processes

MDPI

Article

Effects of Calcium Sulfate and Chitosan on Textural Modification and Microstructure of Tofu Made from Lentils (*Lens culinaris*)

Cheng-Hsun Jao [1], Chieh-Yi Lin [1], Chao-Jung Chen [2] and Jung-Feng Hsieh [1,2,*]

[1] Department of Food Science, Fu Jen Catholic University, New Taipei City 242, Taiwan
[2] Ph.D. Program in Nutrition and Food Science, Fu Jen Catholic University, New Taipei City 242, Taiwan
* Correspondence: 075101@mail.fju.edu.tw; Tel.: +886-2-2905-2516

Abstract: This study investigated calcium sulfate and chitosan on the textural modification and microstructure of tofu made from lentils. The addition of varying amounts of calcium sulfate (0–12 mM) and chitosan (0–1.0%) into lentil milk could affect the gel properties of lentil-based tofu. The gel properties, including the hardness and cohesiveness, of lentil-based tofu significantly increased with the addition of 12 mM calcium sulfate, exhibiting a slightly discontinuous network structure and a slightly regular pore network. However, the gel properties including hardness and cohesiveness significantly decreased with the addition of 1.0% chitosan, presenting a slightly continuous network structure with pores. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis showed that the aggregation of the vicilin, legumin acidic unit and legumin basic unit proteins in lentil milk was induced both by 12 mM calcium sulfate and 1.0% chitosan. Our results suggested that calcium sulfate and chitosan could affect the gel properties, such as hardness and cohesiveness, of lentil-based tofu. Therefore, calcium sulfate and chitosan can be used as practical food additives for the development of texture-modified lentil-based tofu.

Keywords: lentil; calcium sulfate; chitosan; gel properties; lentil-based tofu

Citation: Jao, C.-H.; Lin, C.-Y.; Chen, C.-J.; Hsieh, J.-F. Effects of Calcium Sulfate and Chitosan on Textural Modification and Microstructure of Tofu Made from Lentils (*Lens culinaris*). *Processes* **2022**, *10*, 2000. https://doi.org/10.3390/pr10102000

Academic Editor: Jasna Čanadanović-Brunet

Received: 11 September 2022
Accepted: 28 September 2022
Published: 3 October 2022

1. Introduction

The consumption of lentils (*Lens culinaris*) has several positive effects on human health, and lentils are an excellent source of several nutritional factors. They are one of the staple foods in many countries, including the Indian Peninsula, and it is composed of approximately 44.3% starch, 28.6% protein, 4.9% crude fiber and 3.1% ash. The lentil proteins are composed of approximately 16% albumins, 11% glutelins, 3% prolamins and 70% globulins [1]. Globulins consist of vicilin and legumin proteins. Legumin (11 S) is a hexamer with a molecular weight of approximately 320–380 kDa, consisting of six noncovalently interacting peptide pairs. These polypeptide pairs are composed of an acidic subunit (40 kDa) and a basic subunit (20 kDa) linked by a single disulfide bond. Vicilin (7 S) is a trimer with a molecular weight of approximately 150–190 kDa, consisting of three glycosylated subunits with a molecular weight of 50–60 kDa [2]. Recent studies have identified lentil protein as having good functionality features, such as solubility, foaming and gelling properties [3]. Lentils are high in protein and have the potential for use as an alternative to soybean-based foods.

Soy-based foods, such as tofu, are an important plant protein source. The preparation of tofu generally includes the coagulation, molding and pressing of soymilk. Traditionally, coagulants such as calcium sulfate and chitosan have been used for making tofu [4,5]. Calcium sulfate dissolved in water forms calcium ions and sulfate ions [6]. Ca^{2+}-induced soy protein aggregation is thought to arise from effects including ion-specific hydrophobic interactions, electrostatic shielding and crosslinking of adjacent anionic molecules [7]. Chitosan is a polysaccharide produced by the deacetylation of chitin, comprising units

of d-glucosamine and (1,4)-linked 2-amino-deoxy-β-D-glucan. This polysaccharide has been used to produce tofu and to improve its shelf life [8]. Kim et al. [9] also reported that chitosan would be useful as an additive to extend the shelf-life of tofu without a severe deterioration of physicochemical and sensory properties of tofu. The coacervation of soy protein induced by chitosan and soy protein–chitosan interactions plays an important structural control role in tofu [5]. These complex interactions involve hydrogen bonding between the carboxyl groups of soy proteins (COO^-) and the amine groups of chitosan ($-NH_3^+$). Therefore, the addition of chitosan and calcium sulfate affect the gel properties, microstructure and texture of tofu.

As an alternative to soybean-based foods, peas (*Pisum sativum*) have been used to make pea-based tofu-type products. The peas are rarely genetically modified and are an underutilized crop that does not require allergen labeling [10]. In a previous study, we investigated the effects of glucono-δ-lactone and chitosan on the gel properties, texture and microstructure of pea-based tofu-type products. We found that the addition of glucono-δ-lactone and chitosan reduced the hardness and cohesiveness of the pea-based tofu-type product. These food additives could be practical for developing texture-modified pea-based tofu-type products. Because lentils have the potential to be used as an alternative to soybean-based foods, the effects of calcium sulfate and chitosan on the gel properties and texture modification of lentil-based tofu were examined. Lentils also contain many important phytochemicals, such as phenolic compounds and flavonols, which make lentils a functional health food [11].

To investigate the effects of calcium sulfate and chitosan on the gel properties and texture modification of lentil-based tofu, scanning electron microscopy (SEM), sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and texture analysis were conducted. Our objective was to investigate calcium sulfate and chitosan on lentil-based tofu.

2. Materials and Methods

2.1. Preparation of Lentil-Based Tofu

The lentil-based tofu used in the current study was prepared using methods described by Jao et al. [12] with slight modifications. Lentils (300 g, *Lens culinaris*) were soaked in deionized water for 12 h at 4 °C. The seeds were then drained and ground with 600 mL of distilled water using a homogenizer at 32,000 rpm for 2 min. The sample was then passed through a cotton filter with a mesh opening size of 0.125 mm, and the filtrate was collected. To fix the lentil-based tofu, various concentrations (0, 0.1, 0.5, and 1.0%) of chitosan (molecular weight: 0.4–0.6 kDa, deacetylation > 90%; Simpson Biotech Co., Ltd., Taipei, Taiwan) or calcium sulfate (0, 4, 8 and 12 mM; Sxin Long Industry Co., Ltd., Kaohsiung, Taiwan) were added directly to the filtrate before heating the samples in a water bath at 85 °C for 1 h. The lentil-based samples were then maintained at 4 °C for 12 h until the gel properties and microstructure were measured.

2.2. Assessment of Gel Properties

The texture of the lentil-based tofu was measured using a TA-XT2i Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK) in accordance with the methods described by Liu et al. [13]. To measure cohesiveness and hardness, the lentil-based tofu was cut into a cylindrical shape (20 mm height and 30 mm diameter) and then compressed to 50% of its original height at a rate of 0.5 mm/s using a cylindrical plunger *P*/20 (20 mm diameter). Each sample was subjected to six measurements.

2.3. Microstructure of Lentil-Based Tofu

The microstructure of the lentil-based tofu was observed using SEM (TM4000plus, Hitachi, Ltd., Tokyo, Japan). Sample preparation for SEM was conducted in accordance with the methods outlined by Cao et al. [14]. The tofu was cut into cubes (10 mm × 5 mm × 5 mm) and then freeze-dried directly before being sputter-coated with gold. SEM was performed at a voltage of 15 kV with images obtained on film under 300× magnification.

2.4. Preparation of Lentil Milk Samples

Lentil seeds (50 g) were soaked in deionized water at 4 °C for 12 h, followed by grinding with deionized water (300 mL) before being passed through a cotton filter to collect the lentil milk. The effect of adding chitosan (0, 0.1, 0.5 and 1.0%) or calcium sulfate (0, 4, 8 and 12 mM) to lentil milk (10 mL) in terms of coagulation and antioxidant activity were estimated. After incubation, the treated lentil milk samples were subjected to a temperature of 85 °C for 15 min; the samples were separated into a lentil milk pellet fraction (LMPF) and lentil milk supernatant fraction (LMSF) via centrifugation at 4500× *g* for 15 min. The LMSF and LMPF samples were maintained at 4 °C prior to use.

2.5. SDS-PAGE Analysis of LMSF

LMSF samples were analyzed in accordance with the methods outlined by Hsia et al. [15]. Briefly, the LMSF samples were analyzed using a separating gel (12.5%) and a stacking gel (5%). Each LMSF sample (0.1 mL) was mixed with sample buffer (0.2 mL, pH 6.8, 10% glycerol, 0.02% bromophenol blue, 5% β-mercaptoethanol, 2% SDS, and 70 mM Tris-HCl) and heated to 95 °C for 5 min. A protein ladder (6 µL, 10–180 kDa) and samples (8 µL) were loaded into separate wells. After gel electrophoresis, the gels were stained using Coomassie Brilliant Blue R-250 and scanned using an image scanner (Epson Perfection V39; Nagano, Japan). The coagulation of lentil proteins induced by chitosan and calcium sulfate was assessed by the magnitude of changes in the electropherogram.

2.6. Extraction and Analysis of Total Phenolic Content in LMPF Samples

The total phenolic content (TPC) of the LMPF samples with or without chitosan and calcium sulfate was measured in accordance with methods outlined by Hung et al. [16]. LMPF (0.2 g) was extracted using 80% methanol (2 mL) at 60 °C for 1 h. After centrifugation at 12,000× *g* for 15 min, the supernatant (10 µL) was mixed with 10% Folin–Ciocalteu reagent (100 µL) and incubated at 30 °C for 15 min. Then, 1 M Na_2CO_3 (80 µL) was added. The absorbance at 765 nm was measured using a Versa-Max™ microplate reader (Molecular Devices Corporation, Sunnyvale, CA, USA). TPC was represented as gallic acid equivalents (GAEs) per milliliter of LMPF extract (µg GAE/mL).

2.7. Statistical Analysis

Data analysis was performed using SAS® version 9.4 software (SAS Institute, Cary, NC, USA), and the results are expressed as the mean ± standard deviation (SD). One-way analysis of variance was used to calculate the differences among treatments. All measurements were performed in triplicate, except for the analysis of gel properties, which was repeated six times. Significant differences were determined at $p < 0.05$.

3. Results and Discussion

3.1. Effect of Calcium Sulfate and Chitosan on the Gel Properties of Lentil-Based Tofu

The hardness and cohesiveness are the most often evaluated characteristics when determining tofu, and cohesiveness is defined as the extent to which a material can be deformed before it ruptures [17]. Therefore, the influence of calcium sulfate on the gel properties, including the hardness and cohesiveness, of lentil-based tofu was evaluated (Figure 1). The hardness and cohesiveness of lentil-based tofu without calcium sulfate heated at 85 °C for 1 h were 52.5 ± 2.3 g and 14.7 ± 0.4 g, respectively (Figure 1A,B). The hardness results were lower than the results of Byars and Singh [18], who reported that the hardness and cohesiveness of lentil gel were 165 g and 38.8 g force, respectively. We noticed that both the hardness (62.8 ± 1.0 g) and cohesiveness (17.7 ± 0.4 g force) of lentil-based tofu increased significantly with the addition of 4 mM calcium sulfate ($p < 0.05$). The addition of 12 mM calcium sulfate of lentil-based tofu had the highest hardness (67.5 ± 0.8 g), which was approximately 1.3-fold that without calcium sulfate. The hardness and cohesiveness of soy-based tofu increased with the addition of calcium sulfate, and the calcium ions formed bridges with the soybean proteins, resulting in a stronger

gel [19]. Furthermore, the effect of chitosan on the hardness and cohesiveness of lentil-based tofu was also investigated (Figure 1C,D). The hardness (63.7 ± 0.8 g) and cohesiveness (19.0 ± 0.4 g force) of lentil-based tofu reached a maximum when 0.1% chitosan was added. However, excess amounts of chitosan caused a significant decrease ($p < 0.05$) in both the hardness and cohesiveness of lentil-based tofu. The addition of 1% chitosan to lentil-based tofu had the lowest hardness (36.7 ± 2.2 g), indicating that the addition of chitosan could affect the gel properties of lentil-based tofu. Kim et al. [9] indicated that tofu with chitosan had smaller protein aggregates and looser connections between protein aggregates than tofu without chitosan, which could account for the chitosan tofu being softer. According to the above results, chitosan and calcium sulfate can be used as food additives to prepare texture-modified lentil-based tofu.

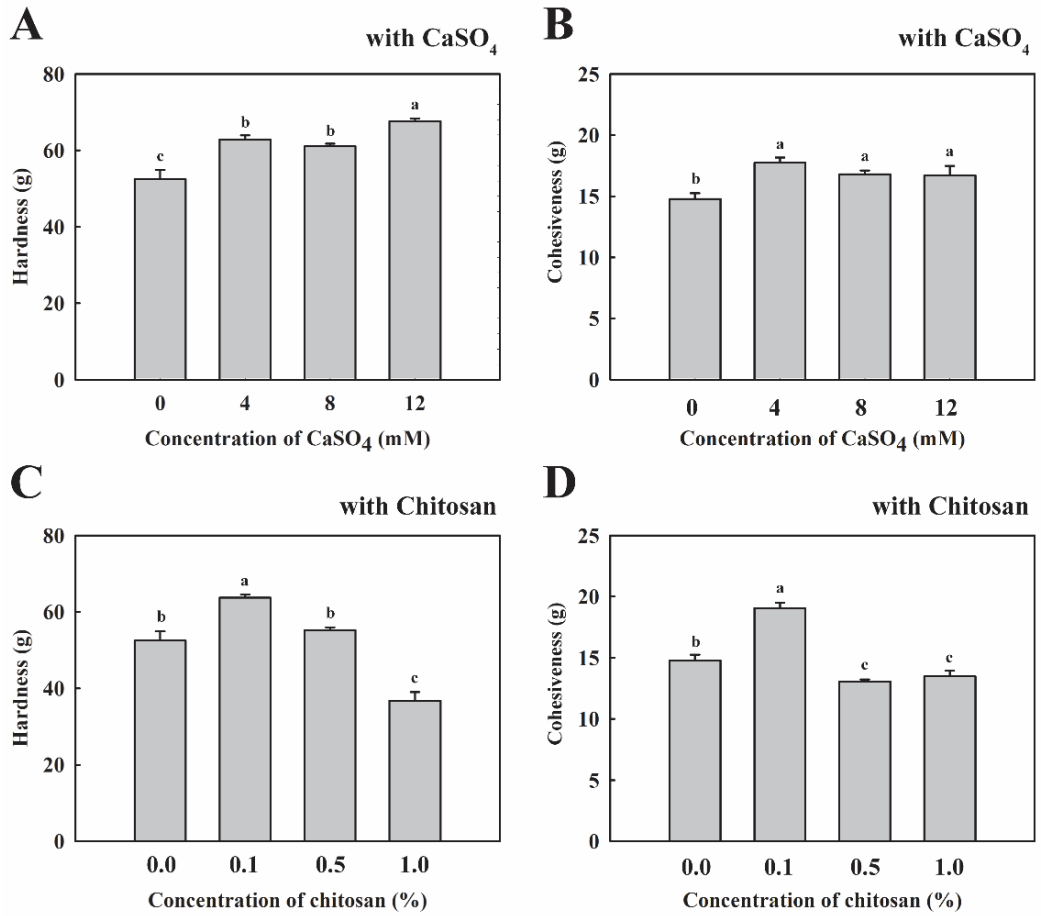

Figure 1. (**A**) Hardness and (**B**) cohesiveness of lentil-based tofu as a function of calcium sulfate content (0, 4, 8 and 12 mM); (**C**) Hardness and (**D**) cohesiveness of lentil-based tofu as a function of chitosan content (0, 0.1, 0.5 and 1.0%). Letters indicate differences at $p < 0.05$. Black arrow: lentil-based tofu.

3.2. Effect of Calcium Sulfate and Chitosan on the Appearance of Lentil-Based Tofu

We further investigated the gelation of lentil-based tofu with/without calcium sulfate (Figure 2). After manufacturing, the lentil-based tofu was stored overnight at 4 °C, and its appearance was observed. Figure 2A displays a photo of lentil-based tofu without calcium sulfate. The results showed that the lentil-based tofu without calcium sulfate (control) was thermally gelled, soft in appearance and texture, and had many pores. Compared to control tofu, lentil-based tofu containing 4 mM calcium sulfate (Figure 2B), 8 mM calcium sulfate (Figure 2C) and 12 mM calcium sulfate (Figure 2D) had a smooth surface and solid structure. Kao et al. [19] reported that a denser and more compact structure maintained by hydrogen bonds and calcium–protein bridges might account for the higher hardness and higher springiness of soy-based tofu at higher concentrations of calcium sulfate. These

results suggested that calcium sulfate helps to increase the hardness and cohesiveness of lentil-based tofu. Therefore, the addition of chitosan and calcium sulfate can affect the structure of tofu made from lentils.

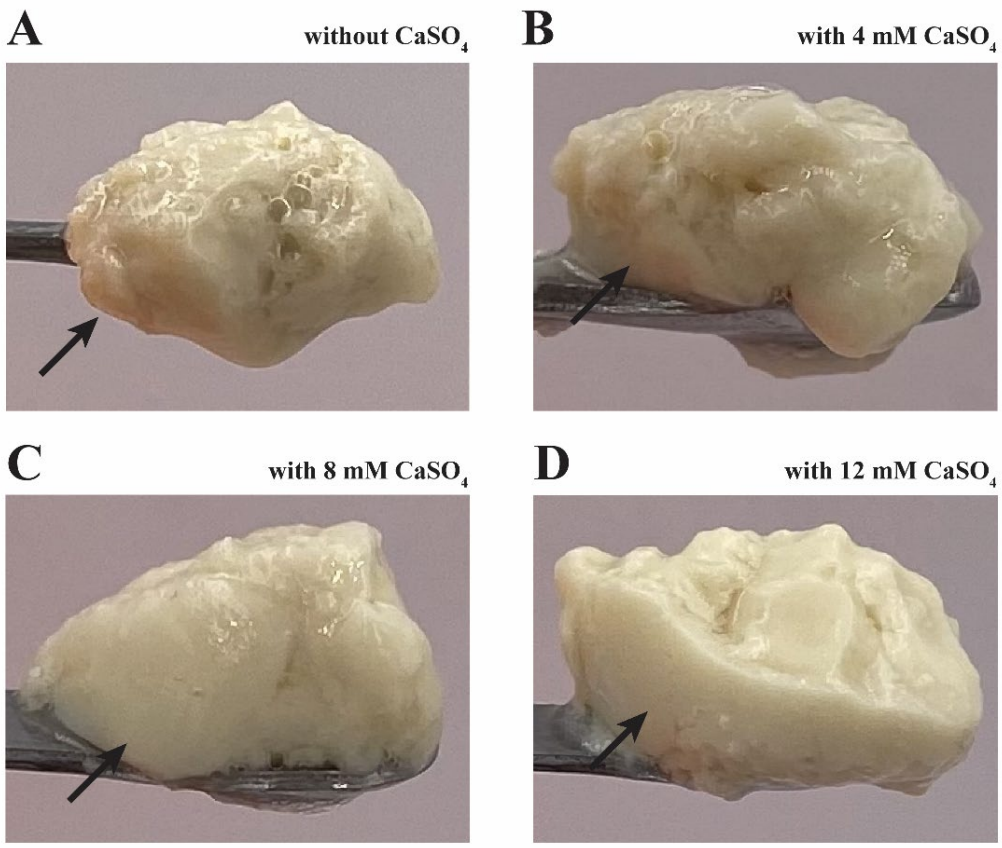

Figure 2. Appearance of lentil-based tofu with/without calcium sulfate: (**A**) without calcium sulfate; (**B**) with 4 mM calcium sulfate; (**C**) with 8 mM calcium sulfate; and (**D**) with 12 mM calcium sulfate.

Figure 3 presents photos of lentil-based tofu with and without chitosan. The appearance of lentil-based tofu without chitosan (Figure 3A), with 0.1% chitosan (Figure 3B), 0.5% chitosan (Figure 3C) and 1.0% chitosan (Figure 3D) was also evaluated. Our findings showed that lentil-based tofu with chitosan was more yellow than tofu without chitosan. Kim and Han [20] indicated that the addition of chitosan to soy-based tofu affected the color of the tofu, making it slightly yellowish. The results also showed that the lentil-based tofu without chitosan was thermally gelled, soft in appearance and texture, and had many pores. With the increase in chitosan added to 1.0%, the lentil-based tofu changed from its original soft appearance and texture to an uneven, porous surface and fragile structure. These results are similar to the results of Jao et al. [12], who reported that pea-based tofu with 1.0% chitosan had a rougher appearance than samples without chitosan. The hardness and cohesiveness of pea-based tofu was significantly reduced after adding 1.0% chitosan to pea milk. According to the above results, the addition of calcium sulfate and/or chitosan was observed to alter the structure of lentil-based tofu.

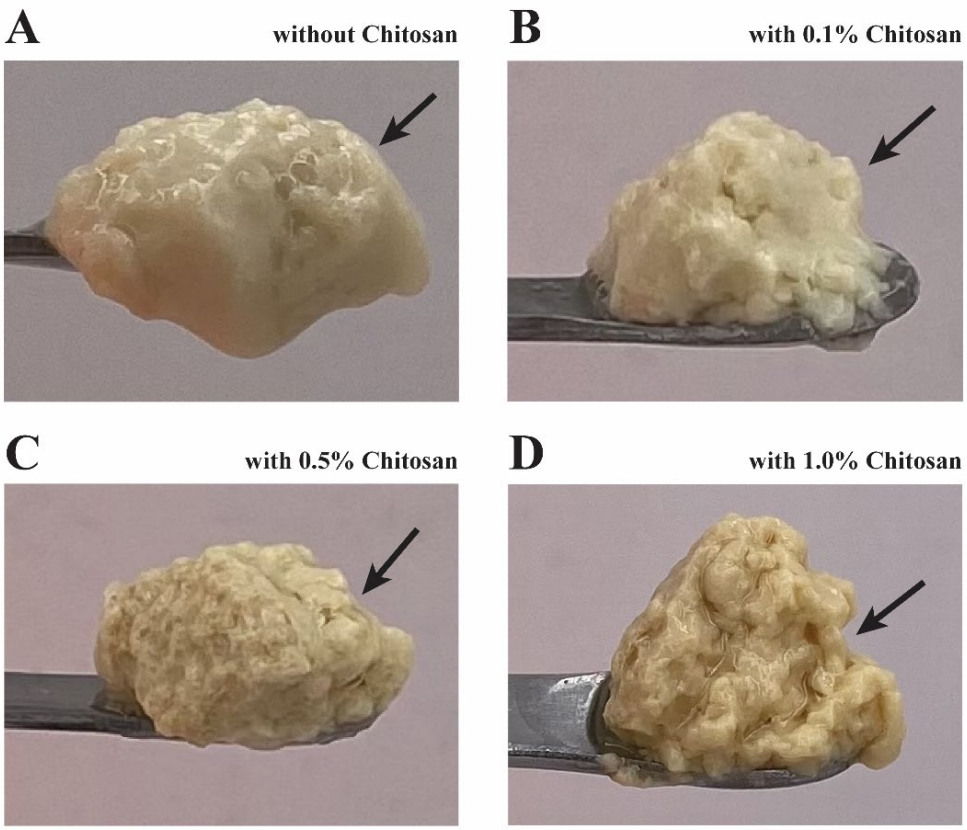

Figure 3. Appearance of lentil-based tofu with/without chitosan: (**A**) without calcium sulfate; (**B**) with 0.1% chitosan; (**C**) with 0.5% chitosan; and (**D**) with 1.0% chitosan.

3.3. Effect of Calcium Sulfate and Chitosan on the Microstructure of Lentil-Based Tofu

The microstructure of lentil-based tofu without/with calcium sulfate is shown in Figure 4. The lentil-based tofu without calcium sulfate presented a discontinuous network structure with pores filled with water (Figure 4A). The lentil-based tofu samples containing 4 mM calcium sulfate (Figure 4B), 8 mM calcium sulfate (Figure 4C) and 12 mM calcium sulfate (Figure 4D) exhibited a slightly discontinuous network structure and a slightly regular pore network. Chen et al. [21] reported that as the amount of calcium sulfate used to make soy-based tofu was increased from 10 to 30 mM, the microstructure became denser and relatively more homogenous. However, the calcium sulfate level was further increased to 40 mM, and the microstructure of the resulting tofu became rougher and coarser and less homogenous. Kao et al. [19] reported that the addition of 0.5% calcium sulfate to soymilk resulted in the formation of cross-linked structures and pores in soy-based tofu. These network structures lead to an increase in tofu hardness. Zhao et al. [22] suggested that it is easier to form a gel with more uniform and denser network structures after adding calcium sulfate to soymilk and heating. According to our findings, lentil proteins aggregated into larger protein particles via calcium sulfate, giving lentil-based tofu a slightly discontinuous network structure and a slightly regular pore network.

Furthermore, Figure 5 illustrates the microstructure of lentil-based tofu without/with chitosan. The lentil-based tofu without chitosan presented a discontinuous network structure with pores filled with water (Figure 5A). The lentil-based tofu samples with 0.1% (Figure 5B), 0.5% (Figure 5C) or 1.0% (Figure 5D) chitosan presented a slightly continuous network structure with pores. Compared with lentil-based tofu without chitosan, the number of pores in lentil-based tofu after adding chitosan was increased. Chitosan has previously been used in the preparation of soy-based tofu and pea-based tofu to modify texture [12,23]. Li et al. [24] also reported that the micrograph of chitosan-containing salt-soluble meat protein gels suggested that chitosan tightly associated and dispersed uniformly into the gel network.

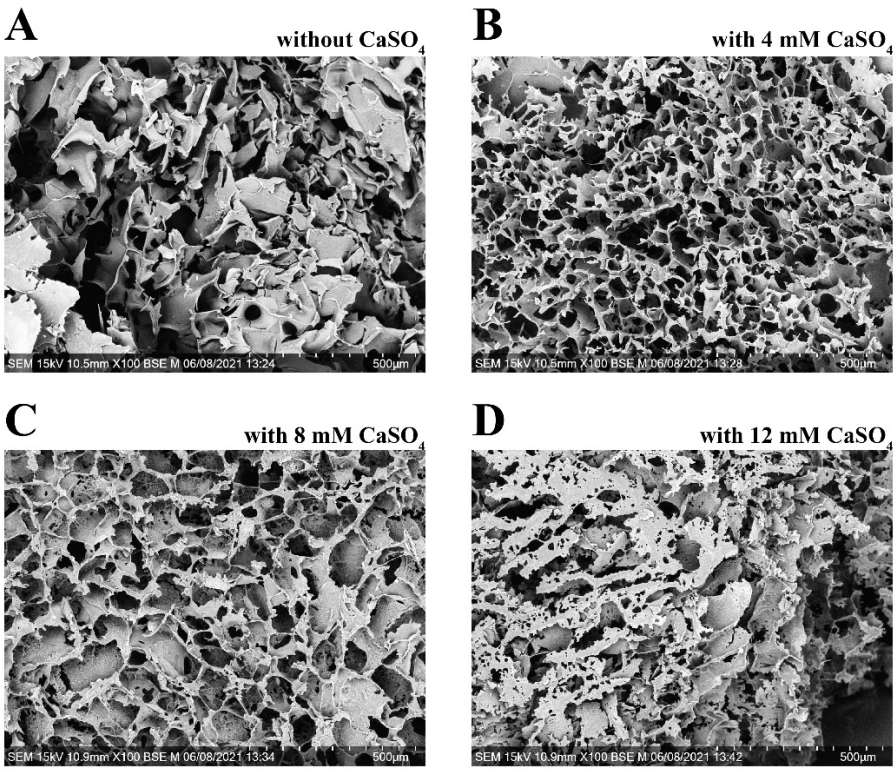

Figure 4. SEM of lentil-based tofu with/without calcium sulfate: (**A**) without calcium sulfate; (**B**) with 4 mM calcium sulfate; (**C**) with 8 mM calcium sulfate; (**D**) with 12 mM calcium sulfate.

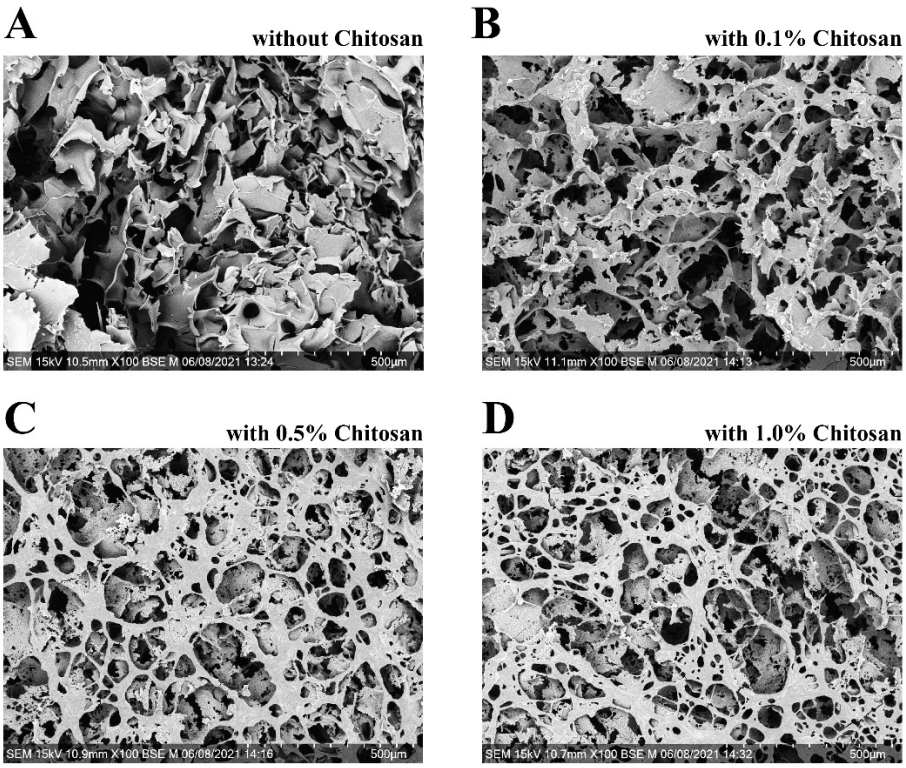

Figure 5. SEM images of lentil-based tofu: (**A**) without chitosan; (**B**) with 0.1% chitosan; (**C**) with 0.5% chitosan; and (**D**) with 1.0% chitosan.

3.4. SDS-PAGE Analysis of Calcium Sulfate and Chitosan on the Coagulation of Lentil Proteins in LMSF Samples

Joehnke et al. [25] reported that lentil globulins consist of two types of proteins: vicilin and legumin. Vicilin consists of three subunits, while legumin consists of six acidic subunits and six basic subunits. Lentil samples were incubated with various amounts of calcium sulfate (0, 4, 8 and 12 mM) incubated at 85 °C for 15 min, and lentil milk samples were separated into the LMSF. As shown in Figure 6A, SDS-PAGE separated the main storage proteins, including vicilin, legumin acidic unit and legumin basic unit proteins, in the LMSF sample without calcium sulfate. The concentrations of the vicilin, legumin acidic unit and legumin basic unit proteins in the LMSF decreased with the addition of 12 mM calcium sulfate. Ono [26] reported that the coagulation of soymilk occurs due to the binding of calcium ions and soymilk proteins, in which the protein molecules bound by calcium ions have been described as being located on the side-chain imidazole group of histidine residues and the side-chain carboxyl groups of aspartic and glutamic acid residues. Therefore, our results suggested that the vicilin, legumin acidic unit and legumin basic unit proteins were aggregated from LMSF into LMPF by calcium sulfate.

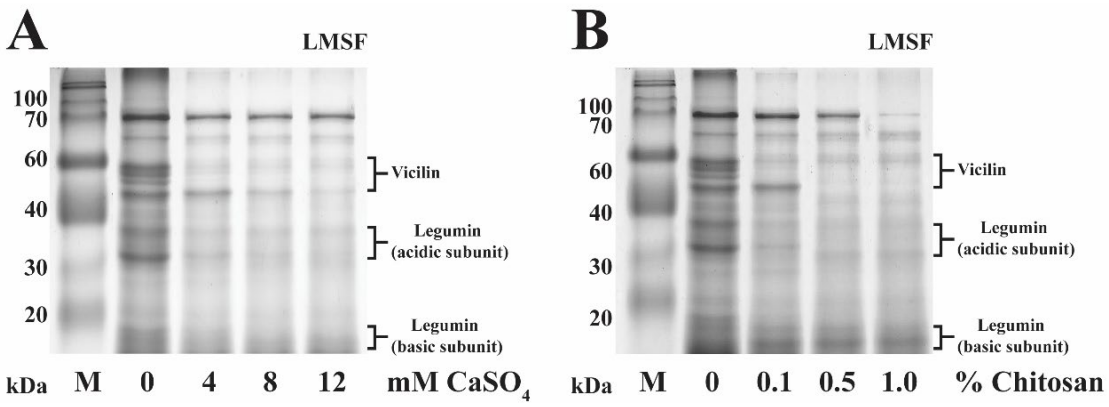

Figure 6. Changes in the SDS-PAGE profiles of lentil milk supernatant fraction (LMSF) with different amounts of calcium sulfate (0, 4, 8 and 12 mM) and chitosan (0, 0.1, 0.5 and 1.0%) incubated at 85 °C for 15 min: (**A**) with/without calcium sulfate; (**B**) with/without chitosan.

Lentil samples were also incubated at 85 °C for 15 min with various amounts of chitosan (0%, 0.1%, 0.5% and 1.0%), and lentil samples were then separated into the LMSF. As shown in Figure 6B, SDS-PAGE separated the main storage proteins, including vicilin, legumin acidic unit and legumin basic unit proteins, in the LMSF sample without chitosan. The concentrations of the vicilin, legumin acidic unit and legumin basic unit proteins in the LMSF decreased with an increase in the amount of chitosan, and these proteins almost disappeared after the addition of 1.0% chitosan. Hsiao et al. [5] reported that soybean proteins in soymilk were aggregated by the addition of 0.5% chitosan. These complex interactions involved hydrogen bonding between the carboxyl groups ($-COO^-$) of soy proteins and the amine groups ($-NH_3^+$) of chitosan [27].

3.5. Effect of Calcium Sulfate and Chitosan on the TPC in LMPF Samples

Xu and Chang [28] reported that lentil has the highest TPC of 7.53 mg gallic acid equivalents/g dry weight among eight legume materials. Zhang et al. [29] indicated that the major phenolic compounds found in lentils include flavan-3-ols, flavonols, stilbenes, anthocyanidins, flavanones and flavones. Phenolic compounds contained in lentil seeds are important components with redox properties and are responsible for antioxidant activity. These phytochemicals can form complexes with bean proteins. Therefore, the phenolic compounds were extracted from the LMPF samples, and the TPC was analyzed. As shown in Figure 7A, the TPCs of the LMPF samples with 0, 4, 8 and 12 mM calcium sulfate were 55.4 ± 1.5, 51.8 ± 2.3, 52.6 ± 1.5 and 53.7 ± 1.8 μg/mL, respectively. These results

suggested that the TPC of the LMPF samples did not increase with the addition of calcium sulfate ($p < 0.05$). However, we noticed that the TPC of the LMPF samples increased significantly with the addition of chitosan ($p < 0.05$). The TPCs of the LMPF samples with 0, 0.1, 0.5 and 1.0% chitosan were 55.4 ± 1.5, 55.6 ± 1.8, 60.6 ± 0.9 and 67.1 ± 1.9 μg/mL, respectively (Figure 7B). These suggested that lentil-based tofu contains many phenolic compounds, making it a functional health food. Hu and Luo [30] reported that chitosan is a positively charged polysaccharide that can combine with phenolic compounds to form polyphenol–chitosan conjugates. These polyphenol–chitosan conjugates have stronger free radical scavenging capability and more enhanced reducing power than chitosan [31]. The above results suggested that the phenolic compounds bonded to lentil proteins form complexes in lentil milk, and a portion of these complexes are then coagulated by 1.0% chitosan into LMPF.

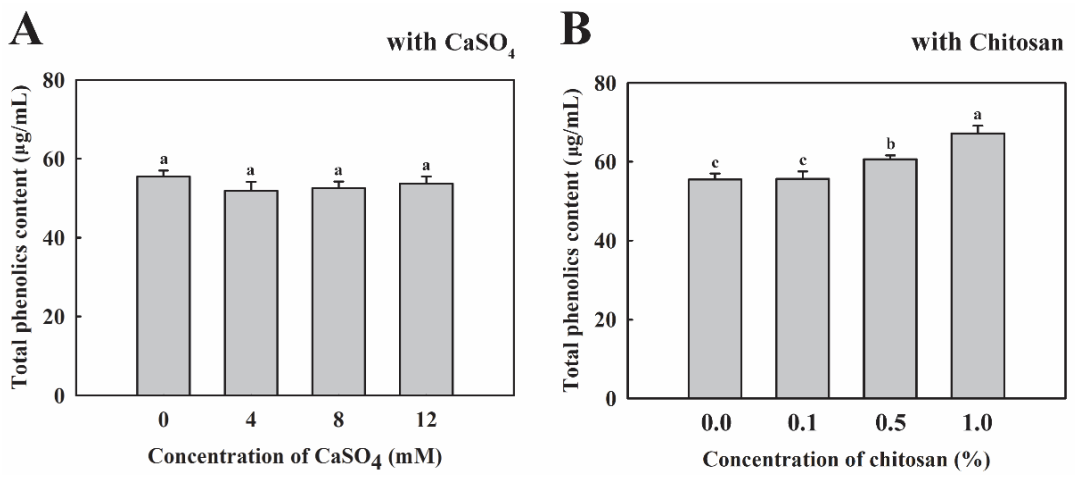

Figure 7. Effects of calcium sulfate (0, 4, 8 and 12 mM) and chitosan (0, 0.1, 0.5 and 1.0%) on the TPC in lentil milk pellet fraction (LMPF): (**A**) with/without calcium sulfate; (**B**) with/without chitosan. Different letters (a, b and c) indicate significant differences ($p < 0.05$).

4. Conclusions

The effects of calcium sulfate and chitosan on the textural modification and microstructure of lentil-based tofu were investigated. Our results showed that lentil-based tofu was formed by heating lentil milk at 85 °C for 1 h. The addition of 12 mM calcium sulfate increased the hardness and cohesiveness of lentil-based tofu, while 1.0% chitosan decreased the hardness and cohesiveness of lentil-based tofu. The SEM results showed that the addition of calcium sulfate and chitosan changed the microstructure of lentil-based tofu. Adding both calcium sulfate and chitosan aggregated the vicilin, legumin acidic unit and legumin basic unit proteins. According to our findings, the gel properties of lentil-based tofu increased after adding calcium sulfate, while the gel properties decreased after adding chitosan. Therefore, calcium sulfate and chitosan can be useful food additives for texture improvement in lentil-based tofu.

Author Contributions: Conceptualization, J.-F.H.; data curation, C.-H.J. and C.-Y.L.; formal analysis, C.-J.C.; funding acquisition, J.-F.H.; methodology, J.-F.H.; supervision, J.-F.H.; validation, C.-Y.L.; writing—original draft, C.-Y.L. and J.-F.H.; writing—review and editing, J.-F.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science and Technology in Taiwan, grant number MOST 109-2221-E-030-003-MY2 and MOST 111-2221-E-030-006-MY3.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We thank the Ministry of Science and Technology in Taiwan for grant support.

Conflicts of Interest: The authors declare no conflict of interest for this article.

References

1. Jarpa-Parra, M. Lentil protein: A review of functional properties and food application. An overview of lentil protein functionality. *Int. J. Food Sci. Technol.* **2018**, *53*, 892–903. [CrossRef]
2. Jo, Y.J.; Huang, W.; Chen, L. Fabrication and characterization of lentil protein gels from fibrillar aggregates and the gelling mechanism study. *Food Funct.* **2020**, *11*, 10114–10125. [CrossRef] [PubMed]
3. Jarpa-Parra, M.; Bamdad, F.; Wang, Y.; Tian, Z.; Temelli, F.; Han, J.; Chen, L. Optimization of lentil protein extraction and the influence of process pH on protein structure and functionality. *LWT-Food Sci. Technol.* **2014**, *57*, 461–469. [CrossRef]
4. Hsiao, Y.H.; Yu, C.J.; Li, W.T.; Hsieh, J.F. Coagulation of β-conglycinin, glycinin and isoflavones induced by calcium chloride in soymilk. *Sci. Rep.* **2015**, *5*, 13018. [CrossRef] [PubMed]
5. Hsiao, Y.H.; Hsia, S.Y.; Chan, Y.C.; Hsieh, J.F. Complex coacervation of soy proteins, isoflavones and chitosan. *Molecules* **2017**, *22*, 1022. [CrossRef]
6. Hu, H.; Li-Chan, E.C.; Wan, L.; Tian, M.; Pan, S. The effect of high intensity ultrasonic pre-treatment on the properties of soybean protein isolate gel induced by calcium sulfate. *Food Hydrocoll.* **2013**, *32*, 303–311. [CrossRef]
7. Ju, Z.Y.; Kilara, A. Aggregation induced by calcium chloride and subsequent thermal gelation of whey protein isolate. *J. Dairy Sci.* **1998**, *81*, 925–931. [CrossRef]
8. No, H.K.; Meyers, S.P. Preparation of tofu using chitosan as a coagulant for improved shelf-life. *Int. J. Food Sci. Technol.* **2004**, *39*, 133–141. [CrossRef]
9. Kim, M.; Son, I.S.; Han, J.S. Evaluation of microbiological, physicochemical and sensory qualities of chitosan tofu during storage. *J. Food Qual.* **2004**, *27*, 27–40. [CrossRef]
10. DePalma, K.; Smith, B.; McDonald, A.G. Synergistic effects of processing parameters on the biochemical and physical properties of tofu made from yellow field pea (*Pisum sativum*), as determined by response surface methodology. *Food Sci. Nutr.* **2021**, *9*, 1132–1142. [CrossRef]
11. Benmeziane-Derradji, F.; Djermoune-Arkoub, L.; Ayat, N.E.H.; Aoufi, D. Impact of roasting on the physicochemical, functional properties, antioxidant content and microstructure changes of Algerian lentil (*Lens culinaris*) flour. *J. Food Meas. Charact.* **2020**, *14*, 2840–2853. [CrossRef]
12. Jao, C.H.; Kuo, M.I.; Chen, C.J.; Hsieh, J.F. Influence of chitosan and glucono-δ-lactone on the gel properties, microstructural and textural modification of pea-based tofu-type product. *Processes* **2022**, *10*, 1639. [CrossRef]
13. Liu, H.H.; Chien, J.T.; Kuo, M.I. Ultra high pressure homogenized soy flour for tofu making. *Food Hydrocoll.* **2013**, *32*, 278–285. [CrossRef]
14. Cao, L.; Lu, W.; Ge, J.; Fang, Y. Modulation of oligoguluronate on the microstructure and properties of Ca-dependent soy protein gels. *Carbohyd. Polym.* **2020**, *250*, 116920. [CrossRef]
15. Hsia, S.Y.; Hsiao, Y.H.; Li, W.T.; Hsieh, J.F. Aggregation of soy protein-isoflavone complexes and gel formation induced by glucono-δ-lactone in soymilk. *Sci. Rep.* **2016**, *6*, 35718. [CrossRef]
16. Hung, Y.C.; Hsiao, Y.H.; Hsieh, J.F. Catechin content and free radical scavenging activity of *Camellia sinensis* twig extracts. *Int. Food Res. J.* **2021**, *28*, 248–254. [CrossRef]
17. Mhatre, S.S.; Jain, S.K.; Murdia, L.K.; Jain, H.K. Effects of different coagulation temperatures on the texture and yield of soy paneer (tofu). *Int. J. Food Eng.* **2008**, *4*, 8. [CrossRef]
18. Byars, J.A.; Singh, M. Rheological and textural properties of pulse starch gels. *Starch-Stärke* **2016**, *68*, 778–784. [CrossRef]
19. Kao, F.J.; Su, N.W.; Lee, M.H. Effect of calcium sulfate concentration in soymilk on the microstructure of firm tofu and the protein constitutions in tofu whey. *J. Agric. Food Chem.* **2003**, *51*, 6211–6216. [CrossRef]
20. Kim, M.; Han, J.S. Evaluation of physico-chemical characteristics and microstructure of tofu containing high viscosity chitosan. *Int. J. Food Sci. Technol.* **2002**, *37*, 277–283. [CrossRef]
21. Chen, K.; Huang, Y.; Li, X.; Wu, Y.; Liu, Y.; Wang, F. Textural properties of firm tofu as affected by calcium coagulants. *J. Food Meas. Charact.* **2021**, *15*, 4508–4516. [CrossRef]
22. Zhao, H.; Li, W.; Qin, F.; Chen, J. Calcium sulphate-induced soya bean protein tofu-type gels: Influence of denaturation and particle size. *Int. J. Food Sci. Technol.* **2016**, *51*, 731–741. [CrossRef]
23. amar Cheba, B. Chitosan: Properties, modifications and food nanobiotechnology. *Procedia Manuf.* **2020**, *46*, 652–658. [CrossRef]
24. Li, X.; Xia, W. Effects of chitosan on the gel properties of salt-soluble meat proteins from silver carp. *Carbohyd. Polym.* **2010**, *82*, 958–964. [CrossRef]
25. Joehnke, M.S.; Jeske, S.; Ispiryan, L.; Zannini, E.; Arendt, E.K.; Bez, J.; Sørensen, J.C.; Petersen, I.L. Nutritional and anti-nutritional properties of lentil (*Lens culinaris*) protein isolates prepared by pilot-scale processing. *Food Chem. X* **2021**, *9*, 100112. [CrossRef] [PubMed]
26. Ono, T. Soy (soya) cheeses. In *Encyclopedia of Food Science and Nutrition*; Caballero, B., Trugo, L.C., Finglas, P.M., Eds.; Academic Press: London, UK, 2003; pp. 5398–5402.

27. Huang, G.Q.; Sun, Y.T.; Xiao, J.X.; Yang, J. Complex coacervation of soybean protein isolate and chitosan. *Food Chem.* **2012**, *135*, 534–539. [CrossRef]
28. Xu, B.J.; Chang, S.K.C. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *J. Food Sci.* **2007**, *72*, S159–S166. [CrossRef]
29. Zhang, B.; Peng, H.; Deng, Z.; Tsao, R. Phytochemicals of lentil (*Lens culinaris*) and their antioxidant and anti-inflammatory effects. *J. Food Bioact.* **2018**, *1*, 93–103. [CrossRef]
30. Hu, Q.; Luo, Y. Polyphenol-chitosan conjugates: Synthesis, characterization, and applications. *Carbohyd. Polym.* **2016**, *151*, 624–639. [CrossRef]
31. Ren, J.; Li, Q.; Dong, F.; Feng, Y.; Guo, Z. Phenolic antioxidants-functionalized quaternized chitosan: Synthesis and antioxidant properties. *Int. J. Biol. Macromol.* **2013**, *53*, 77–81. [CrossRef]