, the red coloration of the whey protein–blueberry mixed fermented gel gradually deepened over the first 7 h of fermentation. Then, the redness decreased, which is related to the interaction between anthocyanins and whey protein at fermentation times of 7.5 and 8.0 h. Malvidin (yellow–red pigments) and delphinidin (blue–purple pigments) derivatives are the most dominant anthocyanins found in blueberry fruits [29]. The presence of the red and blue–purple hues of the blueberry juice may have contributed to the maximum value of Δa found in the blueberry and whey protein fermented gels. This can be attributed to the structural changes of anthocyanin, which affected the color of the gels during fermentation.

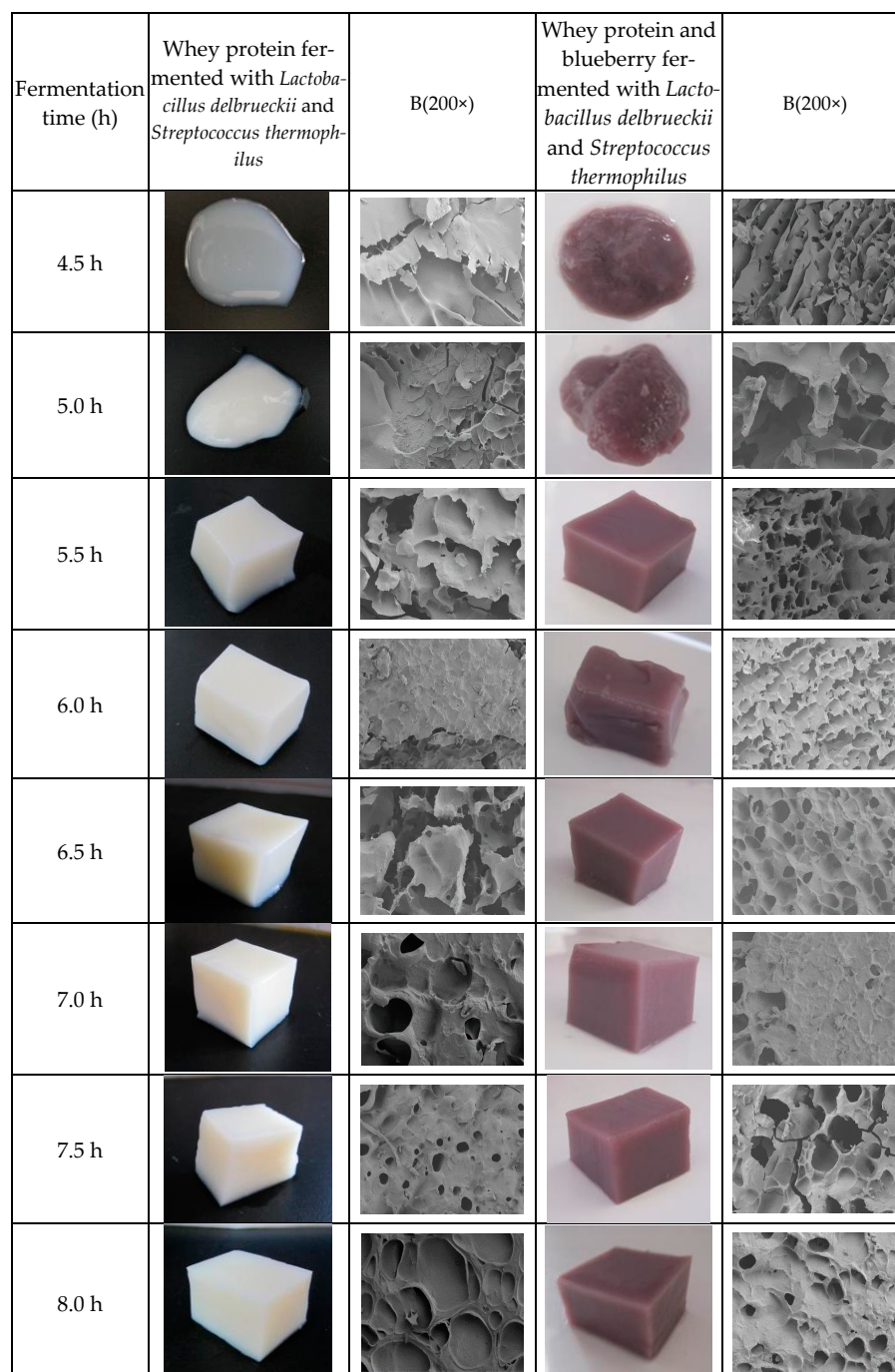


Figure 1. Surface morphological changes of whey protein gels and mixed blueberry juice and whey protein gels fermented by *Lactobacillus delbrueckii* 134 and *Streptococcus thermophilus* grx 02 after different fermentation times, with SEM observations at a magnification of 200×.

Table 1. The color changes of whey protein gels and whey protein–blueberry juice mixed gels fermented by *Lactobacillus delbrueckii* and *Streptococcus thermophilus* after different fermentation times.

Sample (pH 7.0)	Fermentation Time (h)	ΔL	Δa	Δb
Whey protein <i>Lactobacillus delbrueckii</i> + <i>Streptococcus thermophilus</i>	4.5 h	13.086 ± 1.658^b	0.813 ± 0.116^{ab}	4.627 ± 1.403^{ab}
	5.0 h	15.097 ± 0.761^{ab}	0.707 ± 0.182^{ab}	7.040 ± 1.078^a
	5.5 h	16.343 ± 0.887^a	0.462 ± 0.124^b	7.372 ± 0.652^a
	6.0 h	17.600 ± 1.116^a	0.583 ± 0.175^{ab}	6.870 ± 0.426^a
	6.5 h	17.717 ± 0.107^a	0.577 ± 0.171^{ab}	5.610 ± 0.712^{ab}
	7.0 h	15.790 ± 0.255^{ab}	0.937 ± 0.161^{ab}	5.283 ± 0.250^{ab}
	7.5 h	16.807 ± 0.702^a	1.077 ± 0.134^a	6.380 ± 0.508^{ab}
	8.0 h	14.663 ± 0.172^{ab}	0.957 ± 0.109^{ab}	3.963 ± 0.194^b
Blueberry + whey protein <i>Lactobacillus delbrueckii</i> + <i>Streptococcus thermophilus</i>	4.5 h	14.478 ± 0.418^c	6.175 ± 0.320^c	-4.030 ± 0.221^{abc}
	5.0 h	14.430 ± 0.411^c	6.372 ± 0.192^c	-3.773 ± 0.205^{ab}
	5.5 h	14.618 ± 0.101^{bc}	7.188 ± 0.167^{bc}	-4.106 ± 0.274^{abc}
	6.0 h	14.673 ± 0.178^{bc}	7.913 ± 0.333^{ab}	-4.757 ± 0.245^c
	6.5 h	15.990 ± 0.250^a	8.103 ± 0.209^{ab}	-3.840 ± 0.387^{ab}
	7.0 h	16.003 ± 0.146^a	8.313 ± 0.511^a	-4.500 ± 0.044^{bc}
	7.5 h	15.520 ± 0.287^{ab}	7.697 ± 0.193^{ab}	-4.210 ± 0.125^{abc}
	8.0 h	16.450 ± 0.364^a	7.447 ± 0.270^{ab}	-3.487 ± 0.179^a

Note: the mean values are compared within each column. The same letter means no significant difference ($p > 0.05$), a different letter means significant difference ($p < 0.05$).

3.3. Texture Analysis

Flavor and texture are the most relevant drivers of consumer preference [30]. The textural characteristics of the fermented gels are summarized in Table 2. The whey protein gel's elasticity was greatly unaffected by the addition of blueberry juice, as shown in Table 2. The primary pH had an influence on the shape of the whey protein gel. Intermolecular disulfide bonds are preferred at a neutral pH [31]. The protein network structure is a key factor influencing hardness. The stiffest gel was the whey protein gel formed after 8 h of fermentation. More-stable 3D network topologies can be built by considering the disulfide bonds formed. With the extension of fermentation time, the gumminess, cohesiveness, and chewiness of the whey protein gel were increased. The chewiness of the whey protein-mixed blueberry fermented gels was lower than that of the fermented whey protein gels alone after 6.0 h as shown in Table 2, which was related to protein and polyphenol conjugates. Polyphenols contain multiple hydroxyl groups, and when bound to proteins, they introduce additional hydroxyl groups that enhance the hydrophilic environment of the protein, thereby reducing intermolecular forces [32]. The adhesiveness of whey protein–blueberry mixed fermented gel was higher than that of the whey protein gel during the fermentation process. The fermented gel with a mixture of whey protein and blueberry juice exhibited significantly increased adhesiveness and maximum adhesion ($p > 0.05$). The maximum adhesion of the whey protein–blueberry juice mixed gel fermented for 7 h was 1.9 N, which was approximately 1.6 N higher than that of the whey protein gel fermented for 7 h. This was mainly due to the effect of phenolic hydroxyl on the matrix of the fermented gels, which changed the physical properties of the gels. This could perhaps have contributed to the honeycomb-like structure of the whey protein–blueberry mixed fermented gel, as shown in Figure 1. The fermentation process was complex, and the products were also diverse. As shown in Table 2, the hardness of the mixed fermented gels slightly dropped from 6.0 h to 7.0 h. This may be related to the structural changes of the gels brought about through interference by different fermentation products at different times.

Table 2. The textural changes of the gels fermented with *Lactobacillus delbrueckii* and *Streptococcus thermophilus* after different fermentation times.

Sample	Fermentation Time (h)	Hardness (N)	Maximum Adhesion (N)	Adhesiveness (N)	Cohesiveness	Elasticity (mm)	Gumminess (N)	Chewiness (mJ)
Blueberry + whey protein <i>Lactobacillus delbrueckii</i> + <i>Streptococcus thermophilus</i>	4.5 h	0.697 ± 0.039 ^e	−0.125 ± 0.007 ^a	0.826 ± 0.012 ^{de}	0.507 ± 0.02 ^{ab}	41.620 ± 0.208 ^a	0.368 ± 0.016 ^d	15.423 ± 0.592 ^c
	5.0 h	1.151 ± 0.060 ^d	−0.159 ± 0.019 ^{ab}	1.230 ± 0.063 ^{bc}	0.470 ± 0.006 ^{bc}	42.007 ± 0.007 ^a	0.559 ± 0.006 ^c	23.43 ± 0.272 ^b
	5.5 h	1.258 ± 0.019 ^{cd}	−0.143 ± 0.015 ^{ab}	0.890 ± 0.049 ^{de}	0.553 ± 0.023 ^a	42.047 ± 0.003 ^a	0.663 ± 0.033 ^{bc}	27.87 ± 1.394 ^a
	6.0 h	1.499 ± 0.158 ^{abc}	−0.151 ± 0.012 ^{ab}	0.754 ± 0.064 ^e	0.533 ± 0.003 ^a	41.963 ± 0.009 ^a	0.866 ± 0.037 ^a	30.55 ± 1.679 ^a
	6.5 h	1.494 ± 0.068 ^{abc}	−0.167 ± 0.009 ^{ab}	1.141 ± 0.028 ^{cd}	0.517 ± 0.012 ^{ab}	42.000 ± 0.010 ^a	0.748 ± 0.028 ^{ab}	31.393 ± 1.184 ^a
	7.0 h	1.454 ± 0.031 ^{bc}	−0.195 ± 0.039 ^{ab}	1.900 ± 0.019 ^a	0.444 ± 0.003 ^c	41.983 ± 0.003 ^a	0.658 ± 0.060 ^{bc}	28.947 ± 1.634 ^a
	7.5 h	1.757 ± 0.142 ^a	−0.242 ± 0.023 ^b	1.543 ± 0.139 ^b	0.413 ± 0.009 ^c	41.850 ± 0.120 ^a	0.676 ± 0.026 ^{bc}	27.700 ± 0.605 ^a
	8.0 h	1.706 ± 0.027 ^{ab}	−0.206 ± 0.055 ^{ab}	1.510 ± 0.187 ^b	0.443 ± 0.035 ^c	39.913 ± 1.227 ^b	0.757 ± 0.071 ^{ab}	28.113 ± 0.878 ^a
Whey protein <i>Lactobacillus delbrueckii</i> + <i>Streptococcus thermophilus</i>	4.5 h	0.129 ± 0.003 ^f	−0.077 ± 0.002 ^{abc}	0.339 ± 0.010 ^c	0.980 ± 0.006 ^a	41.973 ± 0.018 ^a	0.142 ± 0.006 ^f	5.590 ± 0.306 ^g
	5.0 h	0.789 ± 0.029 ^e	−0.090 ± 0.010 ^{bc}	0.576 ± 0.015 ^b	0.623 ± 0.026 ^{bc}	41.687 ± 0.338 ^a	0.457 ± 0.015 ^e	19.473 ± 0.438 ^f
	5.5 h	1.301 ± 0.117 ^{cd}	−0.077 ± 0.009 ^{abc}	0.535 ± 0.034 ^b	0.647 ± 0.057 ^b	42.027 ± 0.033 ^a	0.777 ± 0.058 ^d	23.837 ± 0.118 ^e
	6.0 h	1.134 ± 0.034 ^d	−0.098 ± 0.005 ^c	0.722 ± 0.011 ^a	0.523 ± 0.034 ^{cd}	41.973 ± 0.007 ^a	0.563 ± 0.006 ^e	23.760 ± 0.312 ^e
	6.5 h	1.402 ± 0.034 ^c	−0.057 ± 0.001 ^a	0.237 ± 0.002 ^d	0.643 ± 0.013 ^b	41.827 ± 0.163 ^a	0.926 ± 0.053 ^c	36.720 ± 0.910 ^c
	7.0 h	1.184 ± 0.001 ^{cd}	−0.071 ± 0.009 ^{ab}	0.342 ± 0.068 ^c	0.450 ± 0.036 ^d	42.007 ± 0.003 ^a	0.781 ± 0.031 ^d	32.437 ± 1.308 ^d
	7.5 h	1.787 ± 0.123 ^b	−0.069 ± 0.005 ^{ab}	0.287 ± 0.023 ^{cd}	0.677 ± 0.023 ^b	41.817 ± 0.143 ^a	1.073 ± 0.067 ^b	48.490 ± 0.978 ^b
	8.0 h	2.212 ± 0.059 ^a	−0.074 ± 0.002 ^{ab}	0.371 ± 0.002 ^c	0.628 ± 0.026 ^{bc}	41.850 ± 0.135 ^a	1.375 ± 0.023 ^a	57.533 ± 0.764 ^a

Note: the mean values are compared within each column. The same letter means no significant difference ($p > 0.05$), a different letter means a significant difference ($p < 0.05$).

3.4. Rheological Characteristics of Fermented Gels

Viscosity is susceptible to changes due to variations in protein aggregation in solutions, as shown in Figure 2(a1,a2). Regardless of the fermentation duration and whether blueberry polyphenols were added or not, the gels exhibited shear thinning, mainly because the protein molecules had fewer connections with each other. As the shear rate increased, the molecular free space between proteins increased [10]. Short- and long-range forces are primarily responsible for the fluidity of WPCs, with the former being predominant. The apparent viscosities of the whey protein–blueberry mixed fermented gels increased rapidly after 5 h of fermentation, and were higher than those of single whey protein fermented gels. When polyphenols are bound to the protein surface, their solubility is reduced by increasing the forces between the protein molecules. However, as the fermentation time increased, more polyphenols were bound to the whey protein surface. Interactions were predicted to be weakened by an increase in protein–protein length and partial protection of charged particles on peptides when polyphenols were attached to their respective surfaces, rendering them susceptible to breakage at low shear rates [10]. This explains the viscosity of the 8 h-fermented whey protein gels, which was consistently higher than that of the whey protein–blueberry juice mixed fermented gels (Figure 2(a1,a2)).

The storage (G') and loss moduli (G'') of the gels after different fermentation times are shown in Figure 2b,c, and reflect the structural development of the fermentation-induced gels. The addition of blueberry juice induced stable hydrogel formation ($G' > G''$). The G' of all gels was larger than the G'' in the range of 0.1–10 Hz. The elastic modulus (G') of all gels became greater with an increase in frequency, showing frequency dependence. The enhancement of G' is consistent with the results of hydrophobic interactions and an increase in disulfide bonds [33]. The G' value of the whey protein gels increased from 1300 to 2300 Pa after 7.5 h of fermentation. Similarly, the G' value of the whey protein–blueberry juice mixed gels increased from 750 to 1485 Pa after the same fermentation period. G' is mainly dependent on covalent cross-linking, and is positively correlated [34]. The G' value of the whey protein fermented gels was higher than that of the whey protein–blueberry juice mixed fermented gels after 7.0 h of fermentation. This was mainly due to protein cross-linking during fermentation, leading to a higher G' value. The enhancement of G' is consistent with the results of hydrophobic interactions and an increase in disulfide bonds [33]. The increase in the G' of the whey protein gels after 7.0 h of fermentation suggests that the gels formed by whole protein extracts became stiffer, but more brittle [14]. The G' and G'' values of the whey protein–blueberry juice mixed fermented gels were higher than those of the whey protein fermented gels at 4.5 h to 5.5 h, and lower than the whey protein fermented gels at 7.0 h to 8 h. This indicates that the blueberry juice could have decreased the stiffness and increased the brittleness of the gel after 7.0 h of fermentation. Figure 1 illustrates the dense porous structure of the whey protein–blueberry juice mixed fermented gels formed at 5 h [35]. The charge-screening mechanism induced by the polyphenols might have been responsible for the decreased mechanical strength of the mixed gels [36]. During fermentation, *L. delbrueckii* and *S. thermophilus* gradually produce acids and protons. In addition, hydrogen bonds also contribute to the interaction of whey protein and polyphenols during the fermentation process [15].

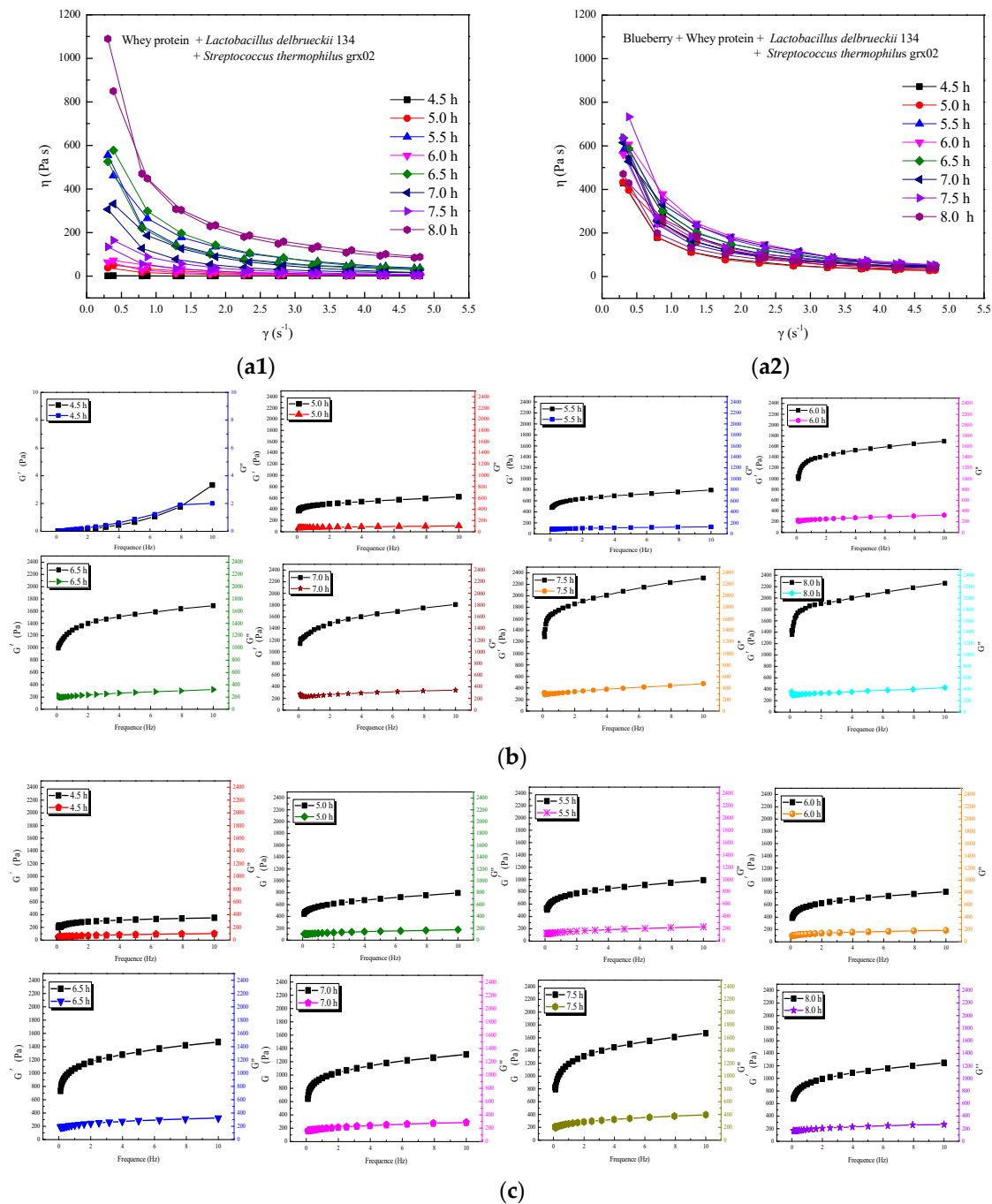


Figure 2. (a1) The apparent viscosity of the whey protein fermented gels formed by fermentation with *Lactobacillus delbrueckii* 134 and *Streptococcus thermophilus* grx 02 after different fermentation times. (a2) The apparent viscosity of fermented gels with a mixture of whey protein and blueberry juice, formed by fermentation with *Lactobacillus delbrueckii* 134 and *Streptococcus thermophilus* grx 02. (b) The frequency sweep of the storage modulus (G') and loss modulus (G'') of whey protein fermented gels formed by fermentation with *Lactobacillus delbrueckii* 134 and *Streptococcus thermophilus* grx 02 after different fermentation times. (c) The frequency sweep of the G' and G'' of whey protein and blueberry fermented gels formed by fermentation with *Lactobacillus delbrueckii* 134 and *Streptococcus thermophilus* grx 02 after different fermentation times.

3.5. Water-Holding Capacity of Gels and Swelling Ratio

The WHC serves as an indicator of the water binding ability of the protein gel network. Decomposition and coagulation lead to protein chain linkage, resulting in a continuous network that traps water within the gel structure, contributing to the WHC. Figure 3a

shows the impact of fermentation time on WHC for different gels. Both systems exhibited a significant increase in WHC after 5 h of fermentation, with no further changes observed beyond this point. As shown in Figure 3a, the WHC of the mixed fermented gel was lower than that of the whey protein fermented gel. This is due to the polyphenols interacting with the whey protein during the fermentation process. The hydroxyl groups of the polyphenols from the blueberry could form more hydrogen bonds with water molecules, preventing the formation of hydrogen bonds with protein molecules. However, the hydrogen bonds formed by water with polyphenols are weaker than those formed with protein, which caused the water retention ability to be lower than that of the whey protein gel. Hydrogen bonds and hydrophobic interactions are the primary chemical forces affecting the WHC of gels [37]. This discrepancy may also be attributed to precipitation, whereby an abundance of hydrogen bonds on one chain could hinder the formation of linkages between polyphenols across peptide chains [22]. The interaction between polyphenols and proteins prevents water molecules from binding to gels [38]. The whiteness of the gels followed a similar trend as that of the WHC with increasing fermentation time, potentially due to the higher water content enhancing the brightness of the whey protein fermented gels. Higher gel strengths correspond to higher WHC values, due to the interactions between proteins forming a compact network [39]. As previously discussed, whey protein gels formed after 8 h of fermentation exhibited optimal hardness characteristics, as shown in Table 2.

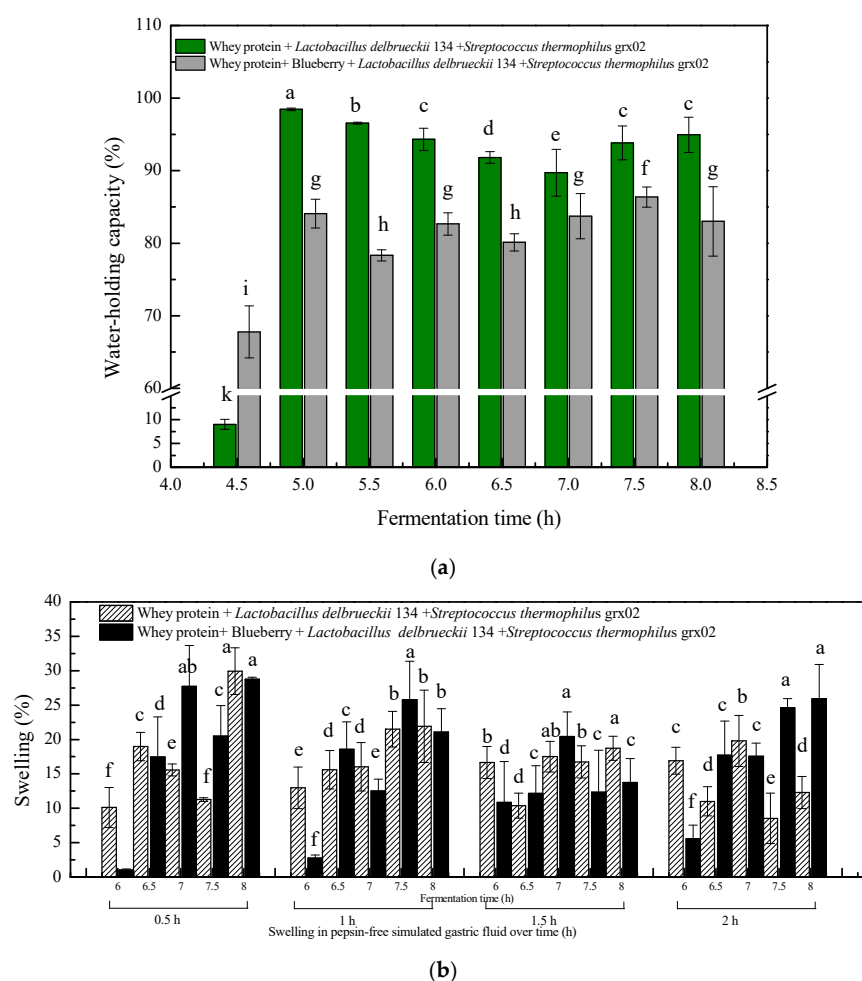


Figure 3. The water-holding capacity (a) of the fermented gels formed by fermentation with *Lactobacillus delbrueckii* 134 and *Streptococcus thermophilus* grx 02 after different fermentation times, and the swelling ratio (b) after in vitro digestion. For each sample, mean values with a common superscript letter for the same swelling time are not significantly different ($p > 0.05$).

As shown in Figure 3b, the swelling rate of the whey protein and blueberry mixed fermented gel, when it was digested for 2 h, was higher than that of the whey protein gel after fermentation for 7.5 h and 8 h. This was due to more polyphenols being exposed on the gel's surface and interacting with water molecules through hydrogen bonding. However, the interaction between whey protein gels and water molecules was lower, due to greater exposure of hydrophobic groups [40]. The gel network formed by whey protein was more dense, mainly due to the hydrophobic interactions and the interaction of disulfide bonds, forming a cross-linking structure. This could also have been a factor contributing to the markedly strengthened textural properties after 8.0 h of fermentation [41]. However, the swelling rate of the mixed fermented gel at 8 h was lower than that of the whey protein gel when the gels were digested for 0.5–1.5 h. This was mainly due to the release of polyphenol. As shown in Figure 4a, the release of polyphenol from the gel after fermentation for 8 h was highest when digested for 2 h.

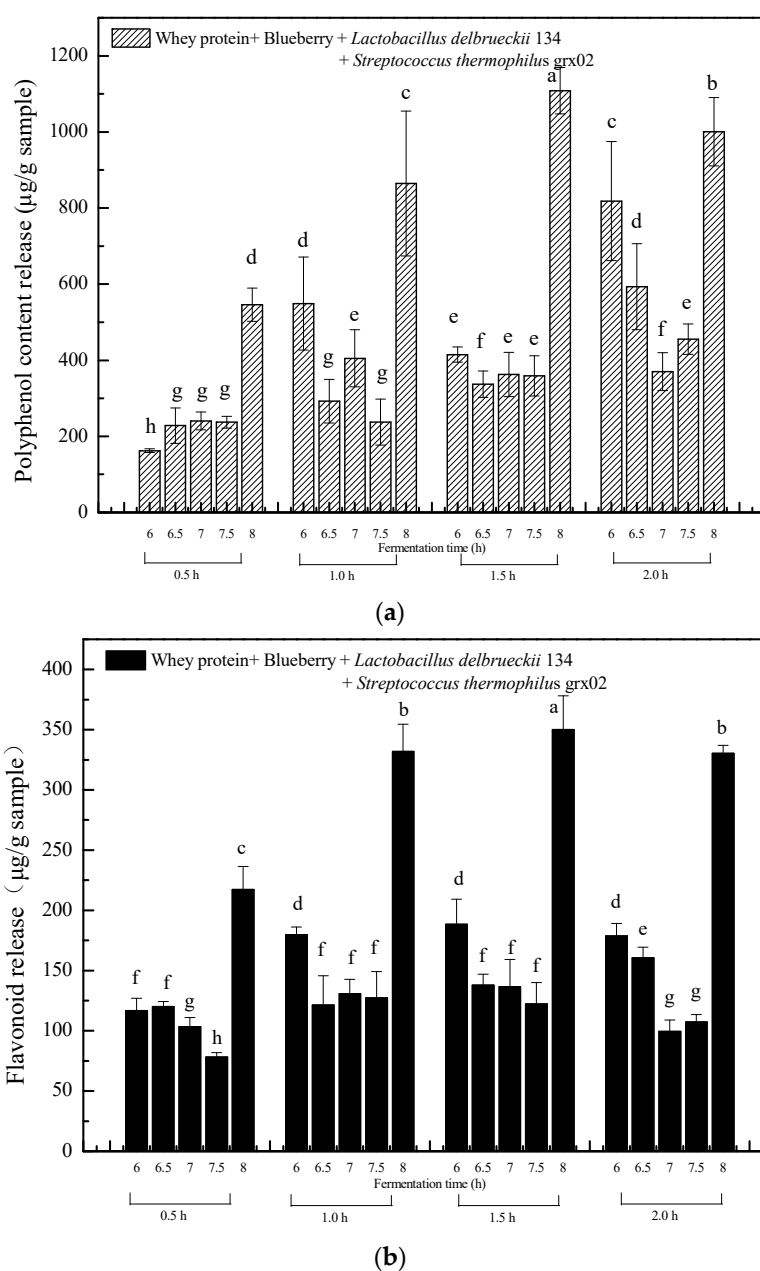


Figure 4. Cont.

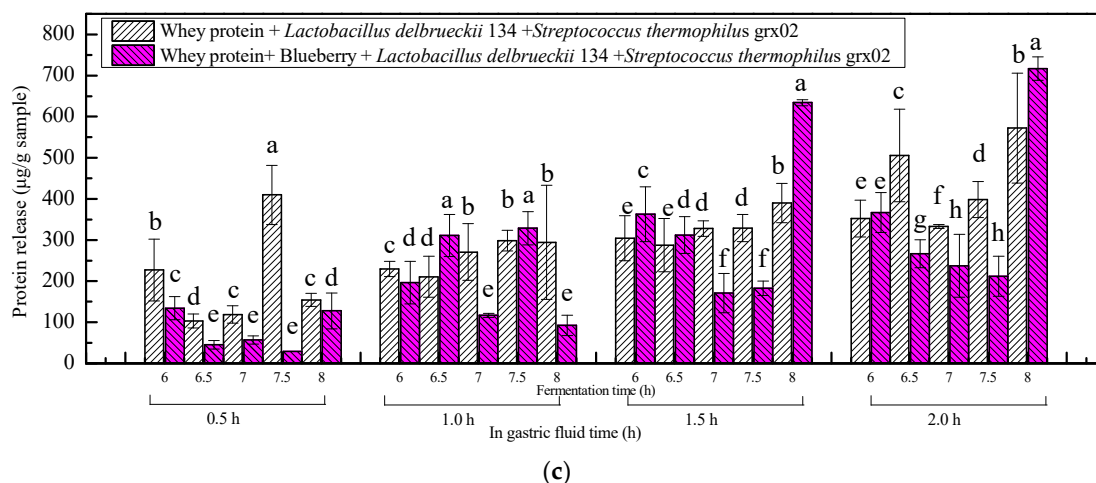


Figure 4. The release of polyphenols (a), flavonoids (b), and protein (c) by the fermented gels formed by fermentation with *Lactobacillus delbrueckii* 134 and *Streptococcus thermophilus* grx 02 after fermentation with different times, as a result of in vitro peptic degradation. For each sample, mean values with a common superscript letter for the same digestion time are not significantly different ($p > 0.05$).

3.6. Polyphenolic, Flavonoid, and Protein Release of Fermented Gels

3.6.1. Polyphenolic Release

As the protein–polyphenol polymer was gradually degraded in the gastric digestive fluid, polyphenol was released from the cross-linking network of the whey protein, and the polyphenol content increased at the end of digestion. As shown in Figure 4a, the polyphenol content of the whey protein–blueberry juice mixed gels fermented for 7 h did not change significantly from 0 to 2 h of digestion, indicating that the gels fermented for 7 h with blueberry juice were more suitable for storing polyphenols. It is possible that polyphenols and proteins have amino acid side chains, polyphenol aromatic rings, or hydrogen bonding interactions that help to generate soluble complexes. These interactions are crucial for the fortification and maintenance of these complexes. With the extension of digestion time, the gel structure was destroyed, and the polyphenol content in the mixed gels fermented for 8 h was the highest. It may be that after the 8 hours of fermentation of whey protein and blueberry by *L. delbrueckii* and *S. thermophilus*, acid production increased, and the polyphenol content in the protein did not decrease. Therefore, the release of polyphenols was increased with the extension of digestion time, and higher than after the other fermentation times, as shown in Figure 3a. The protein precipitated locally, causing the gel framework to collapse and additional pepsin and acid to enter the gel, releasing more polyphenols.

3.6.2. Flavonoid Release

As digestion progressed, the release of flavonoids did not change significantly (Figure 4b). At different digestion times, the release of flavonoids was higher in gel fermented for 8 h than in gel fermented for other times. From 0 to 1.5 h of digestion, the whey protein and blueberry juice fermented for 7.5 h had the lowest flavonoid release in vitro. As mentioned above, the highest hardness of the gel was obtained after 7.5 h of fermentation (Table 1). This was due to the flavonoids being hidden between proteins and their constituent molecules through electrostatic interactions and hydrogen bonding. This shielding effect prevented the flavonoids from being digested by the SGF. When the gels with a mixture of whey protein and blueberry juice were fermented for 8.0 h, the release of flavonoids was higher after 0.5–2 h of in vitro digestion. This is because of the

reduced ability of the protein to bind to flavonoids, which were vulnerable to destruction by the stomach acid environment after 8 h of fermentation. Furthermore, the extension of fermentation time can affect the binding ability of proteins and flavonoids. The ability of flavonoids to be released into the digestive tract is closely related to their antioxidant capacity in the body [42].

3.6.3. Protein Release

Gastric digestion was simulated in pepsin-supplemented SGF to observe the release of protein in the mixed fermented gel. The concentration of protein produced during the breakdown of the gels is shown in Figure 4c. As digestion progressed, the structure of the gels was progressively disrupted and hydrophobic amino acids were exposed, resulting in increased protein release at the end of digestion. During the first 0.5 h, the protein release capacity of the whey protein fermented gels was higher, and the whey protein–blueberry juice mixed fermented gels were more resistant to digestion. Comparing the different fermentation times, the protein release of the whey protein–blueberry juice mixed gels fermented for 7 h was low, which may indicate that the 7 h fermentation time was more conducive to the formation of the whey protein–blueberry juice mixed fermented gels. Furthermore, the gel matrix with the addition of blueberry was stable and hard, and was not easily destroyed by the SGF. The addition of blueberry juice increased the degree of cross-linking of whey protein molecules during fermentation [9]. The small, uniform pores formed by the gel hindered protein digestion, and the amount of protein released increased with an increase in digestion time. In addition, the polymer formed after the fermentation of the blueberry and whey protein gel for 6.5–7 h reduced the protein content during gel digestion [6]. Furthermore, compared with the whey protein gel fermented for 8 h, the whey protein–blueberry juice mixed gel fermented for 8 h had lower digestibility in the first hour, and was significantly degraded within the next 0.5 h. This is attributed to the blueberry polyphenols being bound to the hydrophobic amino acid residues of the whey proteins via hydrophobic forces, thereby decreasing pepsinolysis. Previous studies have highlighted that pepsin preferentially cleaves peptide bonds containing hydrophobic amino acids [20].

3.6.4. Viable Counts in Gels During Storage

The viable counts of the gels were determined from 0 to 10 days, as shown in Figure 5. The viable count of the whey protein fermented gel gradually decreased with the extension of storage time, and that of the fermented gel with a mixture of whey protein and blueberry juice first increased and then decreased. The viable counts of the whey protein fermented gels were higher than those of the whey protein–blueberry juice mixed fermented gels between 0 and 4 days of storage. The viable count of the mixed gel was $7.6 \log 7$ CFU/mL, which was significantly higher than that of the whey protein fermented gels ($5.6 \log 7$ CFU/mL) after 6 days of storage. This could be attributed to the fact that LAB efficiently consumed the proteins in the gels as nutrients to promote their growth during the early stages of storage. The polyphenols and whey proteins formed tighter polymers through hydrogen bonding and hydrophobic interactions. LAB growth in the fermented gels with a mixture of whey protein and blueberry juice was not as vigorous as that in the whey protein gel system. As the proteins were consumed during late storage, the structure of the gel was degraded. LAB had a higher utilization capacity in the mixed gels than in the protein gels after 6 days of storage, leading to a higher relative viable cell count. This result is consistent with the aforementioned change in SEM, as shown in Figure 1, and the uniform porous gel structure was able to preserve the bacteria and retain polyphenols.

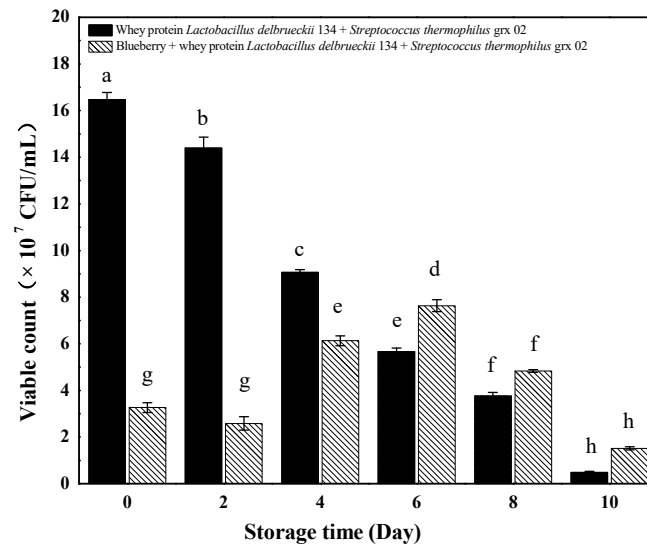


Figure 5. The viable counts of the gels formed by fermentation with *Lactobacillus delbrueckii* 134 and *Streptococcus thermophilus* grx 02 from 0 to 10 days of storage. The same letters in the figure indicate that the difference is not significant ($p > 0.05$), while different letters indicate that the difference is significant ($p < 0.05$).

4. Conclusions

Through visual imaging and electron microscopy, it was determined that the color of the gels fermented by *L. delbrueckii*- and *S. thermophilus*—both the mixed system of whey protein and blueberry juice and the single whey protein system—did not change significantly. Adding the blueberry juice gave the gels a lavender color. The whey protein and blueberry juice created a gel with a smoother surface and smaller pores within a short time. The gel with the highest hardness in terms of texture was the whey protein gel fermented for 8 h. The addition of blueberry juice increased the viscosity of the gel, but had little effect on the other properties of elasticity and hardness. The storage and loss moduli of the gels increased with increasing frequency. The whey protein gel formed after 7.5 h of fermentation had the greatest storage and loss moduli. The apparent viscosity of the fermented gels with a mixture of whey protein and blueberry juice decreased with increasing shearing, and had a rapid increase after fermentation for 5 h. The apparent viscosity of the gels fermented for 8 h was lower than that of the whey protein gels fermented for the same time. This may be because the interaction force between the whey protein molecules and polyphenols was reduced, resulting in reduced viscosity and rheological properties of the fermented gels with a mixture whey protein and blueberry juice. Further studies should evaluate the odor, taste, aroma, and nutritional aspects of the whey protein–blueberry juice mixed fermented gels. The interaction among the whey protein, blueberry juice, and LAB with regard to the regulation of intestinal health should also be investigated, because of the relationship between consumers' acceptance and the sensory attributes of the whey protein–blueberry juice mixed fermented gels. Furthermore, the fermented gels were formed under low temperature, which could protect nutrients. This research can be applied to the area of probiotic jellies or medical biogels. The fermented gels provide not only phenolic compounds and protein, but also probiotics.

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References

- Li, J.; Yang, J.; Li, J.; Gantumur, M.-A.; Wei, X.; Oh, K.-C.; Jiang, Z. Structure and Rheological Properties of Extruded Whey Protein Isolate: Impact of Inulin. *Int. J. Biol. Macromol.* **2023**, *226*, 1570–1578. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zhao, C.; Chen, N.; Ashaolu, T.J. Whey Proteins and Peptides in Health-Promoting Functions—A Review. *Int. Dairy J.* **2022**, *126*, 105269. [\[CrossRef\]](#)
- Yang, X.; Su, Y.; Li, L. Study of Soybean Gel Induced by *Lactobacillus Plantarum*: Protein Structure and Intermolecular Interaction. *LWT* **2020**, *119*, 108794. [\[CrossRef\]](#)
- Pang, Z.; Xu, R.; Zhu, Y.; Li, H.; Bansal, N.; Liu, X. Comparison of Rheological, Tribological, and Microstructural Properties of Soymilk Gels Acidified with Glucono- Δ -Lactone or Culture. *Food Res. Int.* **2019**, *121*, 798–805. [\[CrossRef\]](#) [\[PubMed\]](#)
- Sieuwerds, S.; De Bok, F.A.M.; Hugenholtz, J.; van Hylckama Vlieg, J.E.T. Unraveling Microbial Interactions in Food Fermentations: From Classical to Genomics Approaches. *Appl. Environ. Microbiol.* **2008**, *74*, 4997–5007. [\[CrossRef\]](#) [\[PubMed\]](#)
- Yang, X.; Ke, C.; Li, L. Physicochemical, Rheological and Digestive Characteristics of Soy Protein Isolate Gel Induced by Lactic Acid Bacteria. *J. Food Eng.* **2021**, *292*, 110243. [\[CrossRef\]](#)
- Klost, M.; Giménez-Ribes, G.; Drusch, S. Enzymatic Hydrolysis of Pea Protein: Interactions and Protein Fractions Involved in Fermentation Induced Gels and Their Influence on Rheological Properties. *Food Hydrocoll.* **2020**, *105*, 105793. [\[CrossRef\]](#)
- Ren, Y.; Li, L. The Influence of Protease Hydrolysis of Lactic Acid Bacteria on the Fermentation Induced Soybean Protein Gel: Protein Molecule, Peptides and Amino Acids. *Food Res. Int.* **2022**, *156*, 111284. [\[CrossRef\]](#)
- Wang, W.; Zhang, J.; Yu, Q.; Zhou, J.; Lu, M.; Gu, R.; Huang, Y. Structural and Compositional Changes of Whey Protein and Blueberry Juice Fermented Using *Lactobacillus Plantarum* or *Lactobacillus Casei* During Fermentation. *RSC Adv.* **2021**, *11*, 26291–26302. [\[CrossRef\]](#)
- Chen, D.; Zhu, X.; Ilavsky, J.; Whitmer, T.; Hatzakis, E.; Jones, O.G.; Campanella, O.H. Polyphenols Weaken Pea Protein Gel by Formation of Large Aggregates with Diminished Noncovalent Interactions. *Biomacromolecules* **2021**, *22*, 1001–1014. [\[CrossRef\]](#)
- Zhao, X.; Li, C.; Xue, F. Effects of Whey Protein-Polyphenol Conjugates Incorporation on Physicochemical and Intelligent Ph-Sensing Properties of Carboxymethyl Cellulose Based Films. *Future Foods* **2023**, *7*, 100211. [\[CrossRef\]](#)
- Baba, W.N.; McClements, D.J.; Maqsood, S. Whey Protein-Polyphenol Conjugates and Complexes: Production, Characterization, and Applications. *Food Chem.* **2021**, *365*, 130455. [\[CrossRef\]](#)
- Kar, A.; Bornhorst, G.M. Ultrasound-Treated Hybrid Protein Gels from Pea and Whey: A Comparison of Gastric Breakdown Mechanisms with Commercial Protein-Based Foods. *Food Res. Int.* **2025**, *203*, 115856. [\[CrossRef\]](#)
- Li, A.; Shewan, H.M.; Flanagan, B.M.; Williams, B.A.; Mikkelsen, D.; Gidley, M.J. Impact of Pectin and Alginate Gel Particle Size and Concentration on in Vitro Gut Fermentation. *Food Hydrocoll.* **2025**, *160*, 110808. [\[CrossRef\]](#)
- Yu, Q.; Wang, W.; Liu, X.; Shen, W.; Gu, R.; Tang, C. The Antioxidant Activity and Protection of Probiotic Bacteria in the in Vitro Gastrointestinal Digestion of a Blueberry Juice and Whey Protein Fermentation System. *Fermentation* **2023**, *9*, 335. [\[CrossRef\]](#)
- Wang, Y.; Liu, X.; Shao, Y.; Guo, Y.; Gu, R.; Wang, W. Cheese Whey Protein and Blueberry Juice Mixed Fermentation Enhance the Freeze-Resistance of Lactic Acid Bacteria in the Freeze-Drying Process. *Foods* **2024**, *13*, 2260. [\[CrossRef\]](#)
- Yu, Q.; Lu, G.; Wang, W.; Tang, C.; Gu, R. The Mechanism of Whey Protein and Blueberry Juice Mixed System Fermented with *Lactobacillus* Inhibiting *Escherichia Coli* During Storage. *Sci. Rep.* **2023**, *13*, 6614. [\[CrossRef\]](#)
- Beghdadi, A.; Picart-Palmade, L.; Cunault, C.; Marchesseau, S.; Chevalier-Lucia, D. Impact of Two Thermal Processing Routes on Protein Interactions and Acid Gelation Properties of Casein Micelle-Pea Protein Mixture Compared to Casein Micelle-Whey Protein One. *Food Res. Int.* **2022**, *155*, 111060. [\[CrossRef\]](#)
- Zhang, T.; Xia, Y.; Peng, Q. Steviol Glycosides Improve the Textural Properties of Whey Protein Gel. *J. Food Eng.* **2024**, *369*, 111912. [\[CrossRef\]](#)
- Hazrati, Z.; Madadlou, A. Gelation by Bioactives: Characteristics of the Cold-Set Whey Protein Gels Made Using Gallic Acid. *Int. Dairy J.* **2021**, *117*, 104952. [\[CrossRef\]](#)

21. Deng, R.; Mars, M.; Van Der Sman, R.G.M.; Smeets, P.A.M.; Janssen, A.E.M. The Importance of Swelling for in Vitro Gastric Digestion of Whey Protein Gels. *Food Chem.* **2020**, *330*, 127182. [[CrossRef](#)] [[PubMed](#)]
22. Zahid, H.F.; Ali, A.; Ranadheera, C.S.; Fang, Z.; Dunshea, F.R.; Ajlouni, S. In Vitro Bioaccessibility of Phenolic Compounds and Alpha-Glucosidase Inhibition Activity in Yoghurts Enriched with Mango Peel Powder. *Food Biosci.* **2022**, *50*, 102011. [[CrossRef](#)]
23. Li, L.; Zhang, H.; Liu, Z.; Cui, X.; Zhang, T.; Li, Y.; Zhang, L. Comparative Transcriptome Sequencing and De Novo Analysis of *Vaccinium Corymbosum* During Fruit and Color Development. *BMC Plant Biol.* **2016**, *16*, 223. [[CrossRef](#)] [[PubMed](#)]
24. Chen, Z.; Kang, J.; Zhang, Y.; Yi, X.; Pang, X.; Li-Byarlay, H.; Gao, X. Differences in the Bacterial Profiles and Physicochemical between Natural and Inoculated Fermentation of Vegetables from Shanxi Province. *Ann. Microbiol.* **2020**, *70*, 66. [[CrossRef](#)]
25. Wang, J.; Xu, L.; Lv, Y.; Su, Y.; Gu, L.; Chang, C.; Zhang, M.; Yang, Y.; Li, J. To Improve the Gel Properties of Liquid Whole Egg by Short-Term Lactic Acid Bacteria Fermentation. *Innov. Food Sci. Emerg. Technol.* **2022**, *75*, 102873. [[CrossRef](#)]
26. Qian, Z.; Dong, S.; Zhong, L.; Zhan, Q.; Hu, Q.; Zhao, L. Effects of Carboxymethyl Chitosan on the Gelling Properties, Microstructure, and Molecular Forces of *Pleurotus Eryngii* Protein Gels. *Food Hydrocoll.* **2023**, *145*, 109158. [[CrossRef](#)]
27. Wagner, J.; Andreadis, M.; Nikolaidis, A.; Biliaderis, C.G.; Moschakis, T. Effect of Ethanol on the Microstructure and Rheological Properties of Whey Proteins: Acid-Induced Cold Gelation. *LWT* **2021**, *139*, 110518. [[CrossRef](#)]
28. Yakubu, C.M.; Sharma, R.; Sharma, S.; Singh, B. Influence of Alkaline Fermentation Time on in Vitro Nutrient Digestibility, Bio- & Techno-Functionality, Secondary Protein Structure and Macromolecular Morphology of Locust Bean (*Parkia biglobosa*) Flour. *LWT* **2022**, *161*, 113295. [[CrossRef](#)]
29. Herrera-Balandrano, D.D.; Chai, Z.; Beta, T.; Feng, J.; Huang, W. Blueberry Anthocyanins: An Updated Review on Approaches to Enhancing Their Bioavailability. *Trends Food Sci. Technol.* **2021**, *118*, 808–821. [[CrossRef](#)]
30. Vasile, F.E.; Hryczyński, L.M.; Fernandez, A.B.; Bustos, L.F.; Romero, A.M.; Mazzobre, M.F. Exploring the Sensory Properties of Buffalo (*Bubalus bubalis*) Milk Custards through a Consumer-Based Study Performed with Children. *Int. J. Dairy Technol.* **2023**, *76*, 252–260. [[CrossRef](#)]
31. von Staszewski, M.; Jagus, R.J.; Pilosof, A.M.R. Influence of Green Tea Polyphenols on the Colloidal Stability and Gelation of Wpc. *Food Hydrocoll.* **2011**, *25*, 1077–1084. [[CrossRef](#)]
32. Guo, X.; Wei, Y.; Liu, P.; Deng, X.; Zhu, X.; Wang, Z.; Zhang, J. Study of Four Polyphenol-Coregonus Peled (C. Peled) Myofibrillar Protein Interactions on Protein Structure and Gel Properties. *Food Chem. X* **2024**, *21*, 101063. [[CrossRef](#)] [[PubMed](#)]
33. Yang, S.; Tao, L.; Yan, Z.; Ouyang, K.; Zhang, Q.; Feng, Y.; Zhao, Q. Role of Heat Pretreatment and Transglutaminase Treatment on the Gelling Properties of Pea Protein. *Food Biosci.* **2025**, *66*, 106166. [[CrossRef](#)]
34. Mudau, C.P.K.; Moutkane, M.; Balakrishnan, G.; Nicolai, T.; Chassenieux, C. Heat-Induced Aggregation and Gelation of Rapeseed Proteins. *Food Hydrocoll.* **2025**, *166*, 111338. [[CrossRef](#)]
35. Wang, Z.; Xiao, N.; Guo, S.; Tian, X.; Ai, M. Tea Polyphenol-Mediated Network Proteins Modulate the Naoh-Heat Induced Egg White Protein Gelling Properties. *Food Hydrocoll.* **2024**, *149*, 109514. [[CrossRef](#)]
36. Wu, D.; Zhou, J.; Shen, Y.; Lupo, C.; Sun, Q.; Jin, T.; Sturla, S.J.; Liang, H.; Mezzenga, R. Highly Adhesive Amyloid–Polyphenol Hydrogels for Cell Scaffolding. *Biomacromolecules* **2023**, *24*, 471–480. [[CrossRef](#)] [[PubMed](#)]
37. Ni, X.; Li, M.; Huang, Z.; Wei, Y.; Duan, C.; Li, R.; Fang, Y.; Wang, X.; Xu, M.; Yu, R. Study on the Regulation of Tea Polyphenols on the Structure and Gel Properties of Myofibrillar Protein from Neosalanx Taihuensis. *Food Chem. X* **2025**, *25*, 102243. [[CrossRef](#)]
38. Peng, C.; Liu, B.; Chen, Z. Protective Effects of Enzymatic Hydrolysis Products of Pomelo Peel Cellulose on *Lactobacillus Plantarum* During Freeze-Drying Process. *Appl. Food Res.* **2023**, *3*, 100301. [[CrossRef](#)]
39. Wang, Z.; Chen, F.; Deng, Y.; Tang, X.; Li, P.; Zhao, Z.; Zhang, M.; Liu, G. Texture Characterization of 3d Printed Fibrous Whey Protein-Starch Composite Emulsion Gels as Dysphagia Food: A Comparative Study on Starch Type. *Food Chem.* **2024**, *458*, 140302. [[CrossRef](#)]
40. Wan, Z.; Xiao, N.; Guo, S.; Tian, X.; Ai, M. Synergistic Effects of Tea Polyphenols and Phosphorylation on the Gelation Behavior of Egg White Proteins. *Food Hydrocoll.* **2024**, *149*, 109530. [[CrossRef](#)]
41. Wang, J.; Zhou, L.; Xing, L.; Zhou, G.; Zhang, W. Mechanism of Toona Sinensis Seed Polyphenols Inhibiting Oxidation and Modifying Physicochemical and Gel Properties of Pork Myofibrillar Protein under Oxidation System. *Food Chem.* **2025**, *464*, 141666. [[CrossRef](#)] [[PubMed](#)]
42. Balakrishnan, G.; Schneider, R.G. Quinoa Flavonoids and Their Bioaccessibility During in Vitro Gastrointestinal Digestion. *J. Cereal Sci.* **2020**, *95*, 103070. [[CrossRef](#)]

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