foods

MDPI

*Article*

# Improving the Edible and Nutritional Quality of Roasted Duck Breasts through Variable Pressure Salting: Implications for Protein Anabolism and Digestion in Rats

**Ziwu Gao [1,2], Yinna Zhou [1,2], Dequan Zhang [1,2,*], Ruiyun Wu [1,2], Jiale Ma [1,2], Jinhua He [1,2] and Zhenyu Wang [1,2]**

[1] Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, Beijing 100193, China; gzw96530@163.com (Z.G.); zyn12355@163.com (Y.Z.); wuruiyun814@163.com (R.W.); 17302258026@163.com (J.M.); 15102613432@163.com (J.H.); caasjgsmeat2021_6@126.com (Z.W.)

[2] Integrated Laboratory of Processing Technology for Chinese Meat and Dish Products, Ministry of Agriculture and Rural Affairs, Beijing 100193, China

* Correspondence: zhangdequan@caas.cn

**Abstract:** Variable pressure salting (VPS) is considered a novel salting approach to improve meat quality. This study aimed to investigate the effects of roasted duck's edible and nutritional quality after VPS through serum biochemical indicators and in vivo digestion properties in rats. The results show that roasted duck after VPS led to an increase in the total protein content (57.24 g/L) and blood glucose levels (6.87 mmol/L), as well as a decrease in the blood urea nitrogen content (11.81 mmol/L), in rats. Compared to rats fed base diets and roasted duck after static wet salting (SWS), those ingesting roasted duck after VPS exhibited higher values of apparent protein digestibility (51.24%), pepsin activity (2.40 U/mg), and trypsin activity (389.80 U/mg). Furthermore, VPS treatment improved the textural properties and microstructure of duck breasts shown by a higher immobilized water relaxation area and more ordered protein structures (α-helixes and β-sheets). These improvements enhanced the protein anabolism capacity and in vivo digestion properties in rats. Therefore, VPS represents a beneficial salting method for promoting effective digestion and absorption in rats.

**Keywords:** variable pressure salting; in vivo digestion; water distribution; texture; protein structure

check for updates

**Citation:** Gao, Z.; Zhou, Y.; Zhang, D.; Wu, R.; Ma, J.; He, J.; Wang, Z. Improving the Edible and Nutritional Quality of Roasted Duck Breasts through Variable Pressure Salting: Implications for Protein Anabolism and Digestion in Rats. *Foods* **2024**, *13*, 402. https://doi.org/10.3390/foods13030402

Academic Editor: Junsoo Lee

Received: 30 December 2023
Revised: 23 January 2024
Accepted: 24 January 2024
Published: 26 January 2024

**Copyright:** © 2024 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

Roasted duck is a well-known Chinese meat product celebrated for its distinctive flavor and rich nutritional profile [1]. Salting is a critical step in the processing of roasted duck, as it enhances the color, water retention, and flavor quality of the duck carcasses [2]. During the salting process, the brine enters the raw meat, increasing the osmotic pressure and promoting protein degradation and the release of taste substances (such as free amino acids, free fatty acids, and nucleotides) [3]. However, traditional static wet salting (SWS) has low salting efficiency and cannot respond to industrialized production. Additionally, vacuum tumbling and salting can destroy the shape of the raw meat, compromising the integrity of the whole carcass [4]. Therefore, it is necessary to develop an advanced salting method to improve the salting efficiency and keep the integrity of the raw meat. A novel approach, known as variable pressure salting (VPS), has recently been developed for salt duck carcasses [5]. VPS is applied to raw meat under alternating changes in the vacuum and pressure so that the raw meat and the brine make complete contact.

Previous studies showed that VPS is a potential method for industrial meat curing, as it improved the salting efficiency and raw meat quality [6]. For example, Jin et al. [7] found that VPS shortened the salting time, reduced the cooking loss, and improved the water retention of pork compared to SWS. Jiao et al. [5] reported that VPS reduced shear forces and improved the flavor profile of roasted duck breasts compared to static wet-salted duck breasts. Furthermore, during salting, $Na^+$ and $Cl^-$ disrupt the hydration layer on the

protein's surface, providing more binding sites for pepsin and improving digestibility [8]. Pulsed electric field processing might also improve the digestibility of meat proteins by inducing structural and microstructural changes in the protein and increasing access to meat protein hydrolysis sites [9]. It is reported that VPS treatment improved the in vitro digestibility of roasted duck protein compared to roasted ducks after SWS, indicating that VPS treatment promoted protein degradation [10]. It is worth noting that the apparent protein digestibility depends on the protein structures [11]. Hence, we speculated that VPS could change roasted duck breasts' in vivo digestion properties.

Notably, most studies focused on the in vitro digestibility of meat protein, and research on the in vivo digestion properties still needs to be completed. Detailed research reports are indistinct, especially regarding the effect of the roasted duck after VPS on in vivo digestion properties and possible mechanisms. Therefore, the present study evaluated the effect of roasted duck after VPS on serum biochemical indicators (total protein, blood urea nitrogen, alkaline phosphatase, and blood glucose) and in vivo digestion properties (pepsin and trypsin activities and apparent protein digestibility) in rats. Additionally, the changes in the water distribution, textural properties, and protein structures of raw/roasted duck breasts were explored to explain how VPS affects in vivo digestion properties.

## 2. Materials and Methods

### 2.1. Materials and Chemicals

The thirty six-week-old male specific pathogen-free (SPF) grade Sprague–Dawley (SD) rats and the basal diet used in the experiment were purchased from Beijing Huafukang Bio-Technology Co., Ltd. (Beijing, China) (SYXK 2019-0022). The duck carcasses (two groups of six each, $n$ = 12) were made from cherry valley ducks (41 days old, with an average weight of 1.3 ± 0.2 kg) and randomly selected from Hebei Dongfeng Breeding Co., Ltd. (Cangzhou, China). The duck carcasses were cleaned and packaged in nylon/polyethylene (Magic Seal, Dongguan, China) and chilled at −35 °C for 12 h before being transported to the laboratory via cold-chain logistics within 2 h.

The alkaline phosphatase assay kit, urea assay kit, glucose assay kit, and total protein assay kit were obtained from Shenzhen Rayto Life Science Co., Ltd. (Shenzhen, China). The pepsin activity assay kit and trypsin activity assay kit were obtained from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All other reagents used were of analytical grade.

### 2.2. Processing of Salted Roasted Duck

The salted roasted ducks were prepared according to the method of Jiao et al. [5]. Static wet-salted duck carcasses and variable-pressure salted duck carcasses were roasted in a universal steam oven (Changsha Guli Food Machinery Co., Ltd., Changsha, China) for 50 min at 180 °C. The detailed processing conditions are shown in Table 1. The roasted duck breasts from the salted duck carcasses were minced using a knife mill. Then, the mixtures were freeze-dried, ground into powders, and stored at −80 °C.

**Table 1.** Processing parameters for salted roasted duck.

| Parameters | Static Wet Salting | Variable Pressure Salting |
|---|---|---|
| Temperature/°C | 4 | 4 |
| Vacuum pressure/KPa | Atmospheric pressure | −70~−80 |
| Mass fraction of salt solution/% | 8.9 | 8.9 |
| Pulse ratio | - | 1.5 (54 min vacuum pressure/36 min atmospheric pressure) |
| Salting time/h | 12 | 12 |
| Roasting time/min | 50 | 50 |

"-": Indicates that the treatment was not carried out; atmospheric pressure: 101 KPa.

### 2.3. Animal Treatment

The rats were housed in separate cages in a sterile room and fed water and a sesame-free diet at 23 ± 3 °C and 50 ± 10% relative humidity, with a 12 h light/dark cycle.

The experiment protocol was approved by the animal ethics committee of Beijing Huafukang Bio-Technology Co., Ltd. (Beijing, China) (approval number: SYXK 2019-0022). The approval number from the animal ethics committee is HFK-AP-20221111.

### 2.4. Animal Experiment Design

Thirty SD rats (180 ± 20 g) were randomly divided into three groups ($n$ = 10 rats per group) according to body weight: control check (CK), SWS-Roasted, and VPS-Roasted. The rats in the CK group were fed base diets. The rats in the SWS-Roasted group were fed diets containing 5% (m/m) static wet-salted roasted duck powder. The rats in the VPS-Roasted group were fed diets containing 5% (m/m) variable-pressure salted roasted duck powder. The diets were prepared according to the standard of Nutritional Composition of Combination Feed for Laboratory Animals (GB14924.3-2010) [12]. The composition of the diets is shown in Table S1.

### 2.5. Determination of Serum Biochemical Indicators in Rats

Total protein (TP), blood urea nitrogen (BUN), alkaline phosphatase (AP), and blood glucose (BG) contents were measured using a fully automated biochemical analyzer (Chemray 800, Rayto Life Science Co., Ltd., Shenzhen, China).

### 2.6. Analysis of In Vivo Digestion Properties in Rats

#### 2.6.1. Determination of Pepsin and Trypsin Activities

The pepsin and trypsin activities were determined according to the method described by Xu et al. [13]. Rat stomach tissue (1 mg) and small intestine (1 mg) were weighed, and 0.9% (m/v) saline (9 mL) was added. The mixture was homogenized under ice-bath conditions, and then the supernatant was centrifuged at 3000 r/min for 10 min. The pepsin and trypsin activities were determined in rats using pepsin and trypsin activity assay kits.

#### 2.6.2. Apparent Protein Digestibility Analysis

The protein content in the diet and feces of rats was determined with the method of Terrazas-Fierro et al. [14]. The apparent digestibility of protein was calculated as follows:

$$\text{Apparent protein digestibility } (\%) = \frac{\text{Protein intake} - \text{Fecal protein content}}{\text{Protein intake}} \times 100$$

### 2.7. Low-Field Nuclear Magnetic Resonance (LF-NMR) Analysis

The water distribution was measured following the method of Xu et al. [15] using an LF-NMR Analyzer (NMI20-040H-I, Niumag Electric Co., Ltd., Suzhou, China). Raw and roasted duck breast samples were cut into three strips (2 cm × 1 cm × 1 cm, weighing 5 ± 0.5 g) and placed in a test tube. $T_2$ spectroscopy was conducted on the samples to determine transverse relaxation times using Carr–Purcell Meiboom–Gill (CPMG) sequences. The parameters settings were magnetic field strength: 0.5 T; proton resonance frequency: 23 MHz; SF: 23 MHz; P90: 9 μs; P180: 18 μs; TD: 59,990; TR: 3000 ms; NS: 16; and echo count: 2000.

The H-proton density imaging spectra were obtained using the Nuclear Magnetic Resonance Imaging (NMI) system. The key parameters were repetition time: 2000 MS; performing: 4 repetition times; longitudinal relaxation time: 20 MS; and spin echo time: 20 MS.

### 2.8. Textural Properties and Warner–Bratzler Shear Force (WBSF) Testing

The textural properties were determined according to the method of Wang et al. [16] with minor modifications. The samples were cut into six cubes (1 cm × 1 cm × 1 cm) with a 1 ± 0.2 g mass. The measurement was conducted using a texture analyzer (TA-XT. Plus, Stable Micro Systems Co., Ltd., Godalming, UK) equipped with a P/50 column probe, a pretest speed of 2 mm/s, a test speed of 1 mm/s, and a test deformation of 50%.

The WBSF was measured using the method of Jiao et al. [5]. The sample was cut into six strips (2 cm × 1 cm × 1 cm) according to the direction of the muscle fibers. The measurement was carried out using a texture analyzer (TA-XT. Plus, Stable Micro Systems, Godalming Surrey, UK) with a V-shaped blade slice–shear device, and an HDP BS probe at a pretest speed of 5 mm/s, a test speed of 2 mm/s, and a post-test speed of 10 mm/s. The maximum peak of the WBSF was recorded, and the WBSF is expressed in kilograms (Kg).

### 2.9. Analysis of Microstructure

The raw and roasted duck breasts were cut into cubes (3 cm × 3 cm × 2 mm) and then immersed in 25 mL/L glutaraldehyde for 24 h. The samples were washed three times with 0.1 mol/L phosphate buffer (pH 7.0). A series of ethanol concentrations (300 mL/L, 500 mL/L, 700 mL/L, 800 mL/L, 900 mL/L, 950 mL/L, and 1000 mL/L) were used for dehydration. The specimens were freeze-dried, sputter-coated, and then observed with a scanning electron microscope (SEM) (GeminiSEM 300, Carl Zeiss Co., Oberkochen, Germany).

### 2.10. Determination of Protein Structure

#### 2.10.1. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis

The SDS-PAGE analysis was performed according to Yin et al. [17] with slight modifications. Raw/roasted duck breast samples (2.0 g) of each treatment group were homogenized with 2% (*w*/*v*) SDS (10 mL) using a homogenizer (10,000 r/min, 3 × 30 s). The mixture was centrifuged at 4000× *g* (20 min), and the resulting supernatant was collected as the total protein extraction. The protein solution (2 mg/mL) was combined with a 5× loading buffer. Afterwards, the mixture was boiled at 100 °C for 10 min. Subsequently, the mixture (10 μL) was loaded in each lane of the precast gel (10% polyacrylamide). Electrophoresis was performed using a Mini-Protean Tetra System (Bio-Rad Laboratories, Hercules, CA, USA) under 80 V for 30 min, followed by 120 V for approximately 60 min. After separation, the gel was stained, decolored, and then photographed.

#### 2.10.2. Fourier Transform Infrared (FTIR) Spectroscopy Analysis

The protein secondary structure content was identified via a method delineated by Gangidi et al. [18] using a diamond-attenuated total reflectance (ATR) attachment (Cary 5000, Varian Co., Palo Alto, CA, USA). Raw/roasted duck breasts weighing 5.0 g were subjected to vacuum freeze-drying for 48 h and, subsequently, ground. Subsequently, the treated sample (0.5 g) was scanned. The scanning parameters were measuring range: 600~4000 $cm^{-1}$; number of scans: 64; scanning rate: 0.63 cm/s; and resolution: 32 $cm^{-1}$.

### 2.11. Statistical Analysis

All measurements were independently replicated at least three times with a completely randomized design. The results are presented as the mean ± standard deviation. The statistical analysis was conducted using ANOVA with SPSS 23.0 (SPSS Inc., Chicago, IL, USA), and the Duncan's multiple range test was applied with a significance level set at $p < 0.05$. Data visualization and plotting were conducted using Origin software 2021 (Origin Lab Corporation, Northampton, MA, USA).

## 3. Results and Discussion

### 3.1. Analysis of Differences in Serum Biochemical Indicators in Rats

The total protein (TP) in serum serves various physiological functions, including maintaining blood osmotic pressure and pH and transporting metabolites [19]. Meanwhile, the TP is an essential source of protein for the body, reflecting its protein absorption [20]. As shown in Figure 1A, the TP levels in rats fed with duck breast roasted after VPS were significantly higher ($p < 0.05$) compared to the CK group. This observation demonstrates that duck breast roasted after VPS can promote protein absorption and precipitation in rats. The blood urea nitrogen (BUN) levels reflect the efficiency of the amino acid synthesis

of proteins in the blood, with lower levels indicating enhanced protein anabolism and weakened catabolism in the animal body [21]. As presented in Figure 1B, the BUN content in rats fed with duck breast roasted after VPS was significantly lower than those in the CK and SWS-Roasted groups ($p < 0.05$), suggesting that duck breast roasted after VPS enhanced the protein anabolism in the rats in vivo, leading to a decrease in the synthesis of urea and a lower content of urea in the serum.

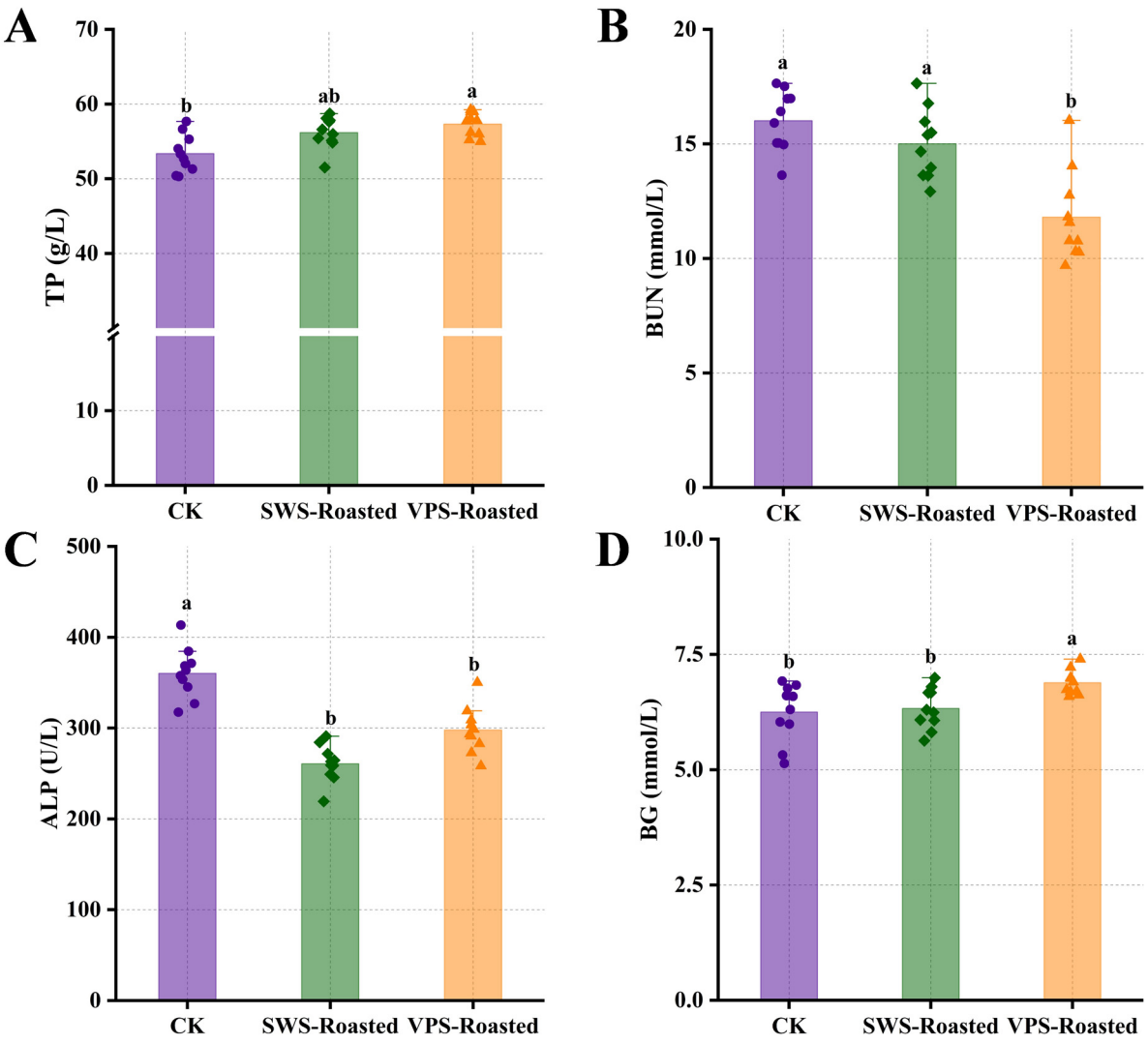



**Figure 1.** Effect of roasted duck treated with different saltings on total protein (TP) content (**A**), blood urea nitrogen (BUN) content (**B**), alkaline phosphatase (ALP) content (**C**), and blood glucose (BG) content (**D**) of rats. CK: base diet, SWS-Roasted: duck roasted after static wet salting, VPS-Roasted: duck roasted after variable pressure salting. Different letters indicate a significant difference ($p < 0.05$).

It is reported that a positive correlation between the alkaline phosphatase (ALP) contents and insulin resistance and high ALP activity may accelerate osteoblast metabolism and apoptosis [22]. The standard reference range for ALP content in rats is 12.04~610.97 U/L [23]. As observed in Figure 1C, the ALP contents of rats in all groups fell within the standard range. Notably, the ALP contents of duck breast roasted after VPS and SWS were significantly lower ($p < 0.05$) than that of the CK group, indicating that slow hepatic metabolism and bone metabolism may be present in rats. Moreover, in the standard range, an increase in blood glucose (BG) indicates that the body absorbs and metabolizes more nutrients [24]. In the fasting state, changes in BG are positively correlated with the body's metabolism [25]. The BG contents of the rats in our experiment were measured in a fasting state. Thus, the

primary source of BG was glucose production from the breakdown of hepatic glycogen from the liver [26]. As illustrated in Figure 1D, the highest BG content (6.93 mmol/L) was observed in the VPS-Roasted group, suggesting that the duck breast roasted after VPS could enhance the catabolism of liver glycogen in rats.

### *3.2. Differences in the Digestion Properties In Vivo*

Pepsin and trypsin are the predominant protein-digesting enzymes in the human body, and their activity can reflect the digestive capacity [27]. As shown in Figure 2A,B, there was a significant increase ($p < 0.05$) in pepsin activity in the rats fed duck breasts roasted after salting at variable pressure and static wet compared to the CK group. Additionally, the trypsin activity in rats fed duck breasts roasted after VPS was significantly higher than in the SWS-Roasted and CK groups ($p < 0.05$). These results indicate that duck breasts roasted after VPS increased the pepsin and trypsin activities in rats. Previous studies pointed out that the substrate concentration in the digestive tract determines the enzyme type and activity and that the substrate induces the secretion of relevant enzymes [28]. The highest feed intake in the rats (Table S2) fed duck breasts roasted after VPS increased the substrate for pepsin and trypsin actions and stimulated the gastric mucosa, thereby inducing the secretion of pepsin and trypsin.

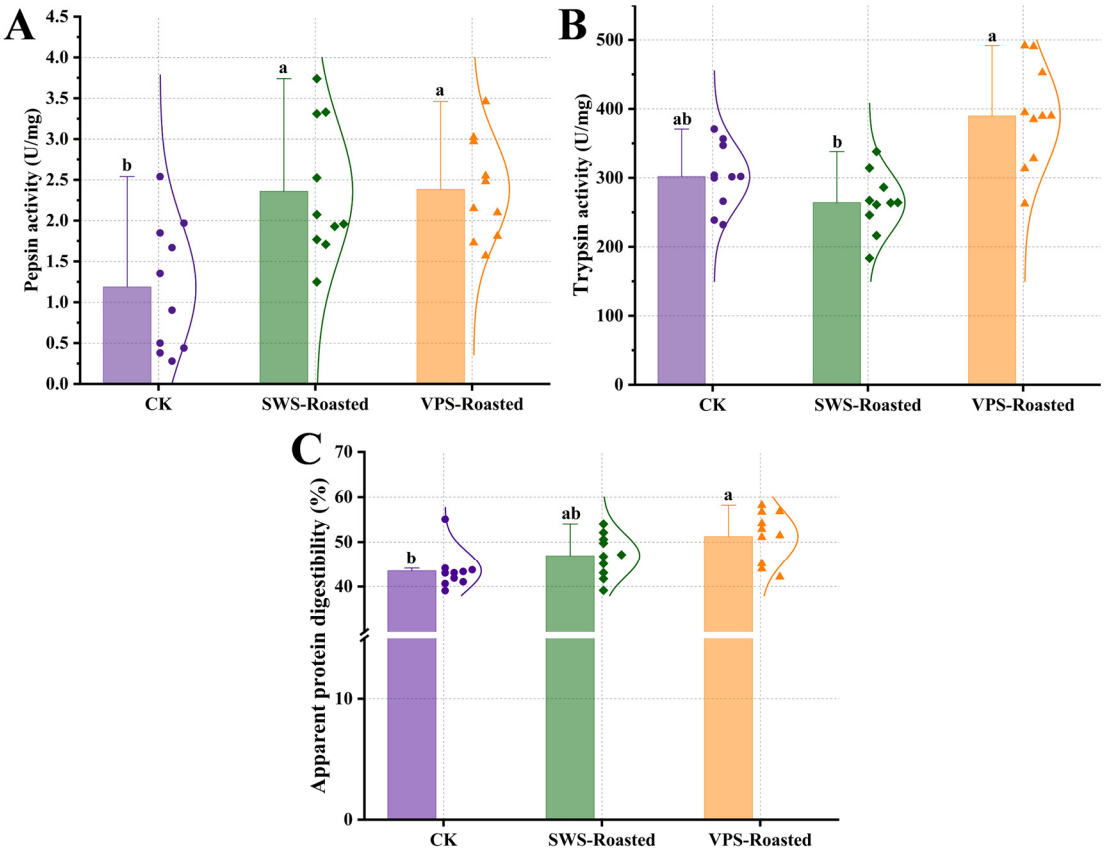



**Figure 2.** Effect of roasted duck treated with different saltings on the pepsin activity (**A**), trypsin activity (**B**), and apparent protein digestibility (**C**) in rats. CK: base diet; SWS-Roasted: duck roasted after static wet salting; VPS-Roasted: duck roasted after variable pressure salting. Different letters indicate a significant difference ($p < 0.05$).

The apparent protein digestibility is a critical indicator of the extent to which the products (amino acids and peptides) of food being digested are absorbed, and it depends on the protein structures [29]. Compared with the CK group, there was a significant increase ($p < 0.05$) in the apparent protein digestibility in the rats fed duck breasts roasted after VPS

(Figure 2C). On the one hand, the duck protein and the soybean meal protein in the CK group contained different types, amounts, and spatial structures of amino acids, leading to differences in their protein properties and digestibility [30]. On the other hand, the VPS enabled raw meat to switch alternately between inhalation and extrusion of the curing solution, resulting in the increased dissociation of meat proteins and smaller molecular weights, facilitating better chewing and digestion [31].

### *3.3. Changes in Water Distribution*

Low-field nuclear magnetic resonance (LF-NMR) reflects the migration pattern of water in different states in meat products by transverse relaxation times ($T_2$) [32]. As observed in Figure 3A, from left to right, $T_{2b}$ (0.1~10 ms), $T_{21}$ (10~100 ms), and $T_{22}$ (100~1000 ms) of relaxation represent bound water, immobilized water, and free water, respectively [33]. In addition, $P_{2b}$, $P_{21}$, and $P_{22}$ represent the relative proportion of $T_{2b}$, $T_{21}$, and $T_{22}$ relaxation areas, respectively (Table 2). By observing the change in the relative proportion, the relative water content in each state of the samples can be intuitively shown, thereby reflecting the change in water-holding capacity.

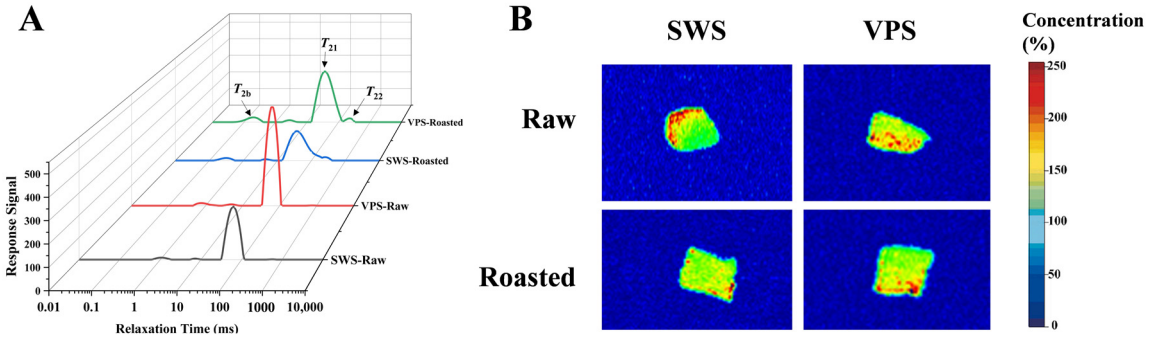



**Figure 3.** Effect of different salting treatments on the $T_2$ transverse relaxation peak response signals (**A**) and H-proton imaging spectra (**B**) of the samples. SWS: static wet salting; VPS: variable pressure salting.

**Table 2.** Effect of different salting treatments on the $T_2$ transverse relaxation peak area percentages ($P_2$) of the samples.

| Index/% | SWS-Raw | VPS-Raw | SWS-Roasted | VPS-Roasted |
|---|---|---|---|---|
| $P_{2b}$ | 4.86 ± 0.95 $^{a}$ | 4.43 ± 0.25 $^{a}$ | 3.49 ± 0.20 $^{b}$ | 3.43 ± 0.37 $^{b}$ |
| $P_{21}$ | 94.91 ± 1.04 $^{b}$ | 95.30 ± 0.64 $^{a}$ | 89.26 ± 1.87 $^{c}$ | 90.75 ± 0.61 $^{bc}$ |
| $P_{22}$ | 0.23 ± 0.05 $^{b}$ | 0.27 ± 0.03 $^{b}$ | 7.25 ± 1.67 $^{a}$ | 5.82 ± 0.97 $^{a}$ |

SWS-Raw: duck after static wet salting; VPS-Raw: duck after variable pressure salting; SWS-Roasted: duck roasted after static wet salting; and VPS-Roasted: duck roasted after variable pressure salting. Different letters within the same row indicate statistically significant differences at a level of $p < 0.05$.

Immobilized water was the primary form of water in the samples and is positively correlated with water retention [34]. According to Table 2, the $P_{21}$ was higher in the VPS-treated group than in the SWS-treated group ($p < 0.05$), suggesting that variable pressure salting improved the water-holding capacity of the duck breasts. The result was related to the salting effect of the VPS, which was efficient, with the higher ionic strength promoting the release of salt-soluble proteins and the formation of tighter three-dimensional protein network structures induced by heat treatment [35]. Moreover, compared with the raw breasts, the percentage of the free water relaxation area ($P_{22}$) in the roasted samples increased significantly ($p < 0.05$). In contrast, the percentage of bound water relaxation area ($P_{2b}$) and immobilized water relaxation area ($P_{21}$) decreased significantly ($p < 0.05$). This phenomenon might be attributed to the weakening water–protein interaction caused by high heating and prolonged heating accelerating the movement of internal moisture out of the muscle tissue [36].

The distribution of H protons in the meat can be intuitively represented by the H-proton density imaging spectra [37]. The H-proton density is positively correlated with the moisture content of the samples, and the red areas in the image indicate the presence of proton [38]. As depicted in Figure 3B, the samples from the VPS treatments exhibited more prominent red images, indicating that more water was retained between the muscle fibers, and the water migration in the breasts was reduced [39]. It is noteworthy that in the SWS-Raw group, the H protons were mainly distributed at the border of the duck breast, whereas H protons spread to the middle of the muscle after VPS. This result further confirmed the homogeneity and effectiveness of variable pressure salting.

### *3.4. Changes in Textural Properties*

As shown in Figure 4, the textural properties of duck breast were significantly affected ($p < 0.05$) by roasting, VPS treatment, and their interaction. Compared to raw duck breast, the samples after roasting exhibited higher hardness values due to the contraction and dehydration caused by high-temperature heating. The hardness values of the VPS treatments were significantly lower than those of the SWS treatments ($p < 0.05$) (Figure 4A), indicating that VPS could improve the tenderness of the duck. Additionally, as presented in Figure 4B,C, the springiness values of the roasted duck breasts in the VPS treatments were higher than those in the SWS treatments, whereas the chewiness value was lower than that in the SWS treatment. These results might indicate that the springiness of the roasted duck due to VPS and the recovery after removing the external force were better than those of SWS.

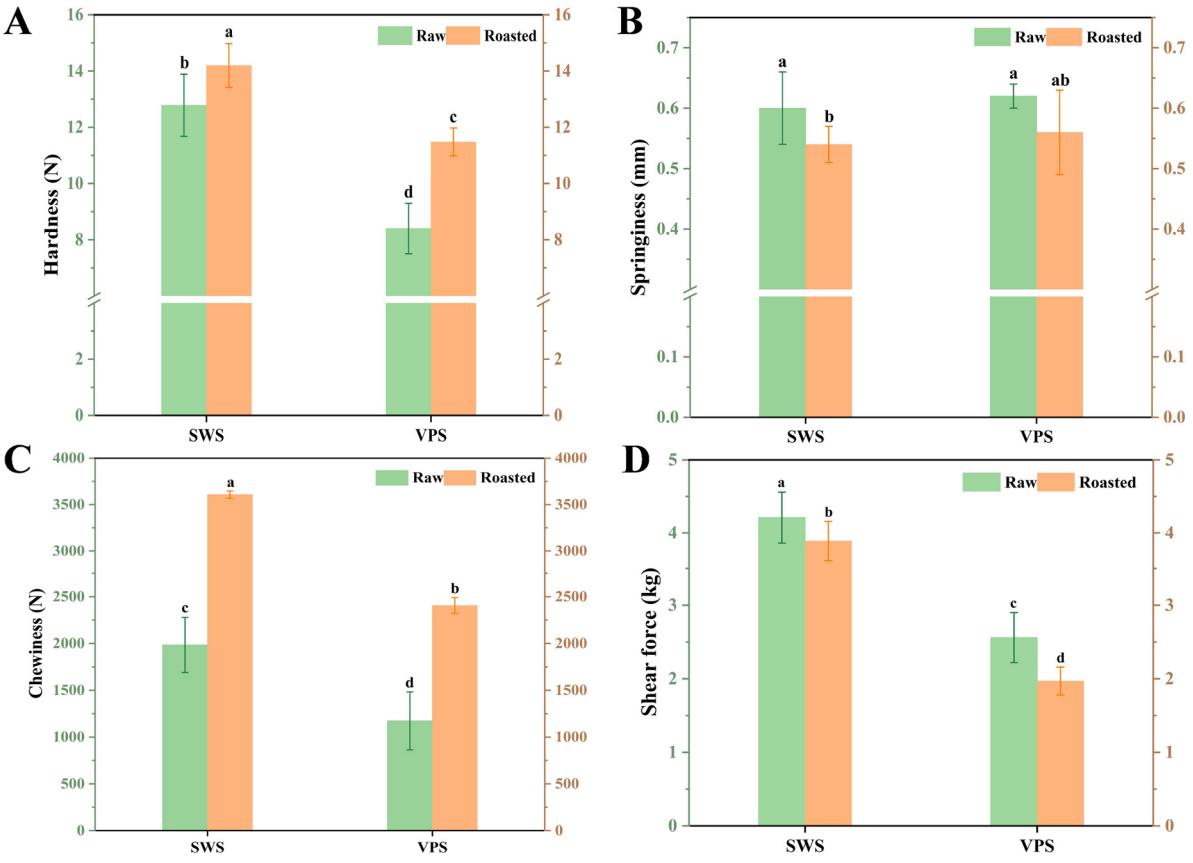



**Figure 4.** Effect of different salting treatments on the hardness (**A**), springiness (**B**), chewiness (**C**), and shear force (**D**) of the samples. SWS: static wet salting; VPS: variable pressure salting. Different letters indicate a significant difference ($p < 0.05$).

According to Figure 4D, the shear force in the VPS samples was significantly lower than in the SWS treatments ($p < 0.05$), which corresponded with Jiao's findings [10]. Gao et al. [40] found that variable pressure tumbling was more effective than atmospheric pressure tumbling at reducing shear forces, possibly due to the changes in myofibrillar skeletal protein structure exacerbated by variable pressure. Moreover, Yang et al. [41] reported that high pressure regulates the degree of protein hydration with water to improve the tenderness of meat gel products. Consequently, it is plausible that VPS may improve the textural properties of duck breasts by altering the skeletal protein structures and increasing the content of immobilized water.

### *3.5. Analysis of Microstructure*

The microstructure of the longitudinal and transverse sections of the samples treated with SWS and VPS is illustrated in Figure 5. The endomysium, which is elastic and maintains the integrity of the muscle, can rupture, indicating the loosening of muscle fiber bundles and an improvement in muscle tenderness [42]. As depicted, the endomysium of the duck breast muscle was destroyed after VPS, the muscle fiber gaps became extensive, and the tenderness improved. This result might be ascribed to the variable pressure expanding the internal space of myofibrils to retain more water [43]. In addition, compared to the raw duck treatments, the muscle fibers of the roasted duck treatments were significantly expanded by heat, resulting in a decreased distance between the muscle bundles, as well as increased hardness, as supported by the textural properties results (Figure 4). Notably, the diameter of the muscle fiber treated with VPS was more extensive than that of the SWS treatments, indicating that VPS could maintain higher levels of water retention and texture quality of the muscles. Therefore, VPS is an efficient salting method for improving the microstructure of the breast and may enhance its digestibility.

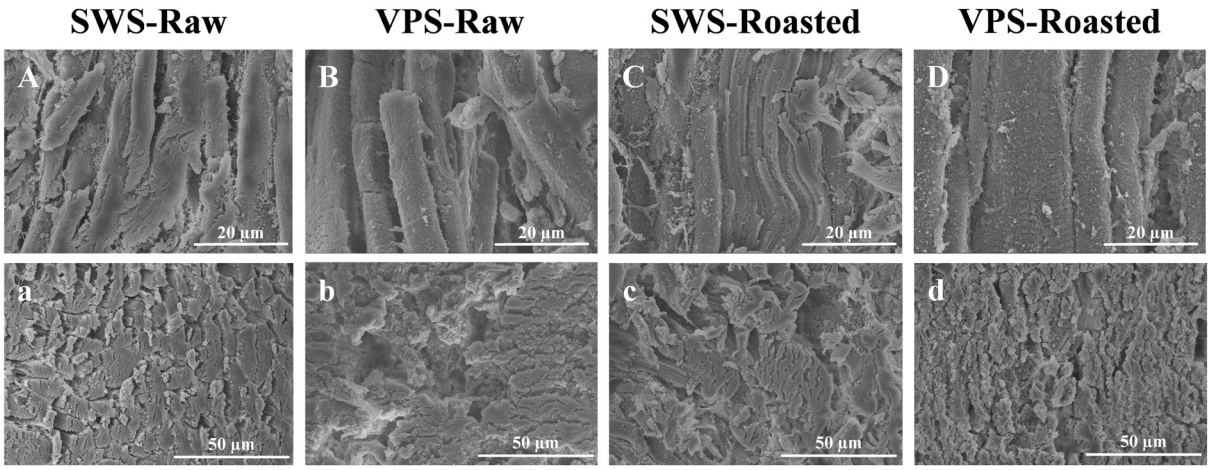



**Figure 5.** Light microscopic images of samples under different salting treatments. (**A**–**D**) Longitudinal sections of myofibrils of the SWS-Raw, VPS-Raw, SWS-Roasted, and VPS-Roasted treatments, respectively. (**a**–**d**) Transverse sections of myofibrils of the SWS-Raw, VPS-Raw, SWS-Roasted, and VPS-Roasted treatments, respectively.

### *3.6. Changes in Protein Structure*

The reduced (with β-ME) and nonreduced (without β-ME) SDS-PAGE results of the total protein from duck breasts treated with VPS and SWS are depicted in Figure 6A. It is evident that the intensity of the protein bands for the roasted samples was weaker than those of the raw samples, indicating that the high temperature generated by roasting promoted the degradation of the duck breast protein [5]. Compared with the SWS-Raw treatment, the VPS-Raw treatment lanes' bands deepened at 10~55 kDa. This result might be attributed to releasing some endogenous proteases in the muscle under the VPS treatment, facilitating protein solubilization and degradation. Cai et al. [44] also suggested that protein degradation

mainly involves deepening at lower molecular weights and weakening bands at higher molecular weights. Furthermore, the intensity of the protein bands of the VPS-Roasted group was weaker than for the SWS-Roasted group. This phenomenon suggests that the protein degradation of duck breasts treated by VPS was more apparent after roasting, which makes it more suitable for digestion and absorption by the human body.

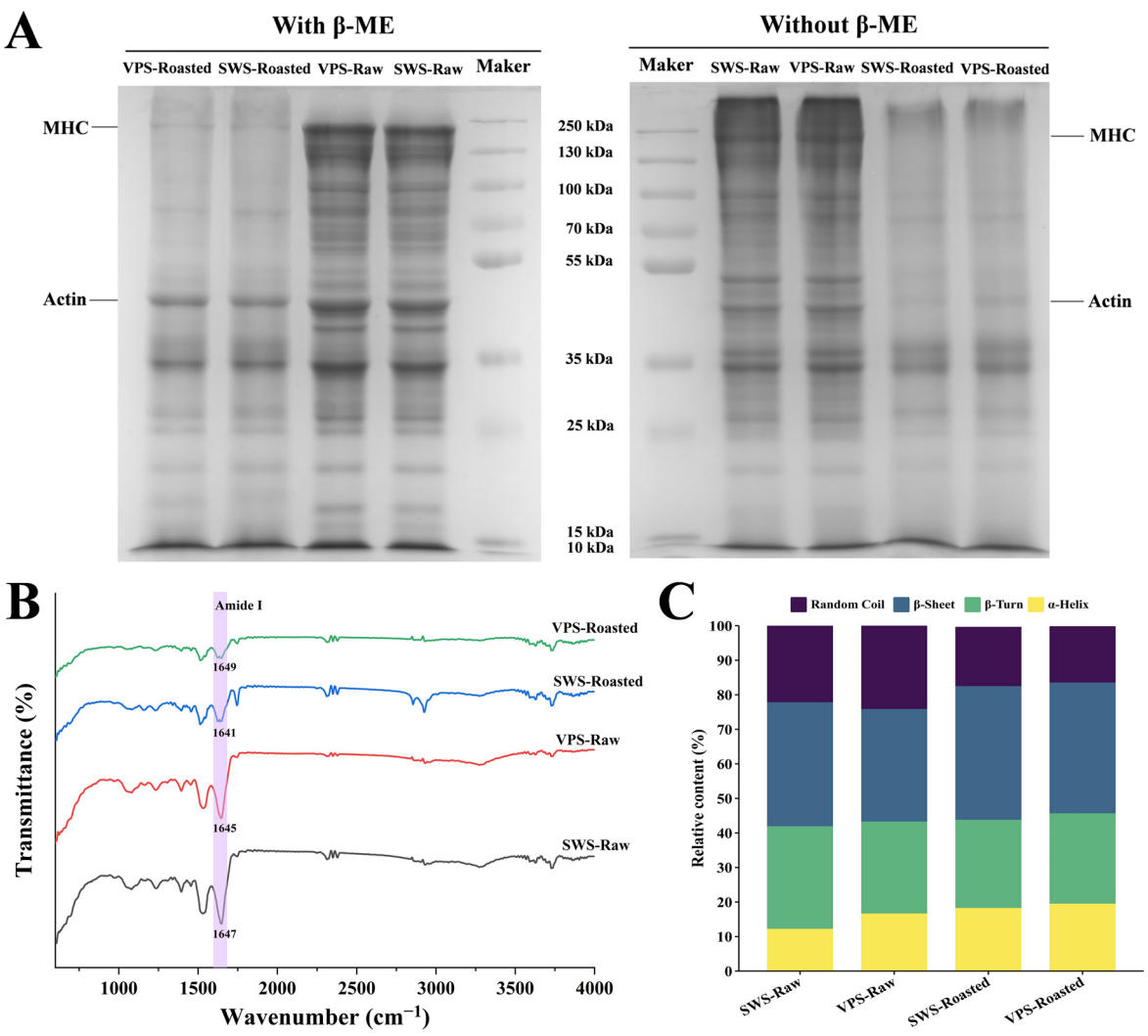



**Figure 6.** SDS-PAGE profiles (**A**) of protein in samples under different salting treatments. Proteins were prepared without and with β-mercaptoethanol (β-ME) addition. Changes in the infrared spectra (**B**) and relative protein secondary structure contents (**C**) under different salting treatments.

As illustrated in Figure 6B, the peak shapes of the FTIR spectra curves were significantly altered after applying different salting methods. The amide I band (1600~1700 $cm^{-1}$) is the main spectral band reflecting the secondary structure of proteins [45]. Higher contents of α-helixes and β-sheets reflect a more ordered structure, whereas more significant proportions of β-turns and random coils represent a more disordered structure [46]. As shown in Figure 6C, the total contents of α-helixes and β-sheets in the VPS-Raw group (49.27%) were higher than in the SWS-Raw group (48.15%), indicating that VPS could maintain the ordered structure of proteins. Moreover, Beattie et al. [47] found that the secondary structure of poorly tenderized muscle contained significantly more β-sheets than that of better-tenderized muscle. Interestingly, after roasting, the content of α-helix in the VPS-Roasted group (19.53%) was higher than in the SWS-Roasted group (18.27%), but the β-sheet content decreased (37.87%). This result implies that the duck breasts roasted after

the VPS treatment had better tenderness than those treated with SWS, consistent with the textural properties (Figure 4).

### *3.7. Correlation Analysis*

The correlations between serum biochemical indicators, in vivo digestion properties, water distribution, textural quality, and protein structure are presented in Figure 7. The peak area of free water ($P_{22}$), chewiness, and protein-ordered structure (α-helix and β-sheet) were found to have a positive correlation with serum biochemical indicators and in vivo digestion properties in the rats, indicating that roasted duck breasts with a higher water content, chewiness, and tenderness were more favorable for digestion and absorption in the body. Interestingly, there was no significant correlation ($p > 0.05$) between hardness, shear force, and in vivo digestion properties, which may be related to the fact that roasted duck breasts were mixed into the feed by stirring as powders. Furthermore, the bound water and immobilized water peak areas ($P_{2b}$ and $P_{21}$) of the roasted duck breasts showed a negative correlation with serum biochemical indicators and in vivo digestion properties of rats ($p < 0.05$). This result could be due to the transformation of bound and immobile water between muscle fibers to free water while preparing roasted duck breast into powders. In summary, the findings above demonstrate that the ordered structure of the protein, water content, and chewiness of the duck breasts roasted after VPS were superior to those roasted after SWS, which is more conducive for digestion in the body.

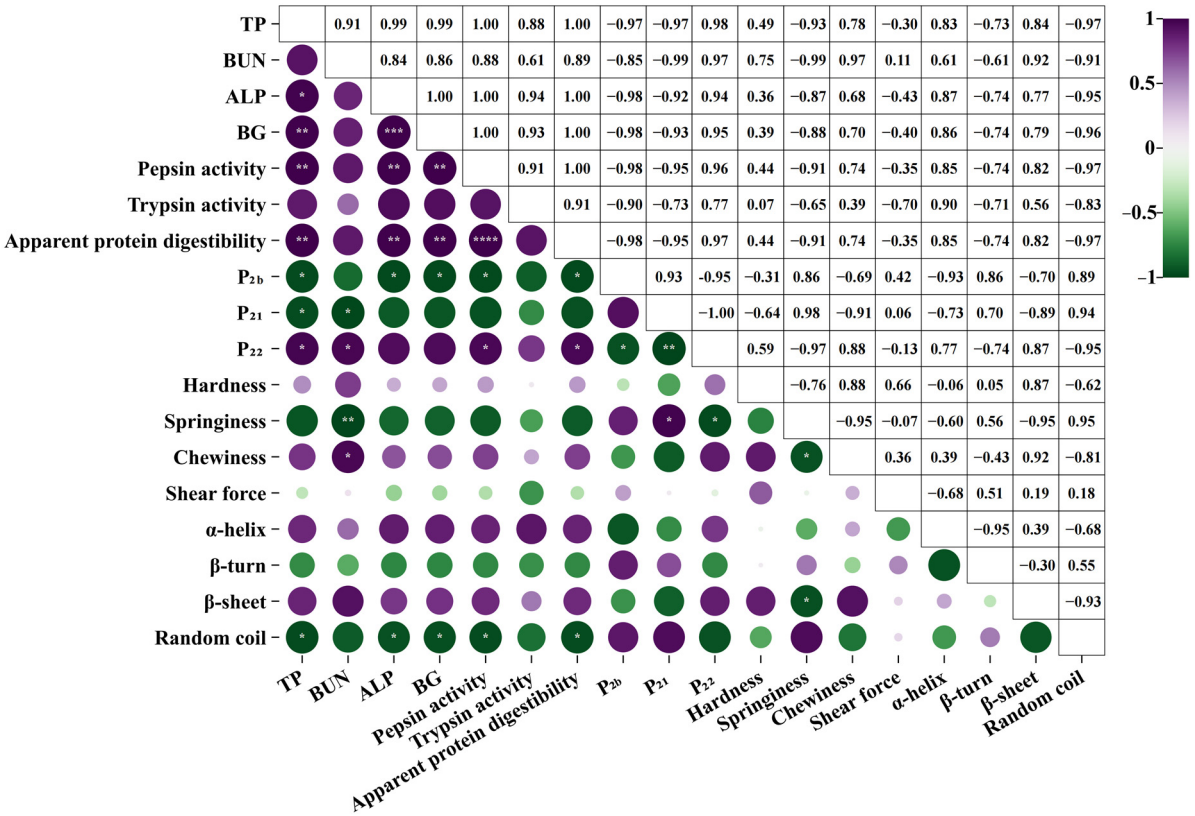



**Figure 7.** Heatmap of the correlation coefficients. The color of the scale bar denotes the nature of the correlation, with violet being a positive correlation and green being a negative. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

## 4. Conclusions

Our study demonstrates that VPS improves the texture quality of duck breasts by altering the protein structure and water distribution and enhancing the protein anabolism and in vivo digestion properties of rats that ingested VPS-treated roasted duck breasts. In detail, compared to SWS, the VPS-treated roasted duck breast promoted growth and

serum biochemical metabolism and significantly increased the apparent protein digestibility, as well as pepsin and trypsin activities, in the rats. Furthermore, the VPS treatment improved the textural properties and microstructure of the duck breasts by regulating protein hydration with water, increasing the ordered protein structures and the immobilized water content to enhance the protein anabolism capacity and in vivo digestion properties in rats. Nevertheless, consuming VPS-treated roasted duck breasts should be moderated to control salt intake. In conclusion, the results initially confirm the feasibility of VPS-treated roasted duck breasts to improve the efficacy of digestion and absorption in rats. In the future, the effect of VPS-treated roasted duck breasts on rat intestinal health should be further investigated.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/foods13030402/s1, Table S1. Diet composition and nutrient levels. Table S2. Effect of roasted duck treated with different salting on daily feed intake of rats (g/d).

**Author Contributions:** Z.G., Investigation, Validation, Conceptualization, and Writing—review and editing; Y.Z., Formal analysis and Methodology; D.Z., Conceptualization, Funding acquisition, Project administration, and Supervision; R.W., Formal analysis and Methodology; J.M., Methodology and Software; J.H., Data curation and Formal analysis; Z.W., Funding acquisition, Project administration, and Supervision. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was financially supported by the Beijing Innovation Consortium of Agriculture Research System (BAIC06-2024-BJJQ-G12); the Key R&D Program of Shandong Province, China (2022TZXD0021); and National Agricultural Science and Technology Innovation Program (G2022-IFST-05, CAAS-ASTIP-2023-IFST).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The raw data supporting the conclusions of this article will be made available by the authors on request. The data are not publicly available due to privacy restrictions.

**Acknowledgments:** The authors would like to thank laboratory colleagues for their advice and support.

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

## References

1. Liu, H.; Wang, Z.; Zhang, D.; Shen, Q.; Pan, T.; Hui, T.; Ma, J. Characterization of key aroma compounds in beijing roasted duck by gas chromatography-olfactometry-mass spectrometry, odor-activity values, and aroma-recombination experiments. *J. Agric. Food Chem.* **2019**, *67*, 5847–5856. [CrossRef] [PubMed]
2. Petit, G.; Jury, V.; de Lamballerie, M.; Duranton, F.; Pottier, L.; Martin, J.L. Salt intake from processed meat products: Benefits, risks and evolving practices. *Compr. Rev. Food Sci. Food Saf.* **2019**, *18*, 1453–1473. [CrossRef] [PubMed]
3. Wang, D.Y.; Zhu, Y.Z.; Xu, W.M. Composition of intramuscular phospholipids and free fatty acids in three kinds of traditional Chinese duck meat products. *Poult. Sci.* **2009**, *88*, 221–226. [CrossRef] [PubMed]
4. Chen, X.; Luo, J.; Lou, A.; Wang, Y.; Yang, D.; Shen, Q.W. Duck breast muscle proteins, free fatty acids and volatile compounds as affected by curing methods. *Food Chem.* **2021**, *338*, 128138. [CrossRef] [PubMed]
5. Jiao, D.; Zhang, D.; Chen, R.; Yang, X.; Shi, H.; Liu, H.; Wang, Z.; Lu, L.; Hui, T. A developed variable pressure-assisted salting process: Improving the textural, flavor, and sensory attributes in roasted duck breast. *LWT* **2022**, *167*, 113800. [CrossRef]
6. Jin, G.; He, L.; Wang, Q.; Liu, C.; Jin, Y.; Huang, F.; Ma, M. Pulsed pressure assisted brining of porcine meat. *Innov. Food Sci. Emerg. Technol.* **2014**, *22*, 76–80. [CrossRef]
7. Jin, G.; He, L.; Li, C.; Zhao, Y.; Chen, C.; Zhang, Y.; Zhang, J.; Ma, M. Effect of pulsed pressure-assisted brining on lipid oxidation and volatiles development in pork bacon during salting and drying-ripening. *LWT* **2015**, *64*, 1099–1106. [CrossRef]
8. Liu, Y.; Yu, J.; Zhu, J.; Peng, W.; Chen, Y.; Luo, X.; Chen, C.; Li, L. Effects of salt-induced changes in protein network structure on the properties of surimi gels: Computer simulation and digestion study. *Int. J. Food Sci. Technol.* **2021**, *56*, 3914–3923. [CrossRef]
9. Bhat, Z.F.; Morton, J.D.; Mason, S.L.; Jayawardena, S.R.; Mungure, T.; Bekhit, A.E.D.A. Cooking does not impair the impact of pulsed electric field on the protein digestion of venison (*Cervus elaphus*) during in-vitro gastrointestinal digestion. *Int. J. Food Sci. Technol.* **2021**, *56*, 3026–3033. [CrossRef]
10. Jiao, D.; Zhang, D.; Liu, H.; Wang, Z.; Hui, T. Optimization of dynamic variable pressure curing of duck carcass for roast duck. *Meat Res.* **2021**, *35*, 14–22. [CrossRef]

11. Bax, M.L.; Buffière, C.; Hafnaoui, N.; Gaudichon, C.; Savary-Auzeloux, I.; Dardevet, D.; Santé-Lhoutellier, V.; Rémond, D. Effects of meat cooking, and of ingested amount, on protein digestion speed and entry of residual proteins into the colon: A study in minipigs. *PLoS ONE* **2013**, *8*, e61252. [CrossRef]
12. *GB14924.3-2010*; Laboratory Animals-Nutrients for Formula Feeds. Standardization Administration of the People's Republic of China, General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China: Beijing, China, 2010.
13. Xu, Z.; Zhao, F.; Chen, H.; Xu, S.; Fan, F.; Shi, P.; Tu, M.; Wang, Z.; Du, M. Nutritional properties and osteogenic activity of enzymatic hydrolysates of proteins from the blue mussel (*Mytilus edulis*). *Food Funct.* **2019**, *10*, 7745–7754. [CrossRef]
14. Terrazas-Fierro, M.; Civera-Cerecedo, R.; Ibarra-Martínez, L.; Goytortúa-Bores, E.; Herrera-Andrade, M.; Reyes-Becerra, A. Apparent digestibility of dry matter, protein, and essential amino acid in marine feedstuffs for juvenile whiteleg shrimp *Litopenaeus vannamei*. *Aquaculture* **2010**, *308*, 166–173. [CrossRef]
15. Xu, Y.; Zhang, D.; Xie, F.; Li, X.; Schroyen, M.; Chen, L.; Hou, C. Changes in water holding capacity of chilled fresh pork in controlled freezing-point storage assisted by different modes of electrostatic field action. *Meat Sci.* **2023**, *204*, 109269. [CrossRef] [PubMed]
16. Wang, Y.; Liu, R.; Ge, Q.; Wu, M.; Xu, B.; Xi, J.; Yu, H. Effects of branched-chain amino acids and *Lactobacillus plantarum* CGMCC18217 on volatiles formation and textural properties of dry-cured fermented sausage. *Int. J. Food Sci. Technol.* **2021**, *56*, 374–383. [CrossRef]
17. Yin, Y.T.; Zhou, L.; Cai, J.M.; Feng, F.; Xing, L.J.; Zhang, W.G. Effect of malondialdehyde on the digestibility of beef myofibrillar protein: Potential mechanisms from structure to modification site. *Foods* **2022**, *11*, 2176. [CrossRef] [PubMed]
18. Gangidi, R.R.; Proctor, A.; Pohlman, F.W. Rapid determination of spinal cord content in ground beef by attenuated total reflectance Fourier transform infrared spectroscopy. *J. Food Sci.* **2003**, *68*, 124–127. [CrossRef]
19. D'Souza, J.M.P.; Pai, V.R.; Harish, S.; Shriyan, C. Relation of serum and salivary total sialic acid and total sialic acid/total protein ratio with clinical parameters of disease progression in preeclampsia. *Indian J. Clin. Biochem.* **2022**, *37*, 113–118. [CrossRef] [PubMed]
20. Hegen, H.; Auer, M.; Zeileis, A.; Deisenhammer, F. Upper reference limits for cerebrospinal fluid total protein and albumin quotient based on a large cohort of control patients: Implications for increased clinical specificity. *Clin. Chem. Lab. Med.* **2016**, *54*, 285–292. [CrossRef] [PubMed]
21. Glaser, N.S.; Quayle, K.S.; McManemy, J.K.; Nigrovic, L.E.; Tzimenatos, L.; Stoner, M.J.; Bennett, J.E.; Trainor, J.L.; Rewers, A.; Schunk, J.E.; et al. Clinical characteristics of children with cerebral injury preceding treatment of diabetic ketoacidosis. *J. Pediatr.* **2022**, *250*, 100–104. [CrossRef]
22. Peng, Z.; Li, Y.; Yan, L.; Yang, S.; Yang, D. Correlation analysis of microbial contamination and alkaline phosphatase activity in raw milk and dairy products. *Int. J. Environ. Res. Public Health* **2023**, *20*, 1825. [CrossRef] [PubMed]
23. Zaher, D.M.; El-Gamal, M.I.; Omar, H.A.; Aljareh, S.N.; Al-Shamma, S.A.; Ali, A.J.; Zaib, S.; Iqbal, J. Recent advances with alkaline phosphatase isoenzymes and their inhibitors. *Arch. Pharm.* **2020**, *353*, e2000011. [CrossRef] [PubMed]
24. Nansel, T.R.; Gellar, L.; McGill, A. Effect of varying glycemic index meals on blood glucose control assessed with continuous glucose monitoring in youth with type 1 diabetes on basalm-bolus insulin regimens. *Diabetes Care* **2008**, *31*, 695–697. [CrossRef]
25. Burgess, S.C.; Jeffrey, F.M.H.; Storey, C.; Milde, A.; Hausler, N.; Merritt, M.E.; Mulder, H.; Holm, C.; Sherry, A.D.; Malloy, C.R. Effect of murine strain on metabolic pathways of glucose production after brief or prolonged fasting. *Am. J. Physiol.-Endocrinol. Metab.* **2005**, *289*, E53–E61. [CrossRef]
26. Liu, Y.G.; Yang, L.L.; Zhang, Y.; Liu, X.C.; Wu, Z.J.; Gilbert, R.G.; Deng, B.; Wang, K.P. *Dendrobium officinale* polysaccharide ameliorates diabetic hepatic glucose metabolism via glucagon-mediated signaling pathways and modifying liver-glycogen structure. *J. Ethnopharmacol.* **2020**, *248*, 112308. [CrossRef] [PubMed]
27. Oberli, M.; Lan, A.; Khodorova, N.; Santé-Lhoutellier, V.; Walker, F.; Piedcoq, J.; Davila, A.M.; Blachier, F.; Tomé, D.; Fromentin, G.; et al. Compared with raw bovine meat, boiling but not grilling, barbecuing, or roasting decreases protein digestibility without any major consequences for intestinal mucosa in rats, although the daily ingestion of bovine meat induces histologic modifications in the colon. *J. Nutr.* **2016**, *146*, 1506–1513. [CrossRef]
28. Jeng, S.-S.; Liao, H.-J.; Wang, M.-S. High Zn concentration in the digestive tract tissues of common carp cyprinus carpio. *Fish. Sci.* **2011**, *68*, 1181–1184. [CrossRef]
29. Pennings, B.; Groen, B.B.L.; van Dijk, J.W.; de Lange, A.; Kiskini, A.; Kuklinski, M.; Senden, J.M.G.; van Loon, L.J.C. Minced beef is more rapidly digested and absorbed than beef steak, resulting in greater postprandial protein retention in older Men. *Am. J. Clin. Nutr.* **2013**, *98*, 121–128. [CrossRef]
30. Peng, Q.; Khan, N.A.; Wang, Z.; Yu, P. Relationship of feeds protein structural makeup in common Prairie feeds with protein solubility, in situ ruminal degradation and intestinal digestibility. *Anim. Feed. Sci. Technol.* **2014**, *194*, 58–70. [CrossRef]
31. Ferreira, V.C.S.; Morcuende, D.; Madruga, M.S.; Silva, F.A.P.; Estévez, M. Role of protein oxidation in the nutritional loss and texture changes in ready-to-eat chicken patties. *Int. J. Food Sci. Technol.* **2018**, *53*, 1518–1526. [CrossRef]
32. Qian, S.; Hu, F.; Mehmood, W.; Li, X.; Zhang, C.; Blecker, C. The rise of thawing drip: Freezing rate effects on ice crystallization and myowater dynamics changes. *Food Chem.* **2022**, *373*, 131461. [CrossRef] [PubMed]
33. Song, Y.; Huang, F.; Li, X.; Han, D.; Zhao, L.; Liang, H.; Rui, M.; Wang, J.; Zhang, C. Water status evolution of pork blocks at different cooking procedures: A two-dimensional LF-NMR $T_1$-$T_2$ relaxation study. *Food Res. Int.* **2021**, *148*, 110614. [CrossRef]

34. Hu, F.; Qian, S.; Huang, F.; Han, D.; Li, X.; Zhang, C. Combined impacts of low voltage electrostatic field and high humidity assisted-thawing on quality of pork steaks. *LWT* **2021**, *150*, 0023–6438. [CrossRef]
35. Bombrun, L.; Gatellier, P.; Carlier, M.; Kondjoyan, A. The effects of low salt concentrations on the mechanism of adhesion between two pieces of pork semimembranosus muscle following tumbling and cooking. *Meat Sci.* **2014**, *96*, 5–13. [CrossRef] [PubMed]
36. McDonnell, C.K.; Allen, P.; Duggan, E.; Arimi, J.M.; Casey, E.; Duane, G.; Lyng, J.G. The effect of salt and fibre direction on water dynamics, distribution and mobility in pork muscle: A low field NMR study. *Meat Sci.* **2013**, *95*, 51–58. [CrossRef]
37. Ouyang, Q.; Liu, L.; Zareef, M.; Wang, L.; Chen, Q. Application of portable visible and near-infrared spectroscopy for rapid detection of cooking loss rate in pork: Comparing spectra from frozen and thawed pork. *LWT* **2022**, *160*, 113304. [CrossRef]
38. Fallah-Joshaqani, S.; Hamdami, N.; Keramat, J. Qualitative attributes of button mushroom (*Agaricus bisporus*) frozen under high voltage electrostatic field. *J. Food Eng.* **2021**, *293*, 110384. [CrossRef]
39. Pearce, K.L.; Rosenvold, K.; Andersen, H.J.; Hopkins, D.L. Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes—A review. *Meat Sci.* **2011**, *89*, 111–124. [CrossRef]
40. Gao, X.; Xie, Y.; Yin, T.; Hu, Y.; You, J.; Xiong, S.; Liu, R. Effect of high intensity ultrasound on gelation properties of silver carp surimi with different salt contents. *Ultrason. Sonochem.* **2021**, *70*, 105326. [CrossRef]
41. Yang, H.; Chen, L.; Tang, H.; Wang, H.; Zhang, J.; Xiao, C.; Tao, F.; Cao, G.; Shen, Q. Tenderness improvement of reduced-fat and reduced-salt meat gels as affected by high pressure treating time. *Innov. Food Sci. Emerg. Technol.* **2021**, *70*, 102687. [CrossRef]
42. Han, M.; Wang, P.; Xu, X.; Zhou, G. Low-field NMR study of heat-induced gelation of pork myofibrillar proteins and its relationship with microstructural characteristics. *Food Res. Int.* **2014**, *62*, 1175–1182. [CrossRef]
43. Li, Y.; Feng, T.; Sun, J.; Guo, L.; Wang, B.; Huang, M.; Xu, X.; Yu, J.; Ho, H. Physicochemical and microstructural attributes of marinated chicken breast influenced by breathing ultrasonic tumbling. *Ultrason. Sonochem.* **2020**, *64*, 1350–4177. [CrossRef] [PubMed]
44. Cai, L.; Nian, L.; Cao, A.; Zhang, Y.; Li, X. Effect of carboxymethyl chitosan magnetic nanoparticles plus herring antifreeze protein on conformation and oxidation of myofibrillar protein from red sea bream (*Pagrosomus major*) after freeze-thaw treatment. *Food Bioprocess Technol.* **2020**, *13*, 355–366. [CrossRef]
45. Wang, J.-Y.; Yang, Y.-L.; Tang, X.-Z.; Ni, W.-X.; Zhou, L. Effects of pulsed ultrasound on rheological and structural properties of chicken myofibrillar protein. *Ultrason. Sonochem.* **2017**, *38*, 225–233. [CrossRef]
46. Jiang, L.; Wang, J.; Li, Y.; Wang, Z.; Liang, J.; Wang, R.; Chen, Y.; Ma, W.; Qi, B.; Zhang, M. Effects of ultrasound on the structure and physical properties of black bean protein isolates. *Food Res. Int.* **2014**, *62*, 595–601. [CrossRef]
47. Beattie, J.R.; Bell, S.E.J.; Borggaard, C.; Moss, B.W. Preliminary investigations on the effects of ageing and cooking on the Raman spectra of porcine longissimus dorsi. *Meat Sci.* **2008**, *80*, 1205–1211. [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.