

## Article

# Application of High Pressure Processing on Ultrasonically Treated Extract from Wild Bitter Gourd

Chang-Yi Huang<sup>1</sup> and Su-Der Chen<sup>2,\*</sup> 

<sup>1</sup> Department of Biotechnology and Animal Science, National Ilan University, Shen-Lung Road, Yilan City 26041, Taiwan

<sup>2</sup> Department of Food Science, National Ilan University, Shen-Lung Road, Yilan City 26041, Taiwan

\* Correspondence: sdchen@niu.edu.tw; Tel.: +886-920518028; Fax: +886-39351892

**Abstract:** Wild bitter gourd extracts, such as saponins, polysaccharides, and peptides, could be used to adjust blood sugar. The objective of this research was to explore the use of high pressure processing (HPP) for sterilization and acceleration of enzyme hydrolysis in the ultrasonic preparation of peptide extracts from wild bitter gourd. The results showed that the wild bitter gourd powder could be extracted via ultrasonic processing with water at 70 °C for only 20 min with a solid to liquid ratio of 1:20 to obtain the total protein content of 1.514 mg/g. The two sterilization methods for wild bitter gourd extract treated with papaya enzyme—for 2 h in the traditional autoclave at 121 °C for 15 min, or under HPP 300 MPa for 5 min—showed no significant effect on protein content, and both sterilization methods were effective. However, the extract sterilized with HPP had a significantly higher ability to scavenge DPPH free radicals. In addition, HPP (300 MPa for 5 min), combined with papaya enzyme to hydrolyze the wild bitter gourd extract, simultaneously pasteurized the extract and acquired the peptides from the wild bitter gourd extract. Therefore, the ultrasonic extraction of wild bitter gourd, combined with HPP and enzyme hydrolysis, could greatly shorten the operation time (to only 5 min) for extracting the active peptides.



**Citation:** Huang, C.-Y.; Chen, S.-D. Application of High Pressure Processing on Ultrasonically Treated Extract from Wild Bitter Gourd. *Processes* **2022**, *10*, 1926. <https://doi.org/10.3390/pr10101926>

Academic Editors: Laifeng Lu, Xia Liu, Qiya Yang and Fangming Deng

Received: 21 August 2022

Accepted: 20 September 2022

Published: 23 September 2022

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2022 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/>).

**Keywords:** wild bitter gourd; high pressure processing (HPP); bitter gourd peptides; antioxidant activity; enzyme hydrolysis

## 1. Introduction

Type I diabetes is caused by a lack of insulin; however, most cases of type II diabetes are caused by insulin resistance or insensitivity, which causes metabolized glucose to be stored in the blood rather than sent to cells for glycolysis, and the glucose is eventually excreted in the urine. In addition to sugar-controlled diets, diabetes can be mitigated through the following three approaches: (1) to inhibit amylase to reduce glucose generation, (2) to increase glucose use by the liver, and (3) to increase insulin secretion or apply insulin-like substances for promoting the transportation of glucose from blood to cells to generate energy through glycolysis [1].

Bitter gourds (*Momordica charantia* L.) are Cucurbitaceae fruits widely distributed in Asia, the Amazon Basin, and East Africa. In numerous cultures, they were a traditional medicine used for treating and preventing specific chronic diseases, including diabetes, menstrual pain, gout, eczema, pneumonia, and tinea.

Wild bitter gourds (*Momordica charantia* L. var. *abbreviata* Ser.), which are the Taiwanese endemic species of bitter gourds, feature effective hypoglycemic ingredients, such as saponins, polysaccharides, and peptides. *M. charantia* saponins, which can be extracted using butanol, inhibit the reaction of glycolytic enzymes to reduce sugar absorption. Therefore, patients with diabetes can drink fresh bitter gourd juice to lower blood glucose concentrations and prevent postprandial hyperglycemia [2]. *M. charantia* saponins also promote glycogen storage and insulin secretion [3]. The hypoglycemic mechanism of *M. charantia* saponins involves activating the AMP-activated protein kinase phosphorylation to

regulate energy metabolism [4]. A study conducted on mice reported that *M. charantia* polysaccharides increased superoxide dismutase content to enhance antioxidation, mitigate streptozotocin-induced kidney and pancreas damage, repair  $\beta$  cells, and lower blood sugar [4]. A new type of protein has been discovered in the water extracted from wild bitter gourd seeds. This protein can be bonded with insulin receptors to regulate blood sugar metabolism [5]. Polypeptide-k, which is also extracted from bitter gourd seeds, inhibits the activities of  $\alpha$ -glucosidase and  $\alpha$ -amylase to impede the degradation of starch and lower glucose content, thereby reducing blood sugar [6]. Therefore, non-thermal processing technology is highly valuable for extracting bioactive ingredients from wild bitter gourds.

The bioactive ingredients in wild bitter gourds can be extracted to increase their concentrations and thus, their hypoglycemic effects, which mitigate the metabolic diseases associated with diabetes. Therefore, the development of plant extraction technology is desirable. Currently, plants are extracted primarily through mechanical stirring, boiling, and Soxhlet extraction (a conventional hot water extraction method) [7,8]. The dry powder of *M. charantia* was extracted by water extraction and alcohol precipitation, and further deproteinized to obtain the polysaccharide which could significantly increase the content of SOD and CAT in the serum, liver, and spleen of mice, and reduce the content of MDA in the liver and spleen, to a certain extent [9]. Although these conventional extraction methods are low-cost, they require a high extraction temperature, long extraction time, and high solvent consumption, and they may cause the loss of bioactive ingredients, thus exhibiting a low extraction efficiency [10]. New extraction technologies, such as supercritical fluid extraction, microwave-assisted extraction, solvent-assisted extraction, squeezing and enzymatic decomposition, pulsed electric field extraction, high pressure extraction, and ultrasonic-assisted extraction (UAE), have been employed to enhance extraction efficiency. Some of the new extraction methods require expensive equipment, but do not increase production capacity. However, UAE achieves satisfactory extraction efficiency with relatively low-cost equipment and can be utilized for cost-efficient mass production and processing [11–14].

Garude et al. [15] extracted the peels of bitter gourds at room temperature using methanol solvent extraction, Soxhlet extraction, and UAE techniques, revealing that the total phenol and flavonoid content, total antioxidant capacity, and scavenging 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical ability were the highest using UAE. Yan et al. [16] extracted polysaccharides from fresh bitter gourds at room temperature through UAE using various solvents, including citric acid, sodium hydroxide, and distilled water, and reported that the types of solvents used profoundly affected the extraction ratio, chemical composition, and monosaccharide composition of the extract. Particularly, the extract obtained with distilled water exhibited the highest uronic acid content (24.22%), as well as the strongest antioxidant properties and  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition capability.

In mass production, bitter gourd extracts and *M. charantia* peptides hydrolyzed using enzymes must be sterilized to extend their shelf life for transportation and storage. Although conventional 121 °C autoclave sterilization is an effective sterilization method, it may affect the nutritional, chemical, biochemical, and sensory properties and active ingredients of food products while lowering their acceptability to consumers. According to Deshaware et al. [17], 65 °C pasteurization could be employed to preserve the desired nutritional and sensory properties of bitter gourds, and it did not significantly affect  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition ( $p > 0.05$ ). As a non-thermal type of sterilization,  $\gamma$ -rays can be employed to enhance the  $\alpha$ -glucosidase inhibition rate by 10% at 2.5 kGy. However, sterilization by  $\gamma$ -irradiation has been banned in the European Union because of concerns regarding hazards caused by residual radiation.

High pressure processing (HPP), another non-thermal sterilization technique, involves using water as the pressure-transmitting medium to apply high hydrostatic pressure (100~1000 MPa) to food products, packed in soft airtight bags or containers, at specified temperature for an appropriate holding time, achieving physical sterilization. HPP sterilization is achieved through damaging the cell membranes of bacteria to change their

permeability, the phospholipid curing on their cell membranes, the denaturation of the protein required for bacterial growth metabolism, and the disruption of bacterial DNA replication and transcription [18,19]. In a high pressure environment, noncovalent bonds in food ingredients (e.g., hydrogen, ionic, or hydrophobic bonds) can be destroyed or formed for enzyme inactivation, starch gelatinization, protein denaturation, and gel property change, thereby enabling food preservation [20,21]. Accordingly, HPP addresses the color change, flavor reduction, and vitamin loss in high temperature pasteurization. HPP is employed to eliminate pathogenic microorganisms in agricultural products and reduce the use of food additives, such as preservatives.

Proteolytic enzymes have been applied to decompose the proteins extracted from wild bitter gourds into *M. charantia* peptides, which exhibit hypoglycemic properties. However, enzymatic hydrolysis is usually time-consuming and incurs high processing costs. There is a lack of research on the use of HPP assisted protease to hydrolyze bitter gourd protein into small molecule peptides. Elisabete et al. [22] compared the effectiveness of 600 MPa and 300 MPa HPP and enzymatic hydrolysis (conducted using  $\alpha$ -amylase and  $\alpha$ -glucosidase), revealing that the total phenol content and antioxidant properties, respectively, of pomegranate extracts treated with 300 MPa HPP were superior. Kitrytė et al. [23] applied HPP, supercritical fluid extraction, and enzymatic hydrolysis on lingonberries, and reported that the extract treated by HPP exhibited the highest anthocyanin content and the strongest antioxidant properties. Moreover, combined HPP and enzymatic hydrolysis yielded an amount of phenolic compounds from grape pomace that was 16 times that obtained by only enzymatic hydrolysis [24]. Therefore, HPP can be applied to facilitate enzymatic hydrolysis activities to boost the release of bioactive compounds, and the extraction ratio is affected by parameters such as extraction temperature and time, enzyme activity, and pressure [25].

The primary objective of this study was to compare the HPP and the conventional high temperature sterilization techniques in regards to their sterilization effectiveness, DPPH free radical scavenging abilities, and total protein content preservation in wild bitter gourd extracts hydrolyzed by papain for 2 h. The effect of HPP-assisted papain hydrolysis on extract sterilization and peptides from the wild bitter gourd extract obtained through hydrolysis was also investigated.

## 2. Materials and Methods

### 2.1. Materials

Hot-air dried wild bitter gourds were purchased from Asakusa Agriculture Processing (Hualien, Taiwan). Bovine serum albumin (BSA), Coomassie brilliant blue G-250, 1,1-Diphenyl-2-picryl hydrazyl (DPPH), ascorbic acid (vitamin C), glacial acetic acid, synthetic antioxidant butylated hydroxyl anisole (BHA), ammonium persulfate, and sodium dodecyl sulfate (SDS) were obtained from Sigma Chemical Corp (St. Louis, MO, USA). The 30% acrylamide: bis solution (29:1) for electrophoresis was purchased from J.T. Barker (Phillipsburg, NJ, USA). Protein maker was obtained from GeneDierX, Inc. (Taoyuan, Taiwan). Tetramethyl ethylenediamine (Temed) was purchased from Merck (Darmstadt, Germany). Protein loading dye was procured from Strong Biotech, Corp. (Taipei, Taiwan). The aerobic count plate (3M Global Headquarters, Maplewood, MN, USA), yeast and mold count plates (3M Petrifilm 6477, 500 EA/CS, MN, USA), and papain (2000 FCCU/mg, Decken Biotech, Taichung, Taiwan) were also obtained for the study.

### 2.2. Equipment

A focused ultrasonic extractor (20k Hz, 1400 W, Ever Great Ultrasonic Co., New Taipei City, Taiwan), spectrophotometer (Model U-2001, Hitachi Co., Tokyo, Japan), benchtop centrifuge (HERMLE Z300, Gosheim, Germany), miniprotein system (Bio-Rad, CA, USA), electrophoresis tank (Bio-Rad, CA, USA), electrophoresis high voltage power supply (Major Science, CA, USA), ultrahigh pressure processing equipment (HPP 600 MPa/6.2L, Kuentai International Co., Yunlin, Taiwan), vertical high-temperature autoclave sterilizer (TM329,

Double Eagle Enterprise, New Taipei City, Taiwan), and digital pocket refractometer (Pocket, 3810, PAL-1, ATAGO Corp., Tokyo, Japan) were employed.

### 2.3. Wild Bitter Gourd Extraction

#### 2.3.1. Focused UAE

The wild bitter gourds was ground to a size of 60 mesh, and 50 g of the ground wild bitter gourd was evenly mixed in 500, 750, and 1000 mL of reverse osmosis (RO) water at solid to liquid ratios of 1:10, 1:15, and 1:20, respectively. Samples were extracted from the solutions at 20k Hz, 1400 W, and 70 °C for 10 min. Each extract was centrifuged at 6000 rpm for 5 min and stored at 4 °C for analysis.

#### 2.3.2. Hot Water Extraction

The wild bitter gourds were ground to a size of 60 mesh, and 50 g of the ground wild bitter gourd was evenly mixed in 500, 750, and 1000 mL of RO water at solid to liquid ratios of 1:10, 1:15, and 1:20, respectively. The solutions were heated at 100 °C, and samples were extracted for 1 h. Each extract was centrifuged at 6000 rpm for 5 min and stored at 4 °C for analysis.

#### 2.3.3. Extraction Ratio

The extracts were measured using a digital pocket refractometer; the results were converted to solid content, and the extraction rates of solid content were calculated.

$$\text{Extraction ratio} = \left( \frac{\text{Brix}}{100} \right) \times \left( \frac{\text{Supernatant weight}}{50} \right) \times 100\%$$

### 2.4. Sterilization Method

#### 2.4.1. High-Temperature Sterilization

The extracts were placed in the 121 °C autoclave for a 15 min sterilization, then cooled and stored at 4 °C for analysis.

#### 2.4.2. High Pressure Processing (HPP)

The extracts were placed in the ultrahigh pressure processing equipment for a 5 min sterilization at 300 MPa, then cooled and stored at 4 °C for analysis.

### 2.5. Wild Bitter Gourd Peptides from UAE Extract Obtained by Enzymatic Hydrolysis

#### 2.5.1. Enzymatic Reaction

Approximately 300 mL of wild bitter gourd extract was evenly mixed with 0.5 g of papain for a 2 h hydrolysis reaction at 60 °C. The extract was then sterilized at 121 °C for 15 min, cooled, and stored at 4 °C for analysis.

#### 2.5.2. HPP Assisted Enzymatic Hydrolysis

Approximately 500 mL of wild bitter gourd extract was evenly mixed with 0.5 g of papain by 300 MPa HPP with a holding time of 5 min and stored at 4 °C for analysis.

### 2.6. Test Methods

#### 2.6.1. Protein Content

The test was conducted in accordance with the Bradford protein binding assay [26] by evenly mixing 800 µL of wild bitter gourd extract with 200 µL of protein assay reagent. The solution was left idle for 10 min of reaction. Absorbance at the 595 nm wavelength was measured three times, and the mean value was calculated. The BSA standard curve was employed to determine the protein concentration in the sample.

### 2.6.2. DPPH Free Radical Scavenging Ability Test

This test was modified from Xu and Chang's method [27], and 2 mL of the supernatant was evenly mixed with 2 mL of 0.2-mM DPPH–MeOH solution and left idle, away from light, at room temperature for 30 min. Absorbance at the 517 nm wavelength was measured and applied to the following equation to calculate the DPPH scavenging rate. The result was compared to that of the control group, which contained 5 mg/mL of ascorbic acid, as well as BHA.

$$\text{DPPH free radical scavenging (\%)} = ((\text{ABS}_{\text{control}} - \text{ABS}_{\text{sample}}) / (\text{ABS}_{\text{control}})) \times 100\%$$

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

Electrode plates were assembled and placed in the electrophoresis tank. SDS running buffer, containing 250 mM of glycine, 25 mM of tris-base, and 0.1% SDS and adjusted to pH 8, was injected into the colloidal pores. In each pore, 10  $\mu\text{L}$  of processed wild bitter gourd extract obtained under different extraction conditions was added (after 50  $\mu\text{L}$  of the extract was evenly mixed with 50  $\mu\text{L}$  of loading dye), and 10  $\mu\text{L}$  of protein marker was employed as a control for protein molecular weight. After the sample loading was completed, electrophoresis was performed. Subsequently, the colloid was immersed in Coomassie brilliant blue dye (0.25 g Coomassie brilliant blue G-250, 10% glacial acetic acid, 45% methanol, 45%  $\text{H}_2\text{O}$ ) and shaken at 60 rpm for 1 h of dyeing. A stain remover (12.5% glacial acetic acid, 50% methanol, 37.5%  $\text{H}_2\text{O}$ ) was then added, and the colloid was shaken at 60 rpm again for 1 to 2 h for destaining until the protein bands were visible. RO water was then used to remove the dye until the colloid returned to its original state for photography or image scanning.

### 2.6.4. Microbiological Test

The extract (1 mL) was serially diluted to 10, 100, and 1000 dilutions with sterilized water. Subsequently, 0.5 mL of each diluted extract was added on an aerobic count plate for total bacteria testing, and a yeast and mold count plate for yeast and mold testing, respectively. They were cultured at 35  $^{\circ}\text{C}$  for 72 h, and the colonies were counted.

### 2.7. Statistical Analysis

The test results are expressed as mean  $\pm$  standard deviation (SD) and analyzed using the Statistical Package for Social Science 14.0 (SPSS Inc., Data Statistical Analysis Corporation, Chicago, IL, USA). Differences between the data were examined through one-way analysis of variance, and the significance of the differences was investigated through Duncan's multiple range test ( $\alpha = 0.05$ ).

## 3. Results and Discussion

### 3.1. Comparison of Ultrasonic Extraction (UAE) and Hot Water Extraction (HWE) of Wild Bitter Gourd

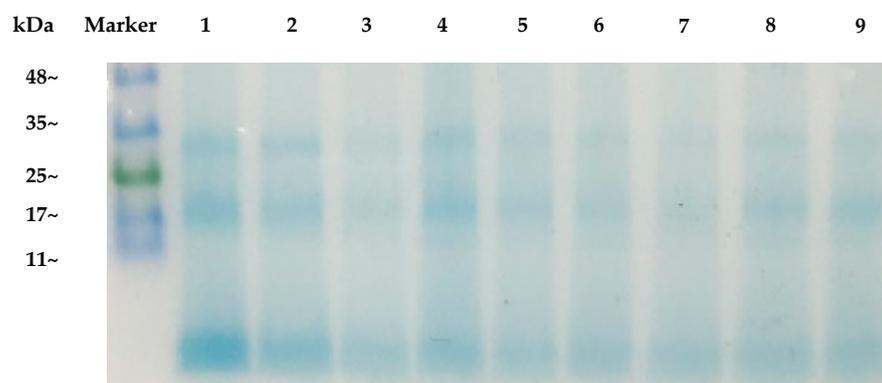
As demonstrated in Table 1, after 10 min of ultrasonic extraction (UAE), no significant differences were observed between the extracts with solid to liquid ratios of 1:10 and 1:15, but extraction at the solid to liquid ratio of 1:20 yielded the highest extraction ratio (23.9%). When the wild bitter gourds were extracted through hot water extraction (HWE), higher solid to liquid ratios led to higher extraction ratios after 60 min. This pattern was similar to that of UAE. At the ratio of 1:20, the extraction ratio of HWE was 25.0%, which was significantly higher than the extraction ratio of UAE.

**Table 1.** Effect of ultrasonic extraction and hot water extraction conditions on yield, total protein, and antioxidant activity of wild bitter gourd.

Extraction Method	Solid–Liquid Ratio	Extraction Time (min)	Extraction Yield (%)	Protein (mg/g)	Ability to Scavenge DPPH (%)
Ultrasonic (UAE)	1:20	10	23.9 ± 0.1 <sup>b</sup>	1.514 ± 0.013 <sup>a</sup>	75.7 ± 0.1 <sup>c</sup>
	1:15	10	22.6 ± 0.1 <sup>c</sup>	1.241 ± 0.003 <sup>c</sup>	75.8 ± 0.1 <sup>c</sup>
	1:10	10	22.4 ± 0.1 <sup>c</sup>	1.198 ± 0.025 <sup>d</sup>	79.2 ± 0.1 <sup>b</sup>
Hot water (HWE)	1:20	60	25.0 ± 0.1 <sup>a</sup>	1.544 ± 0.024 <sup>a</sup>	56.9 ± 0.1 <sup>f</sup>
	1:15	60	22.0 ± 0.1 <sup>c</sup>	1.334 ± 0.031 <sup>b</sup>	60.2 ± 0.1 <sup>d</sup>
	1:10	60	21.0 ± 0.0 <sup>d</sup>	1.302 ± 0.032 <sup>b</sup>	59.6 ± 0.1 <sup>e</sup>
Vitamin C					95.5 ± 0.1 <sup>a</sup>
BHA					95.3 ± 0.1 <sup>a</sup>

Data are expressed as mean ± SD (n = 3). <sup>a–f</sup> Means with different superscript letters in the same column were significantly different ( $p < 0.05$ ).

At the solid to liquid ratio of 1:20, 1.514 mg/g of protein were extracted after 10 min of UAE. At both the solid to liquid ratios of 1:15 and 1:10, the total protein content decreased. When the extracts were obtained by 60 min of HWE at the solid to liquid ratio of 1:20, the total protein content (1.544 mg/g) was higher than that of 1:15 and 1:10. According to the SDS-PAGE results shown in Lanes 1–6 of Figure 1, the extracts obtained through UAE or HWE did not differ significantly in their protein fragments and concentrations. As shown in Table 1, the DPPH free radical scavenging abilities of all the extracts from wild bitter gourd acquired through UAE were larger than 75%, and they were significantly higher than those of the extracts obtained through HWE (about 57–60%). This revealed that HWE—for a long duration—inhibited the extraction of bioactive ingredients. Therefore, 10 min of UAE enabled the quick extraction of protein content equal to that obtained through 60 min of HWE.



**Figure 1.** SDS-PAGE of protein from wild bitter gourd extract under different extraction sterilization conditions. Lane 1–3: ultrasonic extraction (UAE); Lane 4–6: hot water extraction (HWE); Lane 1: 1:20, 10 min; Lane 2: 1:15, 10 min; Lane 3: 1:10, 10 min; Lane 4: 1:20, 60 min; Lane 5: 1:15, 60 min; Lane 6: 1:10, 60 min; Lane 7: extract; Lane 8: extract + 121 °C, 15 min; Lane 9: extract + HPP.

Garude et al. [15] contended that the phenol and flavonoid content in bitter gourd peel extracts acquired through UAE are higher than those in extracts acquired through conventional solvent assisted extraction, but Soxhlet extraction yielded the highest phenol and flavonoid content. Therefore, UAE involved short extraction time and low extraction temperature to prevent the loss and damage of the bioactive ingredients and antioxidant properties of the extract. The yield from UAE could be improved through an appropriate increase in temperature. According to Yan et al. [16], the water extracted from fresh bitter gourds acquired through UAE at room temperature exhibits a high uronic acid content (24.22%), as well as a strong ability to scavenge DPPH free radicals.

### 3.2. Comparison of High Pressure Processing (HPP) and Autoclave Sterilization of Extracts from Wild Bitter Gourd

Although UAE is an effective non-thermal processing technology, extracts must still be sterilized in the mass production process. As shown in Table 2, the UAE temperature was only 70 °C, which was inadequate for high temperature wild bitter gourd extract sterilization. This extract exhibited 1.202 mg/g of protein in total and 80.2% DPPH free radical scavenging ability. After the extract was sterilized in the 121 °C autoclave for 15 min, its total protein content was maintained at 1.234 mg/g, but its DPPH free radical scavenging rate dropped to 73.5%. This revealed that high temperatures decreased the antioxidant properties of the extracts. However, after the extract was treated by 300 MPa HPP, holding for 5 min, its total protein content rose to 1.342 mg/g, and its DPPH free radical scavenging ability was maintained at 79.3%.

**Table 2.** Effect of different sterilization methods on total protein and antioxidant activity of extracts from wild bitter gourd.

Extract from Wild Bitter Gourd	Protein (mg/g)	Ability to Scavenge DPPH (%)
Original (not sterilized)	1.202 ± 0.017 <sup>c</sup>	80.2 ± 0.1 <sup>b</sup>
Autoclave (121 °C, 15 min)	1.234 ± 0.011 <sup>b</sup>	73.5 ± 0.1 <sup>d</sup>
HPP (300 MPa, 5 min)	1.342 ± 0.024 <sup>a</sup>	79.3 ± 0.2 <sup>c</sup>
Vitamin C		95.6 ± 0.1 <sup>a</sup>
BHA		95.2 ± 0.1 <sup>a</sup>

Data are expressed as mean ± SD (n = 3). <sup>a-d</sup> Mean with a different superscript letter in the same column was significantly different ( $p < 0.05$ ).

The total bacterial (Table 3), as well as yeast and mold (Table 4) colonies, were observed to verify extract sterilization effectiveness under different sterilization conditions. The results indicated that the non-sterilized extract exhibited as high as 523,000 CFU/mL of bacteria (i.e., higher than  $10^5$ ) and over 1000 CFU/mL of yeast and mold. Therefore, wild bitter gourd extracts must be sterilized to satisfy food safety demands. After the extracts were treated by high temperature sterilization in the autoclave at 121 °C for 15 min, no bacterial, yeast, or mold growth was observed. After the extract was treated with 300 MPa HPP for 5 min, there was also no mold or yeast growth observation, but the bacteria count was still 10 CFU/mL, which decreased from the original total bacteria count of over  $10^5$  CFU/mL. The shelf life of the extract after treatment by either the autoclave or HPP can be extended by 4 °C refrigeration storage. The extract can be further dried for storage by freeze-drying or hot air-assisted radio frequency (HARF) drying [28].

**Table 3.** Total bacterial counts of wild bitter gourd extract treated using different treatments.

Extract from Wild Bitter Gourd	Total Bacterial Colonies			CFU/mL
	1:10	1:100	1:1000	
Original	TNTC	TNTC	523 *	523,000
Autoclave (121 °C, 15 min)	0	0	0	<10
HPP (300 MPa, 5 min)	1	0	0	10

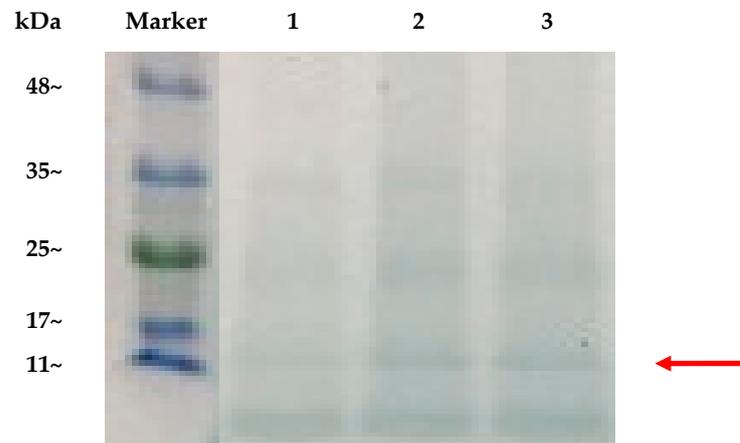
\* It indicates an estimated value. TNTC: too many colonies to count.

**Table 4.** Mold and yeast count of wild bitter gourd extract treated by different methods.

Extract from Wild Bitter Gourd	Mold and Yeast Colonies			CFU/mL
	1:10	1:100	1:1000	
Original	TNTC	TNTC	TNTC	>1000
Autoclave (121 °C, 15 min)	0	0	0	<10
HPP (300 MPa, 5 min)	0	0	0	<10

TNTC: too many colonies to count.

According to the SDS-PAGE protein electrophoresis results listed in Lanes 7–9 of Figure 2, the protein fragments and concentration of the extract were not substantially affected by sterilization. Therefore, wild bitter gourd protein was not substantially affected by high temperature; however, the DPPH free radical scavenging ability was impeded by high temperatures.



**Figure 2.** SDS-PAGE of peptides from wild bitter gourd extract under different hydrolysis conditions. Lane 1: enzyme hydrolysis 2 h; Lane 2: enzyme hydrolysis 2 h + 121 °C, 5 min; Lane 3: HPP assisted enzyme hydrolysis (300 MPa, 5 min). The arrow shows the small molecule fragment.

In analyses of the effects of 600 MPa HPP for 3 min and 85 °C for 5 min of heat treatment on the quality of apple juice, it was observed that the heat treatment led to a drastic change in the color of the juice, as well as polyphenol oxidase and peroxidase inactivation, whereas HPP preserved more than 50% of the enzyme activity. The sugar and acid concentrations were not significantly affected by either HPP or heat treatment. Moreover, the overall appearance and freshness of the apple juice processed through HPP were the same as those of fresh apple juice [29]. HPP significantly enhanced the antibacterial activities of pomegranate waste extract against eight types of pathogens [22]. Unlike the conventional heat sterilization technique, HPP enables relatively comprehensive preservation of the physical properties of food products, such as vitamins; it also inhibits the browning caused by the Maillard reaction. The results of the current study were consistent with the findings of many previous studies. In addition to effective sterilization, room temperature HPP facilitated an increase in the protein content of the wild bitter gourd extract, thus enhancing extraction efficiency without decreasing the DPPH free radical scavenging ability.

### 3.3. Effect of HPP-Assisted Enzymatic Hydrolysis on the Quality of Peptides in Extracts from Wild Bitter Gourd

The peptides in extracts from wild bitter gourd are effective hypoglycemic ingredients because of their short chains [6]. Therefore, papain was added to the wild bitter gourd extract at room temperature for 2 h of protein hydrolysis to increase its peptide content. However, HPP increased the reaction between the enzyme and the substrate at low pressure and temperature, thereby accelerating protein hydrolysis by papain. Moreover, the activity of the enzyme increases as the temperature or pressure increases until it is inactivated because its temperature or pressure tolerance is breached [25]. Therefore, HPP can be applied to assist enzymatic reactions for simultaneous wild bitter gourd extraction and sterilization to decompose protein into peptides within a short time. In the current study, wild bitter gourd extracts were divided into three groups. The first group was hydrolyzed for 2 h using papain before undergoing 300 MPa HPP for 5 min; the second group was also hydrolyzed for 2 h using papain and then heated in the 121 °C autoclave for 15 min; the third group simultaneously underwent 300 MPa HPP for 5 min and papain hydrolysis to

produce wild bitter gourd peptides. As illustrated in Table 5, the total protein content of the HPP assisted enzymatic hydrolysis group and the HPP group (1.386 mg/g) showed no significant differences from that of the autoclave group (1.382 mg/g).

**Table 5.** Effect of HPP assisted enzyme hydrolysis on total protein content and antioxidant activity of wild bitter gourd extract.

Enzyme Reaction and Sterilization Condition	Protein (mg/g)	DPPH Scavenging Ability (%)
Enzyme hydrolysis 2 h	1.152 ± 0.013 <sup>b</sup>	82.2 ± 0.1 <sup>c</sup>
Enzyme hydrolysis 2 h + autoclave (121 °C, 15min)	1.382 ± 0.015 <sup>a</sup>	75.1 ± 0.1 <sup>d</sup>
HPP assisted enzyme hydrolysis (300 MPa, 5 min)	1.386 ± 0.011 <sup>a</sup>	74.6 ± 0.1 <sup>e</sup>
Vitamin C		95.6 ± 0.1 <sup>a</sup>
BHA		95.2 ± 0.1 <sup>b</sup>

Data are expressed as mean ± SD (n = 3). <sup>a–e</sup> Mean with a different superscript letter in the same column was significantly different ( $p < 0.05$ ).

According to the SDS-PAGE electrophoresis results indicated in Figure 2, peptide fragments obtained by enzymatic hydrolysis were observed in both the HPP assisted enzymatic hydrolysis group and the HPP group (11 kDa, indicated by the arrow). The peptide fragments acquired after 2 h of papain hydrolysis were not affected by high-temperature sterilization or HPP. However, the DPPH free radical scavenging activity in the autoclave group dropped from 82.2% to 74.6% (Table 5). This indicates that HPP assisted enzymatic hydrolysis can be applied for simultaneous peptide generation and sterilization in processing wild bitter gourd extract, thus saving time and reducing cost.

#### 4. Conclusions

The peptides in wild bitter gourds could be extracted through UAE at only 70 °C and for only 10 min at the solid to liquid ratio of 1:20 for maximal extraction. Wild bitter gourd extracts could be effectively sterilized by 300 MPa HPP for 5 min, and HPP assisted papain hydrolysis could be employed to simultaneously sterilize the extract and hydrolyze protein into 11-kDa peptides. Accordingly, UAE combined with HPP can improve the quality of wild bitter gourd extracts, enhancing their unique competitiveness and potential as hypoglycemic health food products.

**Author Contributions:** Conceptualization, supervision and project administration, S.-D.C.; analysis and original draft preparation, C.-Y.H. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

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

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data and samples presented in this study are available on request from the corresponding author. Data is contained within the article.

**Acknowledgments:** Thanks to Yu-Hong Zheng for his assistance with experimental statistics and Yu-Shun Lai for its assistance in SDS-PAGE protein electrophoresis analysis.

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

#### References

- Senanayake, G.V.K.; Maruyama, M.; Shibuya, K.; Sakono, M.; Fukuda, N.; Morishita, T.; Yukizaki, C.; Kawano, M.; Ohta, H. The effects of bitter melon (*Momordica charantia*) on serum and liver triglyceride levels in rats. *J. Ethnopharmacol.* **2004**, *91*, 257–262. [[CrossRef](#)] [[PubMed](#)]
- Oishi, Y.; Sakamoto, T.; Udagawa, H. Inhibition of increases in blood glucose and serum neutral fat by *Momordica charantia* saponin fraction. *Biosci. Biotechnol. Biochem.* **2007**, *71*, 735–740. [[CrossRef](#)]
- Zhu, Y.; Dong, Y.; Qian, X. Effect of superfine grinding on antidiabetic activity of bitter melon powder. *Int. J. Mol. Sci.* **2012**, *13*, 14203–14218. [[CrossRef](#)]

4. Wang, Q.; Wu, X.; Shi, F.; Liu, Y. Comparison of antidiabetic effects of saponins and polysaccharides from *Momordica charantia* L. in STZ-induced type 2 diabetic mice. *Biomed. Pharmacother.* **2019**, *109*, 744–750. [[CrossRef](#)] [[PubMed](#)]
5. Lo, H.; Ho, T.; Lin, C. *Momordica charantia* and its novel polypeptide regulate glucose homeostasis in mice via binding to insulin receptor. *J. Agric. Food Chem.* **2013**, *63*, 2461–2468. [[CrossRef](#)]
6. Ahmad, Z.; Zamhuri, K.F.; Yaacob, A. In vitro anti-diabetic activities and chemical analysis of polypeptide-k and oil isolated from seeds of *Momordica charantia* (bitter melon). *Molecules* **2012**, *17*, 9631–9640. [[CrossRef](#)]
7. Cai, Z.; Qu, Z.; Lan, Y.; Zhao, S.; Ma, X.; Wan, Q.; Jing, P.; Li, P. Conventional, ultrasound-assisted, and accelerated-solvent extractions of anthocyanins from purple sweet potatoes. *Food Chem.* **2016**, *197*, 266–272. [[CrossRef](#)] [[PubMed](#)]
8. Gil-Chávez, G.J.; Villa, J.A.; Fernando Ayala-Zavala, J.; Basilio Heredia, J.; Sepulveda, D.; Yahia, E.M.; González-Aguilar, G.A. Technologies for extraction and production of bioactive compounds to be used as nutraceuticals and food ingredients: An overview. *Compr. Rev. Food Sci. Food Saf.* **2013**, *12*, 5–23. [[CrossRef](#)]
9. Chen, F.; Huang, G.; Huang, H. Preparation, analysis, antioxidant activities in vivo of phosphorylated polysaccharide from *Momordica charantia*. *Carbohydr. Polym.* **2021**, *252*, 117179. [[CrossRef](#)]
10. Caldas, T.W.; Mazza, K.E.; Teles, A.S.; Mattos, G.N.; Brígida, A.I.S.; Conte-Junior, C.A.; Borguini, R.G.; Godoy, R.L.; Cabral, L.M.; Tonon, R.V. Phenolic compounds recovery from grape skin using conventional and non-conventional extraction methods. *Ind. Crops Prod.* **2018**, *111*, 86–91. [[CrossRef](#)]
11. Bakht, M.A.; Geesi, M.H.; Riadi, Y.; Imran, M.; Ali, M.I.; Ahsan, M.J.; Ajmal, N. Ultrasound-assisted extraction of some branded tea: Optimization based on polyphenol content, antioxidant potential and thermodynamic study. *Saudi J. Biol. Sci.* **2019**, *26*, 1043–1052. [[CrossRef](#)]
12. Dhanani, T.; Shah, S.; Gajbhiye, N.; Kumar, S. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arab. J. Chem.* **2017**, *10*, S1193–S1199. [[CrossRef](#)]
13. Pradal, D.; Vauchel, P.; Decossin, S.; Dhulster, P.; Dimitrov, K. Kinetics of ultrasound-assisted extraction of antioxidant polyphenols from food by-products: Extraction and energy consumption optimization. *Ultrason. Sonochem.* **2016**, *32*, 137–146. [[CrossRef](#)]
14. Zinoviadou, K.G.; Galanakis, C.M.; Brnčić, M.; Grimi, N.; Boussetta, N.; Mota, M.J.; Saraiva, J.A.; Patras, A.; Tiwari, B.; Barba, F.J. Fruit juice sonication: Implications on food safety and physicochemical and nutritional properties. *Food Res. Int.* **2015**, *77*, 743–752. [[CrossRef](#)]
15. Garude, H.S.; Ade, V.R.; Gadhave, R.K. Comparative study of various methods for extraction of antioxidants compound from bitter melon peel. *Adv. Life Sci.* **2016**, *5*, 11189–11192.
16. Yan, J.K.; Yu, Y.B.; Wang, C.; Cai, W.D.; Wu, L.X.; Yang, Y.; Zhang, H.N. Production, physicochemical characteristics, and in vitro biological activities of polysaccharides obtained from fresh bitter melon (*Momordica charantia* L.) via room temperature extraction techniques. *Food Chem.* **2020**, *337*, 127798. [[CrossRef](#)]
17. Deshaware, S.; Gupta, S.; Singhal, R.; Variyar, P.S. Influence of different pasteurization techniques on antidiabetic, antioxidant and sensory quality of debittered bitter melon juice during storage. *Food Chem.* **2019**, *285*, 156–162. [[CrossRef](#)]
18. Meyer, R.S.; Cooper, K.L.; Knorr, D.; Lelieveld, H.L.M. High-pressure sterilization of foods. *Food Technol.* **2000**, *54*, 67–70.
19. Butz, P.; Garcia, A.F.; Lindauer, R.; Dieterich, S.; Bognar, A.; Tauscher, B. Influence of ultra high pressure processing on fruit and vegetable products. *J. Food Eng.* **2003**, *56*, 233–236. [[CrossRef](#)]
20. Knorr, D. Effects of high-hydrostatic-pressure processes on food safety and quality. *Food Technol.* **1993**, *47*, 156–161.
21. Barba, F.J.; Esteve, M.J.; Frigola, A. High pressure treatment effect on physicochemical and nutritional properties of fluid foods during storage: A review. *Compr. Rev. Food Sci. Food Saf.* **2012**, *11*, 307–322. [[CrossRef](#)]
22. Alexandre, E.M.C.; Sara, S.; Santos Sónia, A.O.; Silvestre, A.J.D.; Maria, F.D.; Saraiva, J.A.; Pintado, M. Antimicrobial activity of pomegranate peel extracts performed by high pressure and enzymatic assisted extraction. *Food Res. Int.* **2019**, *115*, 167–176. [[CrossRef](#)] [[PubMed](#)]
23. Kitrytė, V.; Kavaliauskaitė, A.; Tamkutė, L.; Pukalskienė, M.; Syrpas, M.; Venskutonis, P.R. Zero waste biorefining of lingonberry (*Vaccinium vitis-idaea* L.) pomace into functional ingredients by consecutive high pressure and enzyme assisted extractions with green solvents. *Food Chem.* **2020**, *322*, 126767. [[CrossRef](#)] [[PubMed](#)]
24. Teles, A.S.C.; Chavez, D.W.H.; Coelho, M.A.Z.; Rosenthal, A.; Gottschalk, L.M.F.; Tonon, R.V. Combination of enzyme-assisted extraction and high hydrostatic pressure for phenolic compounds recovery from grape pomace. *J. Food Eng.* **2020**, *288*, 110–128.
25. Eisenmenger, M.; Reyes-De-Corcuera, J.I. High pressure enhancement of enzymes: A review. *Enzyme Microb. Technol.* **2009**, *45*, 331–347. [[CrossRef](#)]
26. Compton, S.J.; Jones, C.G. Mechanism of dye response and interference in the Bradford protein assay. *Anal. Biochem.* **1985**, *151*, 369–374. [[CrossRef](#)]
27. 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*, 159–166. [[CrossRef](#)]
28. Huang, C.Y.; Cheng, Y.H.; Chen, S.D. Hot air-assisted radio frequency (HARF) drying on wild bitter melon extract. *Foods* **2022**, *11*, 1173. [[CrossRef](#)]
29. Yi, J.; Kebede, B.T.; Dang, D.N.H.; Buve, C.; Grauwet, T.; Loey, A.V.; Hu, X.; Hendrickx, M. Quality change during high pressure processing and thermal processing of cloudy apple juice. *LWT* **2017**, *75*, 85–92. [[CrossRef](#)]