

Article

Effects of $\text{CaCO}_3/\text{NaHCO}_3$ on Porous Structure and Quality Characteristics of Protein–Starch Gel Network Formed via Extrusion

Feng Guo [†], Huan Zhou [†], Hui Hu, Anna Hu, Qiang Wang ^{*} and Jinchuang Zhang ^{*} 

Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences/Key Laboratory of Agro-Products Processing, Ministry of Agriculture and Rural Affairs, Beijing 100193, China

^{*} Correspondence: wangqiang06@caas.cn (Q.W.); zhangjinchuang1002@163.com (J.Z.)

[†] These authors contributed equally to this work.

Abstract

CaCO_3 and NaHCO_3 , respectively serving as chemical leavening agents, can promote the expansion of protein or starch extrudates, thereby forming a porous structure. However, the characteristics of this porous structure under the combined regulation of CaCO_3 and NaHCO_3 remained unclear. The results indicated that increasing the proportion of NaHCO_3 promoted the expansion of the extruded protein–starch gel network, with its expansion ratio significantly increasing from 2.29 to 3.17 ($p < 0.05$). This expansion resulted in larger pores, which corroborated the observed significant increase in water holding capacity (WHC). Conversely, an increase in the proportion of CaCO_3 led to a denser porous structure accompanied by a reduction in WHC. Meanwhile, the extrudate with a $\text{CaCO}_3/\text{NaHCO}_3$ ratio of 0:2 exhibited the lowest hardness, measuring 8.87 N. As the proportion of NaHCO_3 increased, the pH shifted toward the alkaline range. This increase in pH enhanced the flexibility of the protein structure, leading to a significant rise in the proportion of disordered structures in the protein secondary structure, such as random coil and β -sheet, which facilitated the formation of an elastic gel network. In conclusion, both CaCO_3 and NaHCO_3 significantly modulated the porous structure of the protein–starch gel network formed during extrusion. This provides a new perspective for investigating the relationship between the protein–starch gel network and the quality characteristics of extruded products.

Keywords: protein; starch; $\text{CaCO}_3/\text{NaHCO}_3$; porous structure; quality



Academic Editor: Maria
Valentina Dinu

Received: 26 December 2025

Revised: 5 February 2026

Accepted: 16 February 2026

Published: 20 February 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons Attribution \(CC BY\) license](https://creativecommons.org/licenses/by/4.0/).

1. Introduction

With the continuous growth of the global population, the demand for dietary protein is steadily increasing, which is expected to lead to a shortage of protein resources. The utilization of alternative proteins (e.g., from plants, microorganisms, insects, or food processing by-products) can significantly alleviate this challenge [1]. As the most abundantly available category, plant proteins can be extracted from protein-rich crops including cereals, legumes, and oilseeds [2]. Peanuts, as an oilseed crop, contain 24–36% protein. The peanut protein derived as a by-product of oil extraction exhibits a high digestibility coefficient (up to 90%), is free from or low in flatulence factors and anti-nutritional factors, and possesses high nutritional value [3]. However, peanut protein is often considered a by-product of peanut oil processing and rarely used. The application of peanut protein in puffed foods is beneficial for enhancing the utilization rate of peanut by-products [4]. Conventional

puffed snacks typically have a high starch content. Their high digestibility results in a high glycemic index (GI), which is detrimental to the dietary health of individuals with obesity or diabetes. Currently, incorporating protein into these snacks presents an effective strategy to mitigate their high GI and enhance their nutritional profile by increasing protein content. One study revealed that explosion puffing of a mixture containing surimi and glutinous rice starch at a 1:4 ratio significantly enhanced the starch's digestibility resistance, leading to a 17% increase in its resistant starch content [5].

Extrusion technology is an efficient physical processing method that integrates multiple unit operations including mixing, shearing, heating, and shaping. This technique offers advantages such as high energy utilization, strong raw material adaptability, and low pollutant emissions, making it widely used in puffed snack production [6]. Starch and protein are the two most important polymers in extruded puffed foods. During extrusion, starch undergoes gelatinization to form a viscous melt. It effectively retains water vapor bubbles, thereby enhancing the expansion puffing of the product. Meanwhile, the protein is subjected to thermal and shear forces within the barrel. This leads to the cleavage of disulfide bonds and protein denaturation. This is followed by the formation of new chemical interactions, such as ionic bonds, hydrogen bonds, and hydrophobic interactions, which facilitate the aggregation and cross-linking of protein molecules [7]. However, during the extrusion process, a higher protein content leads to more extensive filling by starch, resulting in a denser protein–starch gel network. This, in turn, produces pore structures with a hard texture after extrusion, which are unfavorable for chewing and rehydration [8].

Chemical leavening agents such as NaHCO_3 and CaCO_3 decompose upon heating to release CO_2 gas that, combined with steam, provides an expansion force. This expansive force is responsible for the development of a highly porous structure in the extrudate, resulting from enhanced puffing and increased porosity. Zhang et al. [9] investigated the effect of NaHCO_3 level on extrusion puffing. It was found that the expansion ratio of the extrudate first increased and then decreased with higher NaHCO_3 content. The initial increase was attributed to enhanced gas bubble formation within the melt, leading to larger pores. In contrast, the subsequent decrease resulted from the structural collapse and coalescence of pores. In another study, the addition of NaHCO_3 to noodles by extrusion resulted in a uniform porous structure, formed by the CO_2 released from its thermal decomposition. The porosity increased from 4.16 to a maximum of 19.78, which consequently enhanced the water absorption capacity of the noodles [10]. Furthermore, CaCO_3 can act as a nucleating agent, causing steam to form bubbles around its fine particles. This mechanism results in a more uniform distribution of pores within the extrudate, which subsequently influences its textural properties and rehydration capacity [11]. Janve and Hal [12] observed that incorporating calcium carbonate (CaCO_3) into rice flour during extrusion promoted the formation of a uniformly porous structure in the extrudate. This microstructural change consequently led to significant decreases in product density, expansion ratio, and hardness.

While numerous studies have investigated the individual effects of adding either NaHCO_3 or CaCO_3 on the pore structure and quality characteristics of starch-based extrudates, research on the combined effects of NaHCO_3 and CaCO_3 on extrudates is limited. This study investigated the differences in the porous structure and quality characteristics of peanut protein–starch extrudates produced with varying blending ratios of NaHCO_3 and CaCO_3 . Changes in protein secondary structure, rheological properties, and protein–starch interactions were also examined. Furthermore, this study elucidated the formation mechanism of the gel network structure under different $\text{NaHCO}_3/\text{CaCO}_3$ ratios, thereby explaining the reasons for the differences in the pore structure and quality characteristics of the extrudates.

2. Results and Discussion

2.1. Color and Textural Properties Analysis

The color changes in extrudates under different $\text{CaCO}_3/\text{NaHCO}_3$ ratios are shown in Table 1. The high temperature and high shear forces during the extrusion process caused the degradation of the starch structure. The resulting product could undergo a Maillard reaction with the protein structure, ultimately leading to a change in color [13]. With an increasing proportion of NaHCO_3 , the *a* and *b* value increased significantly, indicating a more yellow coloration, which was accompanied by an increase in ΔE . This trend is likely due to the alkaline environment induced by NaHCO_3 , wherein flavonoid compounds present in peanut protein impart a progressively stronger yellow hue to the extrudates under weakly basic conditions [14]. Furthermore, when the proportion of NaHCO_3 exceeded that of CaCO_3 , a significant increase in the expansion ratio of the protein extrudates was observed, accompanied by a concurrent decrease in density (Figure 1a). Specifically, the expansion ratio increased from 2.29 ± 0.05 to 3.17 ± 0.05 , while the density decreased from a maximum of $0.31 \pm 0.01 \text{ g/cm}^3$ to $0.20 \pm 0.01 \text{ g/cm}^3$. This indicated that a higher NaHCO_3 ratio promotes the puffing of extrudates, which is likely attributable to its thermal decomposition during extrusion, releasing carbon dioxide gas that provides the expansion force. Simultaneously, Figure 1b indicated that the hardness of the extrudates significantly decreased from $23.44 \pm 2.08 \text{ N}$ to $8.87 \pm 0.71 \text{ N}$ with an increasing NaHCO_3 ratio, while the fracture distance markedly reduced from $0.35 \pm 0.04 \text{ mm}$ to $0.10 \pm 0.03 \text{ mm}$ ($p < 0.05$). These textural changes corroborate that the enhanced expansion degree facilitates a reduction in hardness and an improvement in crispiness. This finding was consistent with the results reported by Huang et al. [15].

Table 1. Color changes in peanut protein–starch extrudates with different $\text{CaCO}_3/\text{NaHCO}_3$ ratios (2:0, 1.5:0.5, 1:1, 0.5:1.5, 0:2).

Color	2:0	1.5:0.5	1:1	0.5:1.5	0:2
L	$85.55 \pm 0.04 \text{ c}$	$87.29 \pm 0.22 \text{ a}$	$86.24 \pm 0.13 \text{ b}$	$85.17 \pm 0.28 \text{ c}$	$84.16 \pm 0.30 \text{ d}$
a	$0.87 \pm 0.01 \text{ e}$	$1.55 \pm 0.02 \text{ d}$	$2.07 \pm 0.04 \text{ c}$	$2.94 \pm 0.02 \text{ a}$	$2.69 \pm 0.08 \text{ b}$
b	$9.45 \pm 0.05 \text{ d}$	$9.32 \pm 0.17 \text{ d}$	$10.90 \pm 0.16 \text{ c}$	$14.47 \pm 0.07 \text{ b}$	$17.31 \pm 0.24 \text{ a}$
ΔE	$8.65 \pm 0.04 \text{ d}$	$7.87 \pm 0.10 \text{ e}$	$9.76 \pm 0.13 \text{ c}$	$13.56 \pm 0.13 \text{ b}$	$16.52 \pm 0.16 \text{ a}$

Note: Different letters in the same row indicate significantly different ($p < 0.05$).

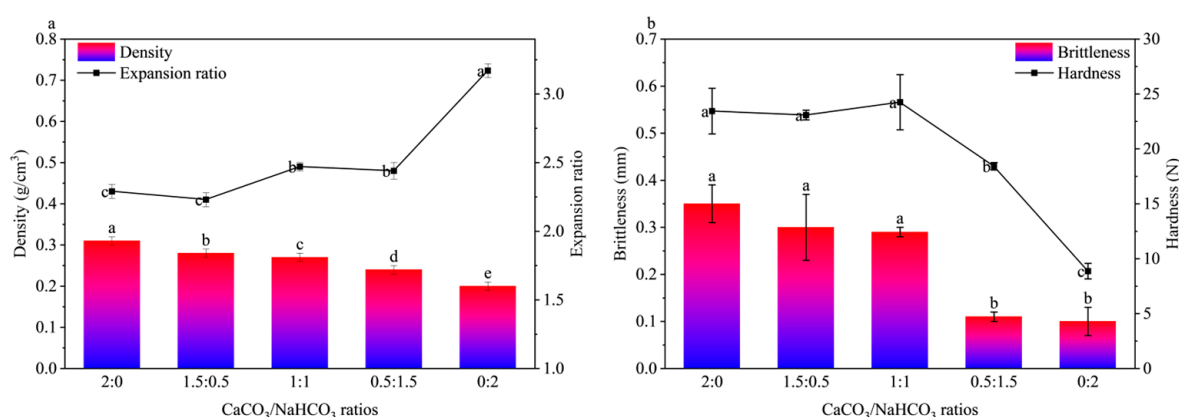


Figure 1. (a) Density and expansion ratio; (b) brittleness and hardness of peanut protein–starch extrudates with different $\text{CaCO}_3/\text{NaHCO}_3$ ratios (2:0, 1.5:0.5, 1:1, 0.5:1.5, 0:2). Different letters in the figures denote statistically significant differences ($p < 0.05$).

2.2. Macro- and Microstructure Analysis

As illustrated in the macroscopic structure images (Figure 2), an increased NaHCO_3 ratio promoted a gradual enlargement in the radial expansion of the extrudates. At a

CaCO_3 to NaHCO_3 ratio of 0:2, surface shrinkage was observed. This phenomenon was likely attributable to the high NaHCO_3 proportion, which led to an excessive accumulation of CO_2 gas during expansion [16]. This gas acted synergistically with vaporized water, increasing the escape velocity of the expanding gases from the extrudates. Consequently, severe internal collapse occurred, adversely affecting the surface smoothness of the extrudates. Furthermore, a higher NaHCO_3 ratio resulted in a noticeable enlargement of the porous structure, as evidenced by the SEM images (Figure 2). Conversely, a higher CaCO_3 proportion yielded a dense and uniformly distributed porous structure. As the CaCO_3 ratio decreased, a loose pore morphology became dominant, accompanied by an increase in pore size. Therefore, NaHCO_3 played a crucial role in the puffing of extrudates, whereas CaCO_3 appeared to inhibit expansion and suppress the increase in pore size.

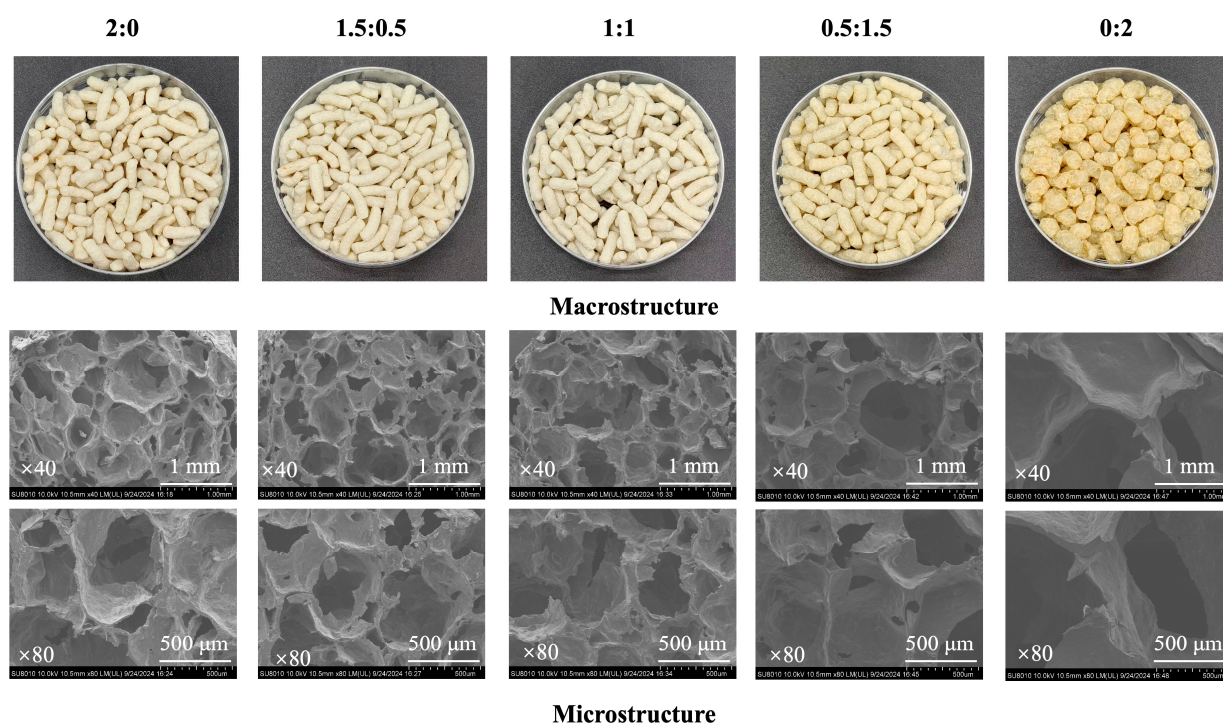


Figure 2. Macro- and microstructure of peanut protein–starch extrudates with different $\text{CaCO}_3/\text{NaHCO}_3$ ratios (2:0, 1.5:0.5, 1:1, 0.5:1.5, 0:2).

2.3. WHC and pH Value Changes

It is indicated in Figure 3 that the WHC of the extrudates generally increased with a higher NaHCO_3 ratio, reaching a maximum of 5.75 ± 0.26 g/g at a CaCO_3 to NaHCO_3 ratio of 1:1. Concurrently, the pH value exhibited a gradual increase from 7.07 ± 0.02 to a maximum of 9.31 ± 0.04 . It was proposed that the WHC of extrudates was enhanced by the moderate addition of CaCO_3 and NaHCO_3 (1:1), thereby improving their rehydration properties. This was likely due to the distinct yet complementary roles played by these two chemical agents during extrusion: (1) NaHCO_3 increased the pH of the protein–starch system. The alkaline conditions induced protein denaturation, exposing more binding sites for starch and facilitating the formation of a gel network that effectively trapped water molecules. (2) CaCO_3 promoted the formation of a denser porous structure within the extrudates. This structure created capillary action, which helped to immobilize water molecules, leading to increased WHC and improved rehydration [17].

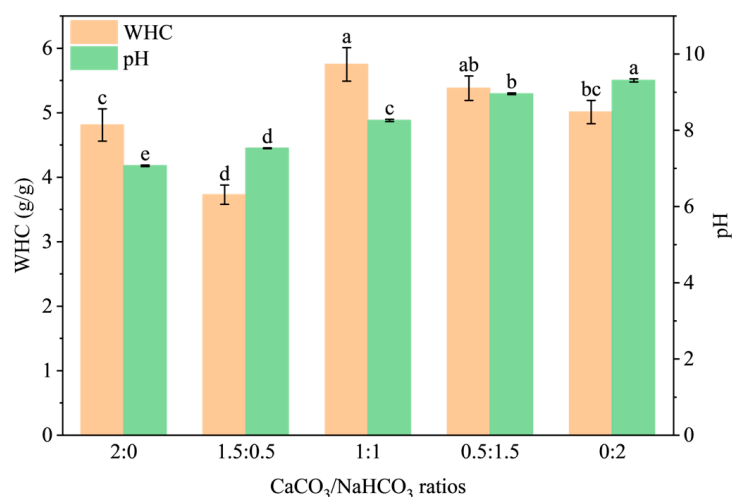


Figure 3. Water holding capacity and pH value changes in peanut protein–starch extrudates with different CaCO₃/NaHCO₃ ratios (2:0, 1.5:0.5, 1:1, 0.5:1.5, 0:2). Different letters in the figures denote statistically significant differences ($p < 0.05$).

2.4. Rheological Properties

The K and n values presented in Table 2 were obtained by fitting the power-law model to the steady shear data of the extrudates, representing their flow characteristics. When the CaCO₃/NaHCO₃ ratio was 0:2, the apparent viscosity was higher than all other groups, with the largest K value of 117.38 ± 1.72 Pa·s and the smallest n value of 0.22 ± 0.002 . This indicates that a higher proportion of NaHCO₃ increased the viscosity of the extrudate, likely due to enhanced protein interactions under weakly alkaline conditions, which reduced its fluidity. In contrast, for ratios of 1.5:0.5 and 1:1, the K and n values reached their lowest and highest levels, respectively, suggesting improved flow behavior of the extrudate. As shown in Figure 4a, the viscosity of all extrudates decreased with increasing shear rate, exhibiting typical shear-thinning behavior characteristic of pseudoplastic non-Newtonian fluids [18].

Table 2. The steady shear flow curves of the peanut protein–starch extrudates were fitted to a power-law model to obtain the consistency index (K), flow behavior index (n), and correlation coefficient (R^2).

CaCO ₃ /NaHCO ₃	K /Pa·s	n	R^2
2:0	31.71 ± 1.34 c	0.37 ± 0.002 c	0.99
1.5:0.5	15.98 ± 0.38 d	0.43 ± 0.001 b	0.99
1:1	17.24 ± 1.28 d	0.48 ± 0.002 a	0.99
0.5:1.5	65.92 ± 3.41 b	0.36 ± 0.000 c	0.99
0:2	117.38 ± 1.72 a	0.22 ± 0.002 d	0.97

Note: Different letters in the same column indicate significantly different ($p < 0.05$).

Furthermore, the storage modulus (G') and loss modulus (G'') of the extrudates were used to indirectly evaluate their viscoelastic properties (Figure 4b,c), providing insight into the interactions between proteins and starch in the extrudates. Under the influence of CaCO₃/NaHCO₃, both G' and G'' of the extrudates increased, with G' exceeding G'' , indicating that elasticity predominates in the gel system. At a CaCO₃/NaHCO₃ ratio of 1:1, both G' and G'' reached their maximum values, indicating an increase in the modulus of the extrudate system. The loss factor ($\tan \delta$) values were all below 1, suggesting a solid-like behavior of the system [19]. Notably, $\tan \delta$ decreased initially and then increased with angular frequency (ω) (Figure 4d), which may be attributed to the predominance of elastic behavior at low frequencies and viscous behavior at high frequencies.

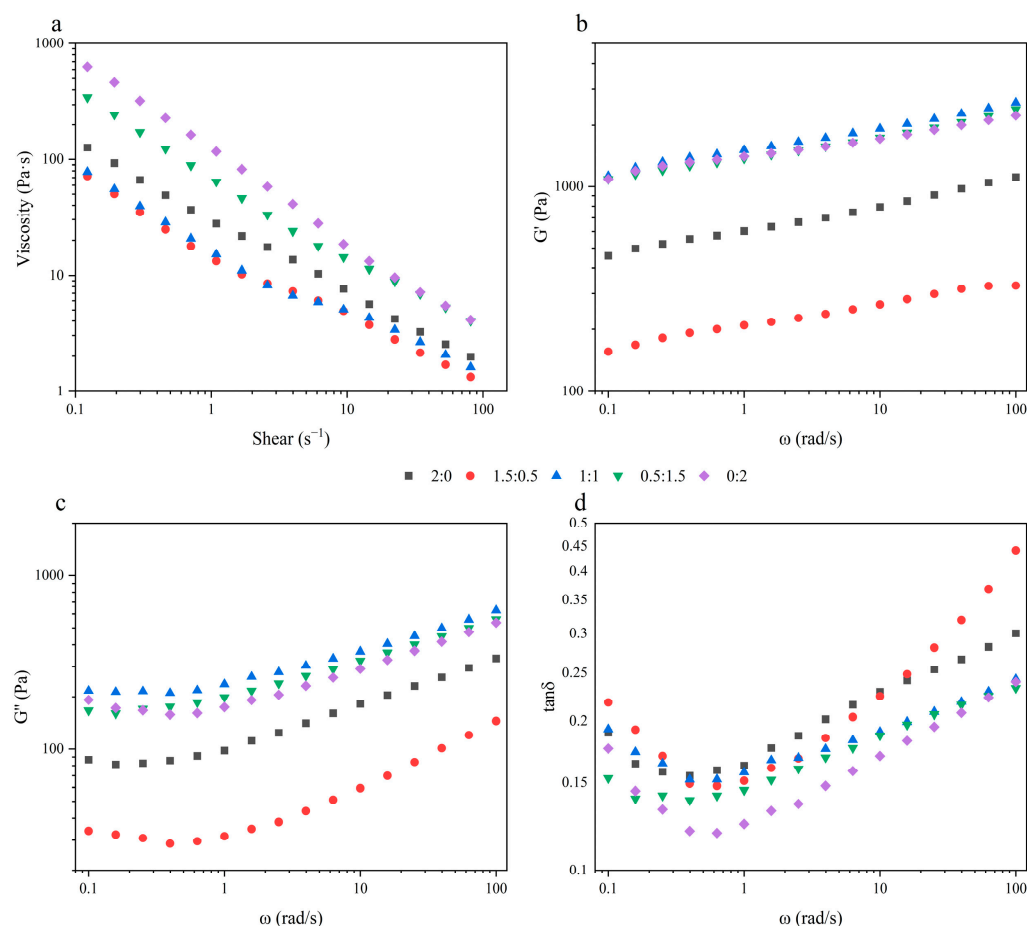


Figure 4. Shear stress-viscosity relationship (a), variation in storage modulus (b), variation in loss modulus (c), and loss angle (d) of peanut protein–starch extrudates with different $\text{CaCO}_3/\text{NaHCO}_3$ ratios (2:0, 1.5:0.5, 1:1, 0.5:1.5, 0:2).

2.5. Secondary Structure of Protein via FTIR

The amide I region ($1700\text{--}1600\text{ cm}^{-1}$) in Fourier transform infrared spectroscopy provides information on the secondary structure of protein in extrudates and can be used to investigate structural changes in protein [20]. The α -helix and β -sheet are generally regarded as rigid secondary structures in protein conformation. As shown in Table 3, with increasing NaHCO_3 addition, the relative content of β -sheet in the extrudate protein secondary structure decreased from $40.57 \pm 1.63\%$ to $36.49 \pm 0.19\%$, while the α -helix content increased from $20.54 \pm 0.41\%$ to $22.77 \pm 0.34\%$. This shift may be attributed to the alkaline pH shift induced by higher NaHCO_3 levels, which could promote the partial conversion of α -helix to β -sheet. When the pH moves away from the isoelectric point toward the alkaline range, enhanced electrostatic repulsion may disrupt intermolecular and intramolecular protein interactions, leading to a more flexible protein conformation [21]. Previous studies have reported a decrease in the proportion of rigid β -sheet structures under alkaline pH conditions, which is consistent with the findings of this work [17]. And then, the random coil and β -turn of the secondary structure of proteins in the extruded products were regarded as the disordered structure of the protein structure. The sum of these two increased as the $\text{CaCO}_3/\text{NaHCO}_3$ decreased, indicating that the protein structure became disordered. This might be attributed to the gradual increase in pH caused by the increasing sodium bicarbonate, which led to the denaturation of the protein structure.

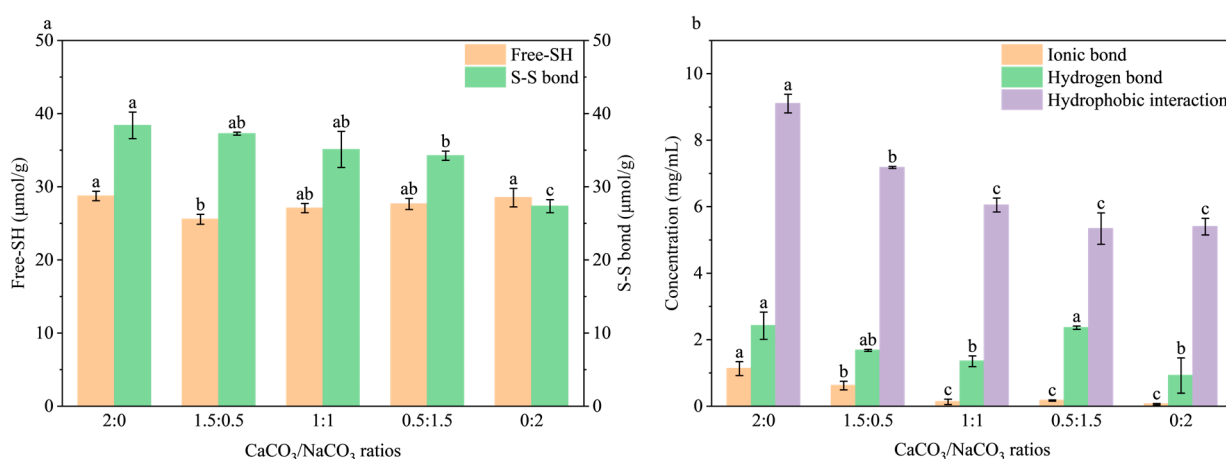
Table 3. The relative content of protein secondary structure of peanut protein–starch extrudates with different $\text{CaCO}_3/\text{NaHCO}_3$ ratios (2:0, 1.5:0.5, 1:1, 0.5:1.5, 0:2).

$\text{CaCO}_3/\text{NaHCO}_3$	β -Sheet/%	Radom Coil/%	α -Helix/%	β -Turn/%
2:0	40.57 ± 1.63 a	25.91 ± 1.18 a	20.54 ± 0.41 b	12.96 ± 0.03 b
1.5:0.5	40.50 ± 0.14 a	23.98 ± 0.35 b	20.94 ± 0.17 b	14.57 ± 0.38 a
1:1	37.45 ± 0.27 b	26.13 ± 0.14 a	22.77 ± 0.34 a	13.64 ± 0.76 ab
0.5:1.5	36.84 ± 0.34 b	26.39 ± 0.53 a	22.62 ± 0.08 a	14.14 ± 0.27 a
0:2	36.49 ± 0.19 b	27.50 ± 0.95 a	22.48 ± 0.93 a	13.51 ± 0.17 ab

Note: Different letters in the same column indicate significantly different ($p < 0.05$).

2.6. Changes in Chemical Bonds and Interaction Forces

Alterations in free sulfhydryl groups and disulfide bonds can serve as indicators of structural changes in protein. The changes in their respective concentrations can be used to determine whether protein denaturation has occurred. As illustrated in Figure 5a, a gradual increase in the proportion of NaHCO_3 resulted in no significant change in free sulfhydryl content, whereas the disulfide bond content decreased significantly from 38.28 ± 1.82 $\mu\text{mol/g}$ to 27.34 ± 0.90 $\mu\text{mol/g}$. It suggested that the combined effect of NaHCO_3 and extrusion treatment led to the cleavage of disulfide bonds that stabilize the polypeptide chains, resulting in a certain degree of protein denaturation. When the $\text{CaCO}_3/\text{NaHCO}_3$ ratio was 0:2, the disulfide bond content reached its minimum, which may be attributed to the elevated pH causing substantial protein denaturation and subsequent depolymerization of the protein structure.

**Figure 5.** (a) Free-SH and S-S bond, (b) ionic bond, hydrogen bond, and hydrophobic interaction of peanut protein–starch extrudates with different $\text{CaCO}_3/\text{NaHCO}_3$ ratios (2:0, 1.5:0.5, 1:1, 0.5:1.5, 0:2). Different letters in the figures denote statistically significant differences ($p < 0.05$).

As shown in Figure 5b, the interactions between protein and starch in the extrudates are primarily non-covalent, mainly hydrogen bonding and hydrophobic interactions. With increasing NaHCO_3 addition, the hydrogen bond strength decreased significantly from 2.42 ± 0.41 $\mu\text{mol/g}$ to 0.92 ± 0.53 $\mu\text{mol/g}$, while hydrophobic interactions declined from 9.10 ± 0.28 $\mu\text{mol/g}$ to 5.40 ± 0.25 $\mu\text{mol/g}$ ($p < 0.05$). The reduction in hydrogen bonding may be attributed to the elevated pH induced by NaHCO_3 , which increased the surface charge of proteins and enhanced electrostatic repulsion, thereby hindering hydrogen bond formation with starch [22]. Additionally, the gelatinization of starch during extrusion disrupts its ordered structure, leading to an abnormal distribution of hydroxyl groups and a consequent decrease in available hydrogen bonding sites. Regarding the weakening of hydrophobic interactions, the spatial hindrance imposed by starch may reduce the exposure of hydrophobic residues in proteins, resulting in diminished hydrophobic interactions.

2.7. Discussion on the Mechanism of Regulating Pore Structure and Quality Characteristics in Protein–Starch Gel Networks Under the Influence of $\text{CaCO}_3/\text{NaHCO}_3$ Ratio

During the extrusion process, protein and starch undergo changes in their molecular structures. Starch acted as a filler, enhancing the continuity of the protein gel network and forming a composite protein–starch gel network structure. Under the regulation of CaCO_3 and NaHCO_3 , the protein–starch gel network exhibited distinct structural characteristics. Firstly, NaHCO_3 served as a source of carbon dioxide expansion within the gel network. It generated gas during extrusion, which created large pores in the gel structure after extrusion. This increased the crispness of the extrudates, reduced their hardness, and enhanced their rehydration capacity. In contrast, CaCO_3 primarily provided calcium ion nucleation sites. These calcium ions formed bridges with specific amino acid residues in the proteins, such as glutamate and aspartate, thereby increasing the rigidity of the protein–starch gel network. This reinforcement helped prevent pore collapse during gas escape and promoted the formation of a dense pore structure. Furthermore, as the proportion of NaHCO_3 increased, the pH shifted toward alkalinity. This increase in pH enhanced the flexibility of the protein structures, facilitating the formation of an elastic gel network. In summary, both CaCO_3 and NaHCO_3 played significant regulatory roles in shaping the pore structure of the protein–starch gel network formed during extrusion. This provided a new perspective for studying the relationship between the protein–starch gel network and the quality characteristics of extruded products.

3. Conclusions

Our study revealed the distinct regulatory effects of CaCO_3 and NaHCO_3 on the protein–starch gel network formed during extrusion. Specifically, an increased proportion of NaHCO_3 promoted network expansion, resulting in larger pores, which corresponded to a decrease in extrudate hardness and an increase in water holding capacity (WHC). In contrast, CaCO_3 acted primarily as a nucleating agent that increased gel rigidity, leading to denser pores, higher extrudate hardness, and poorer rehydration capacity. The gel network was found to be stabilized primarily by non-covalent interactions (e.g., hydrogen bonds and hydrophobic interactions). Overall, this work elucidates how the combined regulation by CaCO_3 and NaHCO_3 drives differences in the formation of protein–starch gel networks, suggesting new avenues for their functional applications.

4. Materials and Methods

4.1. Materials

With a proximate composition of $55.58 \pm 0.79\%$ protein, $5.81 \pm 0.49\%$ fat, $4.85 \pm 0.06\%$ ash, and $5.61 \pm 0.05\%$ moisture, the peanut protein powder used in this study was supplied by Qingdao Longevity Food Co., Ltd. in Qingdao, China. The pea starch used had a purity exceeding 90% and an amylose content of $31.10 \pm 1.21\%$. It was sourced from Chuanfeng Food Co., Ltd. in Jinan, China. NaHCO_3 and CaCO_3 were supplied by Yingjietang Food Co., Ltd. (Nanjing, China). Other chemicals were purchased from Solarbio Technology Co., Ltd. (Beijing, China).

4.2. Extrusion Experiment and Sample Preparation

As described previously, the extrusion parameters were adopted directly from our earlier work [23]. The extrusion was performed using a twin-screw extruder (FMHE36-24, FUMACH, Changsha, China) featuring a 36 mm screw diameter and a length-to-diameter (L/D) ratio of 24. The extrusion process was defined by the following operational parameters: a five-zone heating profile (60, 110, 130, 150, and 180 °C), a feed rate of 26 kg/h, a screw speed of 280 rpm, and a moisture content of 26% (dry basis). CaCO_3 and NaHCO_3

were added to the mixture of peanut protein powder (83%) and pea starch (15%) at a total concentration of 2% (*w/w*). Following a 12 h equilibration period, the mixtures, which contained CaCO₃ and NaHCO₃ in ratios of 2:0, 1.5:0.5, 1:1, 0.5:1.5, and 0:2, were processed. The extrudates were named as 2:0, 1.5:0.5, 1:1, 0.5:1.5, and 0:2. For subsequent analyses, the extrudates were first freeze-dried under vacuum (−40 °C, 12 h). The resulting dried product was then ground and sieved (80-mesh) to obtain a uniform powder.

4.3. Color

The color of the dried extrudates was measured using a spectrophotometer (CS-600, CHN Spec, Hangzhou, China). After being ground into a powder and passed through an 80-mesh sieve, the samples were analyzed for lightness (L), redness (a), and yellowness (b). The instrument was calibrated using a standard white plate with L*, a*, and b* values of 89.73, −0.78, and 1.88, respectively. The total color difference (ΔE) was calculated as follows:

$$\Delta E = \sqrt{(L - L^*)^2 + (a - a^*)^2 + (b - b^*)^2} \quad (1)$$

4.4. Expansion Ratio and Density

The expansion ratio (E_r) was determined from the measured extrudate diameter (d_1), using a Shenhan Vernier caliper (Shenhan Measuring Tool Co., Ltd., Shanghai, China) and the die diameter ($d_2 = 1.5$ mm), according to:

$$E_r = \frac{d_1}{d_2} \quad (2)$$

A DE-300VP density analyzer (Dahong Meituo Measuring Instrument Co., Ltd., Beijing, China) was employed to determine the density of the extrudates.

4.5. Textural Properties

The textural properties of extrudates were analyzed using a TA.XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK), following a published method with minor modifications [24]. Samples were cut into 1 cm segments and tested with a P36R cylindrical probe ($\Phi 36$ mm). Hardness was defined as the peak force (N) required to compress the sample. Brittleness was characterized by the downward displacement (mm) of the probe at the point of fracture, where a smaller value indicates greater brittleness and improved palatability. The test conditions were set as follows: pre-test, test, and post-test speeds of 2 mm/s, a trigger force of 0.15 N, and a compression level of 75%.

4.6. Macro- and Microstructure Observation

The surface of macrostructure of the dried extrudates were evaluated with a camera (D90, Nikon Corp., Tokyo, Japan). Prior to microstructural analysis, the extrudates were sectioned into cylinders approximately 2 mm in height. A gold layer with a thickness of 10 nm was deposited onto the dried samples by sputter coating to provide adequate electrical conductivity. The microstructural images were obtained using a scanning electron microscope (SU8010, Hitachi, Tokyo, Japan) operated at an accelerating voltage of 10 kV, with the following parameters: LM(UL) detector, a working distance of 10.5 mm, and a scan rate of Capture CSS (40).

4.7. Water Holding Capacity (WHC)

The water holding capacity (WHC) was determined as follows. Approximately 0.2 g (W_1) of the extrudate was mixed with 5 mL of deionized water at room temperature using a vortex mixer. (The extrudate was almost insoluble in water and directly

formed a precipitate.) The mixture was then stored at 4 °C for 12 h, followed by centrifugation at 5000 r/min for 15 min. After discarding the supernatant, the precipitate was weighed (W_2). The WHC (W_3) was calculated using the following formula:

$$W_3 = \frac{W_2 - W_1}{W_1} \quad (3)$$

4.8. pH Value

The pH value of the extrudates was determined by homogenizing 2 g of sample with 20 mL of distilled water in a beaker, followed by measurement using a pH meter (Model FE20, Shanghai Mettler Instruments Co., Ltd., Shanghai, China).

4.9. Rheological Properties

The rheological properties were evaluated following Chen et al. [25] with modifications. The extrudates were ground and mixed with distilled water to form a 26% (w/w) suspension. After sealing and equilibrating at 4 °C, the sample was analyzed using a parallel-plate geometry (40 mm diameter, 1 mm gap). Steady-state flow tests were performed by scanning shear rates from 0.01 to 100 s^{-1} at 25 °C. Rheological behavior was modeled using the power law equation as follows:

$$\tau = K\gamma^n \quad (4)$$

where τ , K , γ , and n represent the shear stress (Pa), consistency coefficient, shear rate (s^{-1}), and flow behavior index, respectively.

Frequency sweep tests (0.1–100 rad/s) were conducted at 25 °C within the linear viscoelastic region to assess the viscoelastic properties, as characterized by the storage modulus (G'), loss modulus (G''), and loss tangent ($\tan \delta = G''/G'$).

4.10. Secondary Structure of Protein via Fourier Transform Infrared Spectroscopy (FTIR)

The extrudate was ground into powder and then subjected to FTIR measurement, based on the method of Qadir et al. [26] with minor modifications. The secondary structures of protein and its corresponding extrudates were determined via FTIR using a TENSOR 27 spectrometer (Bruker, Bremen, Germany) equipped with an attenuated total reflectance (ATR) crystal. Spectra were acquired over a range of 4000–500 cm^{-1} at a resolution of 4 cm^{-1} , with 64 scans accumulated per sample to ensure a high signal-to-noise ratio. To quantify the protein secondary structure, the amide I region (1600–1700 cm^{-1}) was analyzed via Gaussian deconvolution and peak fitting using Peak Fit 4.12 software. Individual sub-peaks were assigned to specific structural components: β -sheet (1615–1637 cm^{-1} and 1682–1700 cm^{-1}), α -helix (1646–1664 cm^{-1}), β -turn (1664–1681 cm^{-1}), and random coil (1637–1645 cm^{-1}).

4.11. Free Sulfhydryl and Disulfide Bond Content

The content of free sulfhydryl (Free-SH) and disulfide bonds (S-S bonds) was determined according to the method of Jiang et al. [27] with modifications. The free sulfhydryl (SH) content was determined as follows: First, 50 mg of extrudate powder was homogenized in 10 mL of Tris-glycine buffer (0.086 mol/L Tris, 0.09 mol/L glycine, 4 mmol/L EDTA, 8 mol/L urea) with 30 min of magnetic stirring. Then, after centrifugation (5000 r/min, 10 min), 1 mL of supernatant was combined with 100 μ L of 4 mg/mL Ellman's reagent (DTNB) and incubated in the dark. Finally, absorbance was read at 412 nm. For total SH, 1 mL of the supernatant was mixed with 50 μ L of β -mercaptoethanol and incubated at room temperature for 1 h. The mixture was then vortexed with 10 mL of 12% trichloroacetic acid (TCA) and centrifuged at 5000 r/min for 10 min. The resulting precipitate was resuspended in 10 mL of Tris-glycine buffer, followed by the addition of

100 µL of Ellman's reagent for subsequent measurement. Subsequently, the absorbance at 412 nm was recorded. The concentrations of free and total sulfhydryl groups, denoted as Free-SH and Total-SH, were calculated according to the following formula:

$$SH_{(Free/Total)} (\mu\text{mol/g}) = \frac{73.53 \times A_{412} \times D}{C} \quad (5)$$

where A_{412} represented the measured absorbance at 412 nm, while D and C were defined as the dilution factor and protein concentration (mg/mL), respectively. Based on these parameters, the disulfide bond (S-S) content was calculated using the formula:

$$S-S \text{ bond } (\mu\text{mol/g}) = \frac{SH_{Total} - SH_{Free}}{2} \quad (6)$$

4.12. Protein Molecular Interaction Forces

The protein molecular interactions were analyzed following Yan et al. [28] with modifications. Extrudates (1 g) were treated with 100 mL of four solutions designed to disrupt specific molecular interactions: 0.05 M NaCl (S_1), 0.6 M NaCl (S_2), 0.6 M NaCl + 1.5 M urea (S_3), and 0.6 M NaCl + 8 M urea (S_4). Following vortexing for 2 min and centrifugation at 5000 r/min for 10 min, the protein concentration in the supernatant was determined using the Biuret method. The differences in protein solubility between treatments were used to quantify ionic bonds (S_2-S_1), hydrogen bonds (S_3-S_2), and hydrophobic interactions (S_4-S_3).

4.13. Statistical Analysis

The extrudates were analyzed in triplicate. Data were expressed as mean value $\pm$ standard deviation and were processed using SPSS 26.0 (SPSS Inc., Chicago, IL, USA). Significant differences ($p < 0.05$) were assessed by Duncan's test, and graphical representations were generated with Origin 2024 (Origin Labs, Northampton, MA, USA).

Author Contributions: Conceptualization, F.G. and J.Z.; methodology, F.G.; software, H.Z.; validation, F.G., H.Z. and H.H.; formal analysis, A.H.; investigation, H.Z.; resources, Q.W.; data curation, F.G.; writing—original draft preparation, F.G.; writing—review and editing, J.Z.; visualization, F.G.; supervision, J.Z.; project administration, H.H.; funding acquisition, Q.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the National Key Research and Development Plan of China (2021YFD2100402), the Beijing Natural Science Foundation (6242027), the Key Research and Development Plan of Xinjiang Uygur Autonomous Region (2024B02010), and the Agricultural Science and Technology Innovation Program by CAAS (ASTIP-Y2025QC30).

Data Availability Statement: The data presented in this study are openly available in article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Moura, M.; Martins, B.; de Oliveira, G.; Takahashi, J. Alternative protein sources of plant, algal, fungal and insect origins for dietary diversification in search of nutrition and health. *Crit. Rev. Food Sci. Nutr.* **2023**, *63*, 10691–10708. [CrossRef]
2. Tan, M.; Nawaz, M.; Buckow, R. Functional and food application of plant proteins—A review. *Food Rev. Int.* **2023**, *39*, 2428–2456. [CrossRef]
3. Chu, J.; Zhou, F.; Wu, Z.; Ouyang, W.; Ma, Z.; Liang, S.; Liu, Q. Atmospheric cold plasma treatment of peanut protein: Structural and functional modifications accompanied by microbial reduction. *J. Food Eng.* **2026**, *406*, 112825. [CrossRef]
4. Boukid, F. Peanut protein—An underutilised by-product with great potential: A review. *Int. J. Food Sci. Technol.* **2022**, *57*, 5585–5591. [CrossRef]

5. Wang, D.; Han, W.; Shi, L.; Guo, X.; Chen, S.; Chen, L.; Qiao, Y.; Wu, W.; Li, J.; Wang, L. Effects of explosion puffing on surimi-starch blends: Structural characteristics, physicochemical properties, and digestibility. *LWT-Food Sci. Technol.* **2023**, *183*, 114902. [\[CrossRef\]](#)
6. Lazou, A. Food extrusion: An advanced process for innovation and novel product development. *Crit. Rev. Food Sci. Nutr.* **2024**, *64*, 4532–4560. [\[CrossRef\]](#)
7. Song, J.; Tang, Z.; Zeng, J.; Hassan, M.E.; Cheng, S.; Yu, F.; Wang, Y.; Yu, X.; Wang, P.; Wang, L.; et al. Aggregation mechanism of soybean protein isolate for the formation of plant protein-based meat analogs during high moisture extrusion. *Int. J. Biol. Macromol.* **2026**, *337*, 149524. [\[CrossRef\]](#)
8. Cho, K.; Rizvi, S. 3D microstructure of supercritical fluid extrudates I: Melt rheology and microstructure formation. *Food Res. Int.* **2009**, *42*, 595–602. [\[CrossRef\]](#)
9. Zhang, F.; Yang, B.; Zhang, M.; Qi, J.; Xu, X.; Xiong, G. Influence of sodium bicarbonate and moisture content on physicochemical properties of chicken-based extrudate by twin-screw extruder. *Food Chem.* **2023**, *402*, 134313. [\[CrossRef\]](#)
10. Ji, S.; Ai, Z.; Li, H.; Li, Z.; Suo, B.; Tang, N.; Wang, S.; Yang, Y. Effect of NaHCO₃ on the quality and in vitro starch digestibility of fried-free instant noodles. *Food Res. Int.* **2025**, *220*, 117109. [\[CrossRef\]](#)
11. Lin, Y.; Chen, H.; Chan, C.; Wu, J. Nucleating effect of calcium stearate coated CaCO₃ nanoparticles on polypropylene. *J. Colloid. Interface Sci.* **2011**, *354*, 570–576. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Janve, M.; Singhal, R. Fortification of puffed rice extrudates and rice noodles with different calcium salts: Physicochemical properties and calcium bioaccessibility. *LWT-Food Sci. Technol.* **2018**, *97*, 67–75. [\[CrossRef\]](#)
13. Cui, H.P.; Yu, J.H.; Zhai, Y.; Feng, L.H.; Chen, P.S.; Hayat, K.; Xu, Y.; Zhang, X.M.; Ho, C.T. Formation and fate of Amadori rearrangement products in Maillard reaction. *Trends Food Sci. Technol.* **2021**, *115*, 391–408. [\[CrossRef\]](#)
14. De Camargo, A.; Regitano-d'Arce, M.; Rasera, G.; Canniatti-Brazaca, S.; do Prado-Silva, L.; Alvarenga, V.; Sant'Ana, A.; Shahidi, F. Phenolic acids and flavonoids of peanut by-products: Antioxidant capacity and antimicrobial effects. *Food Chem.* **2017**, *237*, 538–544. [\[CrossRef\]](#)
15. Huang, Y.; Liang, J. Optimization and characteristics of extruded puffed snacks with *Agaricus bisporus* powder and rice flour. *J. Food Process Eng.* **2019**, *42*, E13286. [\[CrossRef\]](#)
16. Campbell, G.; Martin, P. Bread aeration and dough rheology: An introduction. In *Breadmaking: Improving Quality*, 2nd ed.; Cauvain, S.P., Ed.; Woodhead Publishing Series in Food Science Technology and Nutrition; Woodhead Publishing: Cambridge, UK, 2012; pp. 299–336.
17. Jeong, M.; Cho, S. Effect of pH-shifting on the water holding capacity and gelation properties of mung bean protein isolate. *Food Res. Int.* **2024**, *177*, 113912. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Mo, J.; Wang, F.; Xu, Z.; Feng, C.; Fang, Y.; Tang, X.; Shen, X. Characterization and performance of soybean protein modified by tyrosinase. *Int. J. Adhes. Adhes.* **2019**, *92*, 111–118. [\[CrossRef\]](#)
19. Glusac, J.; Davidesko-Vardi, I.; Isaschar-Ovdat, S.; Kukavica, B.; Fishman, A. Gel-like emulsions stabilized by tyrosinase-crosslinked potato and zein proteins. *Food Hydrocoll.* **2018**, *82*, 53–63. [\[CrossRef\]](#)
20. Pei, Y.; Yuan, L.; Zhou, W.; Yang, J. Tyrosinase-Catalyzed Soy Protein and Tannic Acid Interaction: Effects on Structural and Rheological Properties of Complexes. *Gels* **2025**, *11*, 195. [\[CrossRef\]](#)
21. Zhang, W.; Liu, C.; Zhao, J.; Ma, T.; He, Z.; Huang, M.; Wang, Y. Modification of structure and functionalities of ginkgo seed proteins by pH-shifting treatment. *Food Chem.* **2021**, *358*, 129862. [\[CrossRef\]](#)
22. Li, J.; Wang, C.; Li, X.; Su, Y.; Yang, Y.; Yu, X. Effects of pH and NaCl on the physicochemical and interfacial properties of egg white/yolk. *Food Biosci.* **2018**, *23*, 115–120. [\[CrossRef\]](#)
23. Guo, F.; Hu, H.; Zhou, H.; Hu, A.; Wang, Q.; Zhang, J. Changes in starch-assisted expansion porous structure of peanut protein via extrusion and impact on textural and rehydration properties. *Food Struct.* **2025**, *46*, 100474. [\[CrossRef\]](#)
24. Acurio, L.; Salazar, D.; Castillo, B.; Santiana, C.; Martínez-Monzó, J.; Igual, M. Characterization of Second-Generation Snacks Manufactured from Andean Tubers and Tuberous Root Flours. *Foods* **2024**, *13*, 51. [\[CrossRef\]](#)
25. Chen, Q.; Zhang, J.; Zhang, Y.; Meng, S.; Wang, Q. Rheological properties of pea protein isolate-amylose/amylopectin mixtures and the application in the high-moisture extruded meat substitutes. *Food Hydrocoll.* **2021**, *117*, 106732. [\[CrossRef\]](#)
26. Qadir, N.; Wani, I.A. Extrusion assisted interaction of rice starch with rice protein and fibre: Effect on physicochemical, thermal and in-vitro digestibility characteristics. *Int. J. Biol. Macromol.* **2023**, *237*, 124205. [\[CrossRef\]](#)

27. Jiang, Y.; Zhu, Y.; Zheng, Y.; Liu, Z.; Zhong, Y.; Deng, Y.; Zhao, Y. Effects of salting-in/out-assisted extractions on structural, physicochemical and functional properties of *Tenebrio molitor* larvae protein isolates. *Food Chem.* **2021**, *338*, 128158. [[CrossRef](#)] [[PubMed](#)]
28. Yan, B.; Jiao, X.; Zhu, H.; Wang, Q.; Huang, J.; Zhao, J.; Cao, H.; Zhou, W.; Zhang, W.; Ye, W.; et al. Chemical interactions involved in microwave heat-induced surimi gel fortified with fish oil and its formation mechanism. *Food Hydrocoll.* **2020**, *105*, 105779. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.