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The Effect of Soybean Peptides on Improving Quality and the ACE Inhibitory Bioactivity of Extruded Rice

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Abstract: It is crucial to address the dietary problems of hypertensive patients. The effect and mechanism of different contents of soybean protein on cooking quality and angiotensin-converting enzyme (ACE) inhibitory action in the extruded rice were firstly investigated. The results showed that the extruded rice with soybean protein possessed the higher taste value (90.32 ± 2.31), hardness (2.65 ± 0.01 g), and good pasting quality ($p \leq 0.05$). Meanwhile, the soybean protein notably retarded the starch digestibility; the sample with 6% soybean protein showed the fewest rapidly digestible starch (RDS) content (78.82 ± 0.01 mg g^{-1}) and the most slowly digestible starch (SDS) content (8.97 ± 0.45 mg g^{-1}). Importantly, the ACE inhibition rate improved from 17.09 ± 0.01% to 74.02 ± 0.65% in the 6% soybean protein sample because of the production of peptides. The peptide composition of samples were compared, which showed that the effective ACE-inhibitory peptides usually contain 2~20 amino acids, and Pro, Leu, Ile, Val, Phe, and Ala were the main components. Overall, moderate soybean protein would give a good quality and lower ACE activity in extruded food.

Keywords: soybean protein; random peptide; extruded cooking rice; blood pressure; ACE inhibition rate

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1. Introduction

Rice is a staple food that supplies globally two-thirds of the population as the primary food staple. In practice, for pursuing delicious taste, the main nutritionally active component of natural rice is lower than brown rice due to over processing [1]. The rice bran layer and rice embryo are usually removed, which results in less bioactive compounds of flavonoids, phenolic acids, vitamin E, gamma-oryzanol, etc. [2]. Hence, various technologies of rice nutrition fortification were developed such as genetically modified organism-based biofortification, genetics breeding intervention, and post-harvest fortification (extrusion, parboiling, dusting, coating, and sonication) [3,4]. Among them, the extrusion technique has been an effective way to enhance the nutrients by adding fruits, vegetables, medicinal materials, and other nutrients or medicinal ingredients in its formula [5].

Generally, the addition of bioactive compounds not only improves nutrition but also gives some new functional properties to the extruded rice [6–8]. One bioactive compound, soybean peptide, possesses many functions of immune promotion [9,10], anticoagulation [11], and hypocholesterolemic activities [12]. Meanwhile, heating and pressure of extrusion results in the degradation of the protein to peptides or amino acids, and function highly depends on the peptide structure. Hence, the optimal processing parameters and formula of extruded rice is important for controlling the biological activity of extruded rice.

Meanwhile, hyperglycemia and hypertension are the important health problems in the world [13]. Many special peptides were found using functional food for high blood pressure and cardiovascular disease [14]. Some peptides could inhibit the angiotensin-converting enzyme (ACE) activity, which is the key enzyme of anti-hypertension [15–18].

The higher activity of ACE is associated with the higher risk of cardiovascular disease, renal disease and hypertension. For inhibiting the ACE activity, a number of obviously available inhibitory drugs, such as the potent synthetic inhibitory agents Captopril and Lisinopril, have been developed and used widely for the treatment of hypertension [19,20], but the prolonged use of some drugs may cause the progressive side effects [16]. Therefore, safe and nutritious staple food products with inhibitors of ACE need to be developed urgently. However, rare reports on extruded food with anti-hypertensive properties are reported, which were derived from random peptides during extrusion processing. Hence, here, an innovative nutritional fortified rice was developed for anti-hypertensive action by adding soybean protein by using extrusion technology. The effects of soybean protein on taste, palatability, pasting properties, and starch digestibility properties of extruded rice were investigated. Particularly, for illustrating the change rule of soybean protein to random peptides, the difference in the composition and structure of random peptides were analyzed in different soybean protein amounts after extrusion processing. In addition, the main random peptides with ACE inhibition were predicted.

2. Materials and Methods

2.1. Materials

Rice flour was gained from the Tianjin Baiaotai Technology Development Co., Ltd., Tianjin, China. The rice flour contained 7.5% protein, 71.92% total starch content, and 14.19% moisture content. Soybean protein with more than 90% protein and peptides ($\leq$2000 Da) was purchased from Zhongshi Duqing Shandong Biotechnology Co., Ltd., Heze, China.

2.2. Chemicals

Angiotensin-converting enzyme (ACE) from rabbit lung (CAS Number: 9015-82-1), was purchased from Sigma Aldrich (China). Hippuric acid (CAS Number: 495-69-2), Hippuryl-L-histidyl-L-leucine (HHL) (CAS Number: 207386-83-2), porcine pancreatic α-amylase (13 U mg^{-1}), and amyloglucosidase (104 U mL^{-1}) were purchased from Shanghai Yuan Ye Biotechnology Co., Ltd. Pyridine and benzene sulfonyl chloride (BSC) were gained from the Tianjin Baiaotai Technology Development Co., Ltd., China. Glucose assay kits were purchased from Shanghai Rongsheng Biopharmaceutical Co., Ltd., Shanghai, China. Chromatography-grade pure water, acetonitrile, formic acid and trifluoroacetic acid (TFA) were purchased from Fisher Scientific Co., Ltd. (Shanghai, China). Ammonium bicarbonate (ABC) was obtained from Sigma Aldrich (Shanghai, China).

2.3. Experimental Methods

2.3.1. Extruded Process

Twin screw extruders (DS32-VII, Saixin Machinery Co., Ltd., Jinan, China) were used in the experiment. The diameter of the screw was 32 mm, and the length of the barrel was 600 mm (length-diameter ratio: 18:75).

A one-factor-at-a-time experiment was performed by adding 0%, 2%, 4%, 6%, 8%, and 10% soybean protein. The extruded process was run at 110 °C of the barrel temperature and 25% moisture content of the material. The 25% moisture content of the material and 110 °C of the barrel temperature was chosen because of the best starch digestibility properties of extruded cooking rice (Supplementary Material Figures S1 and S2). The control group of extruded rice was added with 0% soybean protein. Then, a suitable range of soybean proteins was chosen according to taste value and palatability, pasting characteristics, starch digestibility in vitro, and ACE-inhibitory activity in extruded rice. All samples were gathered for three replicates.

Furthermore, the process variables affecting the peptide extrude rice were optimized by response surface methodology (RSM). Three factors were varied viz: barrel temperature, material moisture, and soybean protein, and their effects on the extruded rice was investigated. A central composite design (CCD) of RSM was employed to determine the optimal conditions of extruded rice. Three independent variables were used at specified levels and

coded (Table S1). The maximum and minimum values of the independent variables were used that were based on the ability of the twin-screw extruder. The experimental design was arranged with the assistance of the Design-Expert 8.0.6 Trial.

2.3.2. Taste Value and Palatability

The taste value and palatability were measured by a rice taste analyzer (STA-1A, SATAKE Co., Ltd., Hiroshima, Japan) according to previous methods [21]. A total of 30 g samples were placed in a stainless steel pan, subsequently washed with running water for the 30 s, and added 40.5 mL water in the tub, then soaked for 30 min. Next, we transferred the sample to an electric rice cooker and cooked it for 40 min, and lastly kept it warm for 5 min. Finally, 8 g of each sample was transferred to a mold; the hardness, gumminess, balance, and springiness were evaluated.

2.3.3. Pasting Characteristics

The method of analyzing the pasting properties of extruded cooking rice was measured according to the reported method with some modifications, which were assessed by a Rapid Visco analyzer (RVA-4800, New-port Scientific, Sydney, Australia) [22]. A total of 3 g of each sample was slurred with 25 mL distilled water in an aluminum Rapid Visco Analyser (RVA) tank. The mixture was stirred at 960 rpm for 10 s before measurement and then changed to 160 rpm. RVA test processing followed the standard heating mode procedure: 50 °C for 1 min, 95 °C (12 °C/min) for 5 min, then 50 °C (12 °C/min) for 2.5 min.

2.3.4. Starch Digestibility In Vitro

The starch digestibility in vitro of extruded cooking rice was conducted following the previous method with some modifications [23]. Simulated intestinal fluid (SIF) was composed of 1 mL amyloglucosidase in 2 mL of deionized water, and the pancreatin supernatant was prepared by centrifuging (10 min at 2918× *g*) the solution of porcine pancreatic α-amylase that was obtained by 3.89 g of porcine pancreatic α-amylase in 25.7 mL deionized water (pH 7.0). The enzyme solution was served by mixing 18.7 mL pancreatin supernatant and 1 mL of diluted amyloglucosidase. The sample was composed by mixing five balls (10 mm in diameter) and 20 mL of acetate buffer; then, the reaction mixture (pH 5.2) was incubated for 30 min at 95 °C. After the addition of the enzyme solution the sample was incubated in a 37 °C shaking water bath. Aliquots of 1 mL were withdrawn at 0, 20, and 120 min of digestion and mixed with 5 mL of ethanol; the released glucose content was measured using the GOPOD kits. Most starches contain a rapidly digestible portion (RDS, hydrolyzed within 20 min), a slowly digestible portion (SDS, hydrolyzed between 20 and 120 min), and a portion that is resistant to digestion (RS, not hydrolyzed after 120 min).

2.3.5. ACE-Inhibitory Activity Analysis

Hippuryl-L-histidyl-L-leucine (HHL) was used as an artificial substrate to measure the ACE activity, and the amount of produced hippuric acid (HA) was determined according to the method of [24], with slight modifications. We dissolved 1 g of sample in 5 mL borate buffer solution (BBS) containing 300 mM NaCl (pH 8.2) to prepare inhibitor solutions, which was then agitated on a vortex mixer at 1500 rpm for 1 min, and then centrifuged for 10 min at 4085× *g*. The substrate solution (5 mM) was prepared by dissolving 25 mg HHL in 11.26 mL BBS, containing 300 mM NaCl (pH 8.2). Then, 50 µL HHL (5 mM) was added to 125 µL of inhibitor solution containing 300 mM NaCl (pH 8.2). Subsequently, 25 µL of the ACE (0.1 U/mL) was added, and the reaction mixture was incubated for 30 min at 37 °C. Then, 200 µL HCl (1 M) was added to stop the reaction. A total of 0.4 mL of pyridine was added followed by 0.2 mL of benzene sulfonyl chloride (BSC), mixed evenly for 1 min, and cooled on ice. The absorbance was measured at 410 nm.

2.3.6. Identification and Analysis of Endogenous Polypeptides by LC-MS/MS

The composition of endogenous peptides in soybean was identified by LC-MS/MS according to the previous report [25] with slight modifications. Endogenous polypeptides were extracted by the ultrasonic method with 1% formic acid (model: pulse on 5 s; pulse off 15 s; power 180 W). After the extract was centrifugated at 12,000× *g* for 30 min successively, the supernatant was collected for ultrafiltration. Sep-Pak C18 was then used to desalt the sample. The column was equilibrated with 500 µL 0.1% TFA and 1% acetonitrile before sample loading. Then, 200 µL 0.1% TFA and 0.5% acetonitrile were used to desalt. The elution procedure was carried out using 300 µL 0.1% TFA and 80% acetonitrile. The obtained desalted samples were then freeze-dried.

The samples were separated by ultra-high pressure liquid chromatography (NanoAcuity UPLC; Waters Technology Co., Ltd., Shanghai, China) with a nanometer. The eluents were: 0.1% formic acid in MilliQ-treated (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The samples were dissolved in 200 µL A, and 2 µL samples were absorbed by the automatic sampler, then sent to the collecting column (Acclaim PepMap C18, 100 µm × 20 mm; Thermo Scientific and Technology Co., Ltd., Shanghai, China) at a flow rate of 10 L/min for 2 min. The samples on the capture column were separated by chromatography on the analysis column (Acclaim PepMap C18, 75 µm × 250 mm; Thermo Scientific and Technology Co., Ltd., Shanghai, China) at a flow rate of 300 nL/min. The relative liquid gradient was as follows: the elution gradient started with 5% B and increased to 30% B in 105 min. The mobile phase composition was raised from 30% B to 90% B in 5 min. It was maintained at 90% B for 2 min then decreased to 5% in 1 min and maintained at 5% B for 7 min. The samples were eluted with blank solvent for 30 min in mobile phase gradient once.

The ESI source operated with the following parameters: curtain gas was 35 psi; ion spray voltage was 2.0 kV; heating capillary temperature was 300 °C; ion source gas 1 was 40 psi; gas 2 was 50 psi.

The data dependence mode was adopted to automatically switch between MS and MS/MS. The Level 1 scan used Orbitrap, the scan range was set at m/z 350–1600, and the resolution was set 70,000 (m/z 200). Maximum ion introduction time was 50 ms, automatic gain control (AGC) was set at 3×10^6. The top 10 parent ions which conformed to cascade (MS/MS) fragmentation conditions were fractured using Higher Energy C-trap dissociation (HCD) and scanned by Orbitrap at a resolution of 17,500. The scanning range was automatically controlled according to the mass charge ratio of parent ions, and the scanning range was fixed at m/z = 200–2000. The minimum ionic strength value for MS/MS was set at 50,000. In MS/MS, the maximum ion introduction time was 100 ms, AGC control was set to 1.0×10^5, and the parent ion selection window was set 2 Da. For ions with 2, 3 and 4 charge numbers, MS/MS collection was carried out, and dynamic exclusion was set to take place during MS/MS within 10 s for each parent ion, followed by an exclusion time of 30 s.

Uniprot database (Species Glycine Max, Entires 85843, http://www.uniprot.org/uniprot/?query=taxonomy:3847) (accessed on 2 September 2021). All the raw nano-LC-MS/MS data were analyzed by MaxQuant software (version 1.6.5.0, http://www.coxdocs.org/doku.php?id=maxquant:start) (accessed on 2 September 2021) for the database search [26]. For quantitative analysis, we used the IBAQ algorithm.

2.4. Statistical Analysis

All the treatments were analyzed for three replicates. A one-way analysis of variance (ANOVA) was used for the experimental data by SPSS 13.0 software. All data are expressed by mean ± standard deviation. LSD tests were performed to significance level analysis, in which the $p \leq 0.05$ was recognized as the significance level.

3. Results and Discussion

3.1. Effect of Soybean Protein Contents on the Taste and Palatability of Extruded Cooking Rice

The effects of different soybean protein contents on the taste and palatability of extruded cooking rice are shown in Figure 1. The taste value was significantly increased in extruded rice with soybean protein when compared to the control, which possessed the maximum value (90.32 ± 2.31) in extruded rice with 8% soybean protein (Figure 1A). For the hardness, gumminess, and balance, they first increased and then decreased with the increase of soybean protein content (Figure 1B). The extruded rice with 2% soybean protein had the maximum hardness. Meanwhile, the samples with 4% soybean protein had the highest balance values and gumminess than other groups. However, springiness had no significant correlation with soybean protein (Figure 1B). Overall, these data suggested that the samples with soybean protein (4~8%) could possess a good taste and texture in extruded cooking rice.

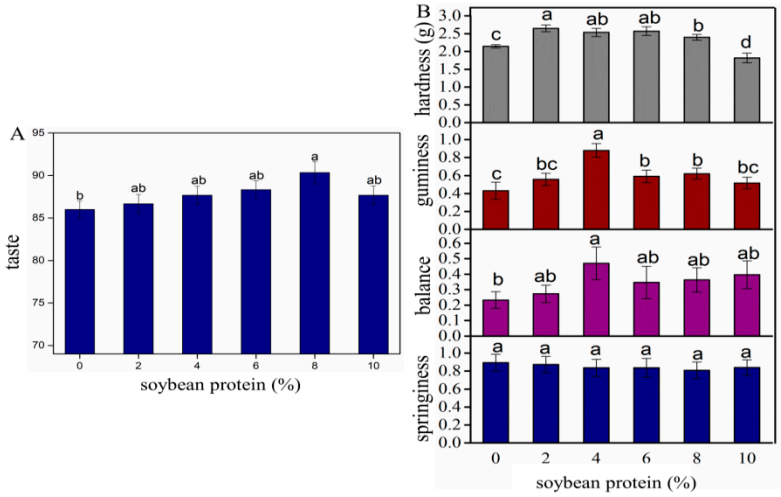

Figure 1. Effect of different soybean protein contents on the quality of extruded cooking rice. (**A**) Taste; (**B**) hardness, gumminess, balance, and springiness. Values are means ± SD. Values followed by the same letters in the same column are not significantly different ($p \leq 0.05$).

Taste value is an important indicator for assessing the eating quality of extruded cooking rice. At the same time, the taste value of extruded cooking rice was greater than 70 taste values, which indicates a super taste quality [27]. Usually, the amylose content and crystallinity are considered to be momentous factors that lead to differences in the eating quality of rice [28,29]. The higher the amylose content, the lower the taste value. Here, the taste value was affected by soybean protein content, which may be because of lower amylose and the interaction of protein and starch in extruded cooking rice [30].

3.2. Effect of Soybean Protein Contents on the Pasting Properties of Extruded Cooking Rice

The pasting properties of samples by Rapid Visco Analysis (RVA) are shown in Table 1. Overall, extruded rice with different doses of soybean protein expressed a gradual decrease in the peak viscosity, through viscosity, breakdown viscosity, and final viscosity, except for the through and final viscosity in the samples with 2% soybean protein. Meanwhile, there was a remarkably lower breakdown viscosity in the sample with 2% soybean protein than in the sample with 4% soybean protein ($p \leq 0.05$). In detail, a slight decrease in viscosity was observed (soybean protein content $\leq$ 4%), followed by a sharp decrease (soybean protein content $\geq$ 6%). However, the pasting temperature and setback viscosity were gradually increased with more soybean protein content. The extruded cooking rice with 8% soybean protein had the minimum pasting time (5.09 ± 0.31), and a sharp increase was observed in 10% soybean protein extruded rice (6.25 ± 0.30). Overall, these data suggested that the samples with soybean protein (6~8%) could possess good pasting properties in extruded cooking rice.

Table 1. Pasting properties extruded cooking rice with different levels of SP.

SP (%)	Peak Visc.	Trough Visc.	Breakdown Visc.	Final Visc.	Setback Visc.	Pasting Temp.
0	115.52 ± 9.9 ^{a}	56.72 ± 9.77 ^{a}	59.95 ± 5.62 ^{a}	103.08 ± 2.56 ^{a}	−13.32 ± 7.53 ^{d}	67.82 ± 7.60 ^{a}
2	94.29 ± 9.28 ^{b}	58.87 ± 9.97 ^{a}	38.44 ± 2.77 ^{c}	106.53 ± 2.29 ^{a}	15.40 ± 4.93 ^{b}	67.94 ± 7.63 ^{a}
4	96.94 ± 4.98 ^{b}	49.65 ± 9.08 ^{a}	47.79 ± 3.43 ^{b}	93.24 ± 2.81 ^{b}	−1.40 ± 8.45 ^{c}	68.07 ± 6.94 ^{a}
6	79.11 ± 4.84 ^{c}	45.58 ± 9.74 ^{ab}	27.07 ± 2.68 ^{d}	84.93 ± 4.72 ^{c}	6.66 ± 5.42 ^{bc}	68.90 ± 7.08 ^{ab}
8	56.12 ± 4.81 ^{d}	30.74 ± 10.02 ^{b}	19.13 ± 3.53 ^{e}	69.90 ± 4.58 ^{d}	15.86 ± 4.86 ^{b}	72.45 ± 7.44 ^{b}
10	40.06 ± 5.03 ^{e}	29.55 ± 10.14 ^{b}	7.93 ± 5.43 ^{f}	72.79 ± 2.87 ^{d}	35.63 ± 5.09 ^{a}	74.25 ± 6.90 ^{b}

All the values are the mean ± SD. Values with different letters within a column are significantly different at $p \leq 0.05$ as evaluated by Tukey's test to analyze the different soybean protein contents in the same column.

As is well known, the pasting properties are linked to the potential molecular interactions in rice [31]. It has been proved that setback viscosity not only depends on amylose content but also on the number of amylose chains and the size of the molecules of amylopectin [32]. The lower breakdown viscosity may be due to the structural remodeling of the branch chains of amylopectin for soybean protein extruded cooking rice, which led to the inhibition of starch pasting [33,34]. Agreeing with previous studies in natural rice, the breakdown viscosity expressed the consistent trend of gumminess [35]. Here, soybean random peptides can restrict starch swelling and retard starch gelatinization; the samples with soybean protein had the lower peak viscosity, through viscosity, and final viscosity, which may result from the soybean protein restricting the starch granules' swelling during gelatinization [18,36]. Moreover, the heating and pressure during the extrusion process promotes the protein–starch interactions, which expose multifarious hydrophobic amino acids (e.g., Pro, Leu, Ile, Val, and Phe) (Table 2). The pasting temperature was improved because these small molecules could be attached or embedded to granular surfaces or intervals of starch by hydrogen bonds or hydrophobic interactions [37]. To sum up, soybean protein reduced the pasting capacity of extruded rice starches owing to the production of a more compact structure, which finally inhibited the gelatinization of starch and reduced the viscosity.

Table 2. Peptide composition and characteristics of samples.

No.	Sequence Length	No. of Type	Relative Amount (%)	Pro	Leu	Ile	Val	Phe	Ala	Trp	Tyr
Sample 1: SP											
1	8	5	3.226	9	2	2	6	1	0	2	0
2	9	18	11.613	18	14	11	6	4	4	2	6
3	10	33	21.290	55	23	19	16	8	12	2	7
4	11	25	16.129	43	15	16	11	15	14	3	2
5	12	17	10.968	34	13	14	11	14	4	1	1
6	13	14	9.032	25	10	14	11	11	4	1	3
7	14	8	5.161	20	4	9	3	6	0	0	1
8	15	8	5.161	18	8	6	7	8	2	1	2
9	16	5	3.226	19	4	1	0	3	0	2	0
10	17	4	2.581	6	7	4	1	3	1	0	1
11	18	3	1.935	16	0	1	1	4	1	2	1
12	20	4	2.581	23	0	1	1	6	1	2	0
13	21	5	3.226	23	2	3	3	7	2	2	1
14	22	4	2.581	13	5	3	2	7	1	0	1
15	23	1	0.645	6	0	0	0	2	0	0	0
16	25	1	0.645	6	0	0	0	2	0	0	0

Table 2. *Cont.*

No.	Sequence Length	No. of Type	Relative Amount (%)	Pro	Leu	Ile	Val	Phe	Ala	Trp	Tyr
Sample 2: extruded rice (6% SP)											
1	8	7	4.667	9	4	1	2	4	0	0	1
2	9	27	18.000	32	15	13	13	9	8	7	1
3	10	35	23.333	44	22	13	24	13	16	14	1
4	11	28	18.667	36	16	13	20	15	16	2	2
5	12	14	9.333	20	9	7	12	6	7	0	1
6	13	10	6.667	12	6	6	7	2	3	0	3
7	14	9	6.000	11	3	7	9	3	0	1	2
8	15	4	2.667	9	2	2	2	2	0	0	0
9	16	2	1.333	6	0	2	2	0	0	0	0
10	18	1	0.667	1	1	0	0	1	0	0	0
11	19	2	1.333	12	0	2	0	2	0	0	0
12	20	1	0.667	0	0	0	0	0	0	0	0
13	21	4	2.667	21	0	2	1	5	0	0	0
14	22	4	2.667	21	0	2	0	5	0	0	0
15	23	1	0.667	6	0	0	0	2	0	0	0
16	25	1	0.667	6	0	0	0	2	0	0	0
Sample 3: extruded rice (0% SP)											
1	8	4	21.053	4	2	0	1	2	0	1	0
2	9	5	26.316	8	0	3	1	0	0	0	1
3	10	7	36.842	8	6	3	1	0	5	4	0
4	11	2	10.526	4	0	1	1	1	1	0	0
5	12	1	5.263	1	0	1	1	0	0	0	0

3.3. Effect of Soybean Protein Contents on the Starch Digestibility Properties of Extruded Cooking Rice

Significant changes of RDS, SDS, and RS with the addition of soybean protein are shown in Figure 2. Interestingly, the SDS contents had gradually increased with the increase of soybean protein content until the content was up to 10%. Notably, at the sample of 6% soybean protein, RDS was the minimum (78.82 $\pm$ 0.10 mg/g), and SDS reached the maximum (8.97 $\pm$ 0.45 mg/g). Meanwhile, RDS contents decreased in all soybean-containing samples when compared to the control, except for the sample with 2%. Nevertheless, with the soybean protein increasing, the RS contents of extruded rice were slightly lower than control group ($p \leq 0.05$). On the whole, the samples with soybean protein of 6% had greater effects on the starch digestibility of extruded rice by increasing SDS content. This finding supported the idea that a higher soybean protein amount results in lower starch digestibility, which is consistent with previous studies [18,38].

As is known to all, high glycemic index (GI) foods are closely related to starch digestibility, which could cause the change of amylopectin to amylose in starch granules during the extruding process [39,40]. The lower the GI of foods, the higher the contents of SDS and RS. The increase of SDS could regulate the body's blood glucose response, enhance the nutritional constituent of foods in diabetic patients, and retard the digestion of rice starch [41,42]. Simultaneously, some interactions between amino acids or peptides and starch will partially restrict the hydrolysis of starch [43]. Moreover, proteins or random peptides are easier to interact or combine with α-amylase for reducing its catalytic activity then directly inhibiting the digestion of starch [40,44]. Here, proteins or peptides may be effectively glued to starch granules, forming a barrier surrounding starch granules, which reduces the starch digestibility rate by increasing the steric hindrance, delaying the internal contact point of the interaction between amylase and starch.

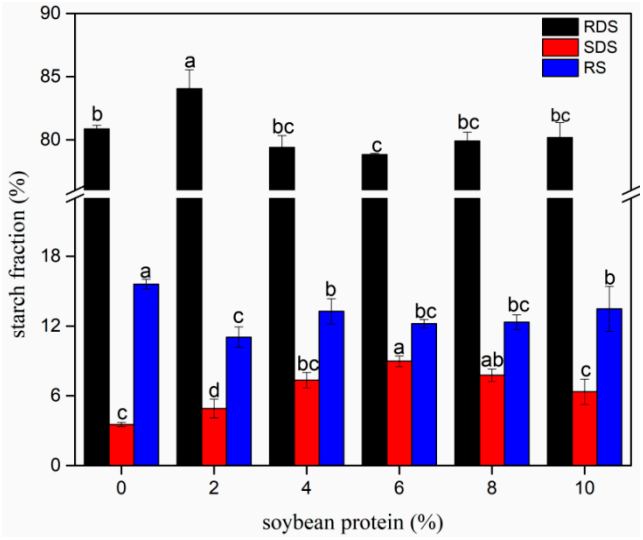

Figure 2. Starch digestibility in vitro extruded cooking rice with different soybean protein additions. Values are means ± SD. Values followed by the same letters in the same column are not significantly different ($p \leq 0.05$).

3.4. Response Surface Methodology (RSM) Analysis

The optimal extrusion process of extruded cooking rice was determined by taking the taste and balance values as the response values (Table S2).

The following quadratic polynomial regression equation for the taste value:

$$Y \text{ (eating value)} = 93 - 0.37A + 1.25B + 0.88C + 1.75AB - 0.75BC - 7A^2 - 4.75B^2 - 0.5C^2.$$

The following quadratic polynomial regression equation for the balance value:

$$Y \text{ (balance value)} = 035 + 0.021A - 1.00 \times 10^{-2}B + 0.011C + 0.025AB + 2.500 \times 10^{-3}AC - 0.066A^2 - 0.12B^2 - 0.046C^2.$$

The variance analysis of items of regression equations on eating values and balance values are shown in Tables S3 and S4, respectively. The response surface plot and contour plot of the interactive effects of soybean protein content, moisture content, and barrel temperature on taste values and balance values are shown in Figures S4 and S5, respectively. Results showed that the predicted process conditions of the regression model analysis obtained by software Design-Expert 8.0 were basically close to each other, taking the taste value and balance value as the response surface within the range of selected factors, indicating that the evaluation results of each factor are basically consistent. Combined with the actual test operation, the optimal extrusion process was determined as follows: 6% soybean protein, 25% material moisture, and barrel temperature of 110 °C, which has the highest taste value (93) and balance value (0.35).

3.5. ACE Inhibitory Action In Vitro of Different Soybean Protein Contents

ACE activity is one of the major therapies for treating hypertension, which shows a direct association with diastolic blood pressure. It was obvious that the ACE inhibition rate of extruded rice with soybean protein (2~10%) was significantly higher (>74.02 ± 0.65%). than the sample of non-soybean protein (17.09 ± 0.01%) (Figure 3) ($p \leq 0.05$), and the ACE inhibition rate of extruded rice was significantly higher than naturally grown rice, which was only 0.03 ± 0.01% ($p \leq 0.05$). Of note, there were no dose effects of the soybean protein content on the ACE inhibition rate (Figure 3). Probably, the retention rate and transformation rate of soybean peptide in the samples was high, which needs to be further explored. Fortunately, the ACE inhibition rate of extruded rice with soybean protein was significantly higher than the sample of non-soybean protein and ordinary rice, which maybe was because of the presence of bioactive peptides [45,46].

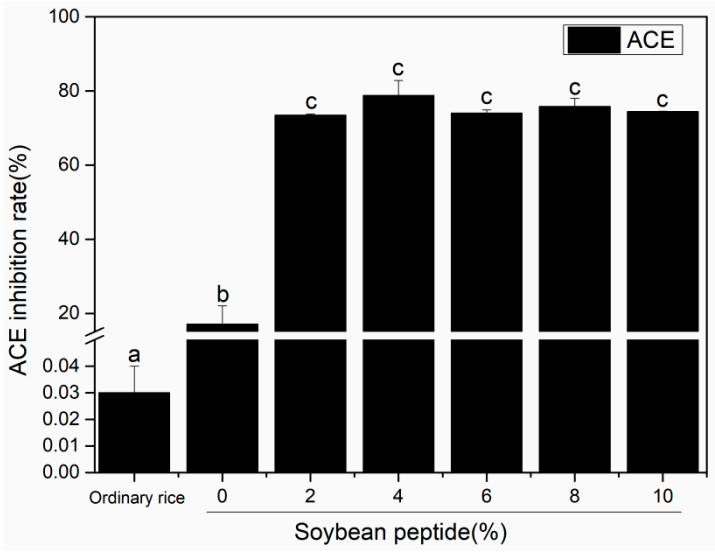

Figure 3. ACE inhibition rate of samples. Ordinary rice and extruded cooking rice with different soybean protein additions. Values are means ± SD. Values followed by the same letters in the same column are not significantly different ($p \leq 0.05$).

Inhibiting the ACE activity level is consistent with decreasing diastolic blood pressure [47]. So, the extruded rice with soybean protein or peptides may be a promising candidate as ACE inhibitor staple food for patients with hypertension [40].

3.6. Peptides Molecular Change of Different Soybean Protein Content during Extrusion

Why does extruded rice have ACE inhibitory action? For illustrating this point, LC-MS/MS was used to identify the difference of molecular changes of the soybean protein in extruded rice. The extruded rice with 6% soybean protein was adopted based on its better ACE inhibition rate and appearance, taste, palatability, and pasting quality. The peptides composition and amino acid sequence were demonstrated in Figure S3 and Table S5. There were 155 peptide sequences in the soybean protein powder (Figure S3A), and 150 peptide sequences in soybean protein extruded rice (Figure S3B), whereas only 19 peptide sequences were found in extruded rice (0% soybean protein) (Figure S3C). From Table 2, the soybean protein power contained a large number of key amino acids in the composition of bioactive polypeptides of ACE inhibitory activity. Here, emerging evidence suggests that the most effective ACE-inhibitory peptides usually contain 2~20 amino acids. In this research, Pro, Leu, Ile, Val, Phe, and Ala were the main components. Additionally, the proportion of small peptides of about 8~13 amino acids was 72% (112/155) and 80% (121/150) in soybean protein and the extruded rice with soybean protein, respectively, but there were few of these kinds of peptides in extruded rice with 0% soybean protein, which is in line with previous research [48]. All these results revealed that as the molecular weight of peptides tends to reduce, more small peptides were produced during the extrusion. Hence, the difference between bioactive peptides may be the main reason for the ACE inhibitory activity, because of the lower amount of small peptides with ACE inhibition properties.

The physicochemical properties and biological activities of peptides are related to chain length, molecular weight, amino acid composition, and sequence [49,50]. It has been reported that peptides in soybean protein may be depolymerized, degraded, conversed, and modified during extrusion processes [51]. Generally, potent ACE inhibitory peptides are short peptides, and their C-terminal plays a key role in their inhibitory activity. According to the AHTPDB database, peptides containing Tyr, Pro, Try, Phe, and Leu at the C-terminal have been elucidated as effective ACE inhibitor peptides. Some ACE inhibitory peptides were also reported by previous research, such as IRW, IPP, VPP, VLPVP, HLPLP, IVGRPRHQG and so on [15,52,53]. In addition, heating during extrusion results in protein segment degradation into small molecular peptides and the production a large number of

hydrophobic amino acids, which enhances the biological activity for ACE [54,55]. In this study, the main hydrophobic amino acids, such as Pro, Leu, Ile, Val, Phe, Ala, Trp, and Tyr (Table 2), accounted for 38.8% of the 8~13 amino acid sequence. The inhibition of ACE is achieved by hydrophobic peptides which have a high affinity for the active sub-sites of ACE, block the reaction of angiotensin-I and kinin with angiotensin-converting enzyme, and inhibit the generation of angiotensin-II and sustained-release peptide, thus lowering blood pressure. Particularly, proline, as a branched-chain amino acid and aromatic amino acid, could enhance the ACE inhibition rate, which is promoted in more kinds of peptides in the soybean protein rice samples [56–58].

4. Conclusions

In conclusion, we first reported and analyzed the effect of soybean protein on cooking quality and ACE inhibitory action by increasing bioactive peptides in the extrusion rice. The results revealed that the optimal amount of soybean protein (6%) could not only give a higher taste value but also improve ACE inhibitory activity and decrease the starch digestibility rate of extruded cooking rice. Meanwhile, multifarious hydrophobic amino acids (e.g., Pro, Leu, Ile, Val, and Phe) were exposed during the heating extruded process. As a whole, small peptides may act as a vital factor in the function of extruded rice for ACE inhibitory activity. The extruded rice with soybean protein could provide a very good novel staple food for people, and includes many soybean peptides with ACE inhibitory bioactivity. This search provides a basis for functional extrusion food research in hypoglycemic and anti-hypertensive people.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr10101921/s1, Figure S1: Starch digestibility in vitro extruded cooking rice with different material moisture contents. Values are means ± SD. Values followed by the same letters in the same column are not significantly different ($p \leq 0.05$); Figure S2: Starch digestibility in vitro extruded cooking rice with different barrel temperatures. Values are means ± SD. Values followed by the same letters in the same column are not significantly different ($p \leq 0.05$); Figure S3: Peptides composition and amino acid sequences of samples tested by LC-MS/MS. (A) The total Ions Chromatography of SP, (B) the total Ions Chromatography of extruded rice (6% SP), (C) the total Ions Chromatography of extruded rice (0% SP); Figure S4: Response surface plot and contour plot showing the interactive effects of soybean protein content, moisture content, and barrel temperature on taste value; Figure S5: Response surface plot and contour plot showing the interactive effects of soybean protein content, moisture content, and barrel temperature on balance value. Table S1: Factors and levels of response surface experiments; Table S2: Factors and levels in the response surface design arrangement and experimental results; Table S3: Variance analysis of items of regression equation on taste value; Table S4: Variance analysis of items of regression equation on balance value; Table S5: Peptides composition and amino acid sequences of samples tested by LC-MS/MS.

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