



Effect of the extrusion process on allergen reduction and the texture change of soybean protein isolate-corn and soybean flour-corn mixtures

Huanyu Zheng^{a,b,c,*}, GuoSen Yan^b, Youngsoo Lee^a, Christina Alcaraz^{a,d}, Susan Marquez^a, Elvira Gonzalez de Mejia^{a,**}

^a Department of Food Science and Human Nutrition, University of Illinois Urbana-Champaign, Urbana, IL, USA

^b College of Food Science, Northeast Agricultural University, Harbin 150030, China

^c National Research Center of Soybean Engineering and Technology, Heilongjiang Green Food Science Research Institute, Harbin 150028, China

^d New Biology National Science Foundation Fellow, Northeastern Illinois University, Chicago, IL, USA

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ABSTRACT

Soy protein and corn meal are widely used as main ingredients in the cereal and snack industries. We studied the effect of extrusion processing on the immunoreactivity of proteins in soy protein isolate-corn meal (SPI-corn) blends and soy flour-corn meal (SF-corn) blends using a full-factorial design with two levels of temperature, moisture, and screw speed (SS). The changes in protein solubility, protein secondary structure and physical properties, such as the expansion ratio, specific volume, water hydration capacity (WHC), texture, and color, were also studied. The western blotting results illustrated that extrusion processing decreased the immunoreactivity of proteins in SPI-corn and SF-corn blends and eliminated certain allergenic proteins in SF-corn extrudates. The enzyme-linked immunosorbent assay (ELISA) results indicated that different extrusion conditions decreased the immunoreactivities of SPI-corn and SF-corn blends in the ranges of 53%–68% and 80%–86%, respectively, compared with the raw material. The protein solubility decreased after extrusion by > 58.7% for SPI-corn extrudates and by 66.3% for SF-corn extrudates compared with the initial materials and decreased significantly for both SPI-corn and SF-corn extrudates with the increase in extrusion temperature ($P < 0.05$). The α -helix structure decreased and β -strand structure increased after extrusion for both SPI-corn and SF-corn blends. Higher moisture significantly decreased the expansion ratio, specific volume and WHC of SPI-corn and SF-corn extrudates, as well as increased the energy required to cut the samples. Thus, the effect of extrusion on allergenic proteins using the human plasma of subjects allergic to soybean and corn showed that moisture had a clear effect on their reduction. Product treated with low moisture (20%), regardless of the starting material used, was the most effective based on the reduction of allergenic proteins.

1. Introduction

Food extrusion has become a very important process in the manufacture of cereals and snacks (Kumar et al., 2018). Soy protein and corn meal are the main ingredients of extrusion processing to produce snacks, pet foods and feedstuff because of their nutritional balance and special flavor (Maziadixon, Alamu, Popoola, & Yomeni, 2017; Neely, Youngsoo, & Soo-Yeun, 2010). Soybean is already an integral part of the human daily diet. It has been suggested that soybean contains several components that can lower the incidence of high plasma cholesterol, cancer, diabetes mellitus, and obesity (Carroll & Kurowska, 1995; Demmer et al., 2016; Jaceldo-Siegl et al., 2017; Kaur, 2014;

Kingsley et al., 2017; Mazotto et al., 2017). Corn contains high levels of fiber that can help lower cholesterol and also reduce the risk of colon cancer (Cagno, Mazzacane, & Rizzello, 2010; Hamid & Kalsoom, 2017; Josep et al., 2011). However, the incidence of allergies makes it difficult for individuals with soy and corn allergies to reap their benefits. Soybean is currently one of the “big 8” common allergens, and an estimated 0.5% of the U.S. population is allergic to soy (Cordle, 2004; Kabourek & Taylor, 2003; Murakami et al., 2018). Corn is considered a rare allergen; however, several allergens have been reported such as the 9-kDa lipid transfer protein (Krishna, Albers, Schafer, Demetrius, & Young, 2011; Pastorello et al., 2000), 16-kDa α -amylase trypsin inhibitor, Zea m 25 (Weichel et al., 2010), 27-kDa (Krishnan et al., 2010;

* Correspondence to: H. Zheng, College of Food Science, Northeast Agricultural University, Harbin 150030, China.

** Correspondence to: E. Gonzalez de Mejia, 228 Edward R. Madigan Laboratory, MC-051, 1201 W. Gregory Dr., Urbana, IL 61801, USA.

E-mail addresses: zhenghuanyu1@163.com (H. Zheng), edemejia@illinois.edu (E.G. de Mejia).

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Sung-Ho et al., 2005) and 50-kDa (Krishnan et al., 2011) γ -zein, vicilin, globulin-2 and endochitinase (Fasoli et al., 2009).

Alteration of the protein structure by physical, chemical, and biochemical changes during food processing can impact the allergenicity of proteins (Ballmer-Weber et al., 2007). Extrusion is a common processing method that plasticizes and machine wets and inflates paste-like and/or protein-rich ingredients by water, pressure, temperature and mechanical forces, leading to chemical reactions and molecular structure changes (Castells, Marín, Sanchis, & Ramos, 2005; Hauck & Huber, 1989; Singha, Muthukumarappan, & Krishnan, 2018). Compared with other processing methods, it is unique because the ingredients are strongly squeezed while being heated. Thus, it can completely change and destroy the structure of food molecules and improve the functional ingredients of food (Carvalho, 2010). Extrusion has been reported to denature undesirable enzymes and inactivate some antinutritional factors (lipoxygenase, hemagglutinins, saponin and urease), all of which affect the full use of nutrients, leading to increased protein and starch digestibility, sterilization of the finished product and retention of the original color and flavor (Bhandari, apos, Arcy, Young, & D'Arcy, 2010; Singha et al., 2018).

There is a need to explore the effect of extrusion processes on the reduction of allergens of blends of corn meal and soy protein. This was aimed to establish the relationship between the conditions of the extrusion process and immunogenicity of soy and corn protein by measuring immunoglobulin E (IgE) binding to serum from both soy and corn allergic patients. We used soy flour (SF)-corn and soy protein isolate (SPI)-corn blends as extended models, as SPI and SF are the main soy protein products widely used in the daily diet. The physical properties, such as expansion ratio, specific volume, texture, water hydration capacity and color, as well as the protein secondary structure changes, were also investigated.

2. Materials and methods

2.1. Materials

Soy protein isolate (SPI) (Archer Daniels Midland Company, Decatur, IL, USA), defatted soy flour (SF) (Archer Daniels Midland Company, Decatur, IL, USA) and corn meal (CC401 Yellow Degermed Cornmeal; Bunge North America, St. Louis, MO, USA) were used as extrusion materials. The protein concentrations of SPI, SF and corn were 94.3%, 56.9% and 7%, respectively, and the fiber concentrations were 0%, 18.0% and 2.0%, respectively. To obtain the same total protein concentration of the soy protein product and corn meal blends, two sample formulations were used: SPI:corn = 1:1 (w/w) and SF:corn = 1.5:1(w/w).

2.2. Experimental design

The experiment adopted a three-factor and two-level design (3×2 design) to investigate the effects of three extrusion conditions of temperature (T), moisture concentration (MC), and screw speed (SS) on the properties of corn and soybean after extrusion repeated twice to ensure accuracy. The extrusion processing conditions used to produce SPI-corn and SF-corn extrudates are presented below (The five values are the temperatures in different areas of the extrusion process):

Sample code	Temperature (°C)					Moisture (%)	Screw speed (rpm)
	Inlet		Die				
1	30	75	120	130	140	20	200
2	30	75	120	130	140	20	400
3	30	75	120	130	140	40	200
4	30	75	120	130	140	40	400
5	30	75	105	105	110	20	200
6	30	75	105	105	110	20	400

7	30	75	105	105	110	40	200
8	30	75	105	105	110	40	400

2.3. Extrusion processing

The material was squeezed in a co-rotating twin-screw extruder with a cylindricity of 111 cm and a screw extent to barrel diameter (L/D) of 37 (Werner Pfleiderer ZSK-30, Ramsey, NJ, USA). Five temperature settings were used along the barrel from the inlet (zone 1) to die (zone 5), maintaining the temperature of the first two areas near the inlet at 30 °C and 75 °C, respectively, and adjusting the last three areas as the design: 105 °C, 105 °C, 110 °C or 120 °C, 130 °C, 140 °C. The premixed material was fed into the inlet using a feed system at a ratio of 20 kg/h (series 7100; K-Tron Corp., Pittman, NJ, USA), controlling the water inflow through a three-action piston pump to regulate the moisture content of the material (U.S. Electric Motors, Milford, CT, USA). The extrudates were formed using an "O"-shaped die with a 2-mm diameter. The extrudates were collected, cooled, sealed and placed into bags, kept away from light at 4 °C until use.

2.4. Purification of β -conglycinin and glycinin

The edulcoration of β -conglycinin and glycinin was adjusted according to the existing method (Wenji, Bringe, Berhow, & Elvira, 2008). One hundred grams of defatted SF was prepared into a 1.5-L suspension with distilled water, and then the pH was adjusted to 7.5 with 2 N NaOH. Next, the sample was centrifuged at 9000g for 0.5 h, 0.98 g/L sodium bisulfite (SBS) was added to the supernatant, and the pH was adjusted to 6.4 with 2 N HCl. After incubation at 4 °C for 12 h, the sample was centrifuged again for 20 min (4 °C, 6500g) and the precipitate was rinsed with ice distilled water and then resolved in distilled water. Next, the solution was purified using Amicon Ultra-0.5 50 K Membrane centrifugal filters (Millipore, USA) to remove proteins larger than 50 kDa to obtain purified glycinin. The purification of β -conglycinin was similar to the above procedure, but the first step was adjusted to pH = 5.9. After centrifugation (9000g, 30 min), the salt concentration of the supernatant was adjusted to 0.25 M with NaCl and the pH was adjusted to 5.0 with 2 N HCl. Next, the sample was incubated at 4 °C for 1 h and then centrifuged again (9000g, 30 min), followed by the addition of ice water to the supernatant (1:2) and adjustment of the pH to 4.8. Finally, the precipitate was collected by centrifugation (6500g, 20 min, 4 °C), washed twice with cold distilled water, and suspended in 25 mL of pH 7.5 distilled water.

β -Conglycinin was obtained after filtration using Amicon Ultra-0.5100 K and 30 K Membrane centrifugal filters (Millipore) to remove proteins larger than 100 kDa and smaller than 30 kDa. Both purified β -conglycinin (> 90% purity) and glycinin (> 95% purity) were stored at -70 °C.

2.5. Soluble protein extraction

Protein extraction with 0.05 M Tris-HCl pH 8.2 buffer was performed as previously described by Song (Song, Frias, Martinez-Villalunga, Vidal-Valverde, & Mejia, 2008). A 50-mg soybean product powder sample and 1 mL of extraction buffer were mixed in an Eppendorf tube, and then the mixture was treated with an ultrasonic bath for 70 min, mixing every 10 min to avoid precipitation (M2510; Branson Ultrasonic Corporation, Danbury, CT). After extraction, the sample was centrifuged at 20,000g 4 °C for 0.5 h (M5417R; Brinkmann Instruments, Westbury, NY). Each sample was extracted three times to ensure reliability. The extracted protein solution was stored in an Eppendorf tube.

2.6. Soluble protein quantification

The protein concentration was determined using the Protein DC Microplate Assay Protocol (Bio-Rad Laboratories, USA). Briefly, 5 μ L of the diluted sample (2%, v/v), 25 μ L of reagent A and 0.2 mL of reagent B (Bio-Rad Laboratories, USA) were added to a 96-well plate, which was incubated for 15 min at room temperature with shaking. Thereafter, the absorbance was measured at 690 nm. A bovine serum albumin (BSA) standard curve was established— $y = 0.170x + 0.0029$, $R^2 = 0.99$, where y = absorbance and x = concentration in mg/mL—and then the protein content of the sample was obtained.

2.7. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

For SDS-PAGE, the Gould gel (Lonza, USA) concentration of the electrophoresis solution was 12%. To eliminate any deposits formed during freezing or thawing, the sample was centrifuged first (20,000g). Next, 50 μ L of the supernatant was mixed well with 50 μ L of Laemmli sample buffer (Bio-Rad Laboratories, Hercules, CA, USA) containing 5% β -mercaptoethanol (Sigma, MO, USA). The sample was boiled for 5 min in a Laemmli buffer system. After cooling at room temperature, the soybean protein extracts with an equivalent of 20 μ g of protein and 4.5 μ g of β -conglycinin and 2.5 μ g of glycinin as controls were loaded onto gels. A broad-range protein molecular weight Precision Plus Protein standard was used (Bio-Rad Laboratories, USA). The gels were run for 50 min (175 V). For electrophoresis, the gel in water/methanol/acetic acid (2:5:5) was soaked for 0.5 h and dyed with Coomassie Brilliant G-250 overnight. Next, the gel was washed three times with 10% acetic acid solution for 5 min each time, followed by a final wash with distilled water. The molecular mass and band strength of each soy protein sample were recorded using an image workstation (Kodak 440 CF, USA).

2.8. Western blot procedures using human plasma

For western blotting, the control was the same as that used in SDS-PAGE. First, 40 mL of pH 8.3 blotting buffer containing 4 mL of methanol, 50 mM Tris base, and 384 mM glycine was prepared, and the unvarnished gel was immersed in it for 3 min. Next, a sponge, filter, gel, Immun-Blot PVDF membrane, filter, and sponge were applied sequentially while avoiding the formation of air bubbles. The western blotting sandwich was transferred at 110 V for 1 h. Thereafter, the membrane was incubated in a buffer containing 5% skim milk powder (SMP) and TBS containing 0.01% Tween (TBST) for 60 min at room temperature, followed by incubation overnight in 20% soybean-positive plasma from donors (Plasma Lab International, Everett, WA; 32.8 IU/mL of soybean allergenic IgE and 19.0 IU/mL of corn allergenic IgE) with TBS containing 3% BSA and 0.1% Tween at 4 °C. The membrane was rinsed 3 times in TBST and then was incubated in goat anti-human IgE alkaline phosphatase (Bethyl Lab, Inc., Montgomery, TX, USA) diluted 1000-fold with 3% SMP in TBST for 60 min at 20 °C. The membrane was washed again and prepared for detection using an Immun-Star Chemiluminescence Alkaline Phosphatase substrate solution (Bio-Rad Laboratories, USA). The intensity of the strip was observed using an imaging system after color development.

2.9. IgE Immunoreactivity by immunoassay competitive antigen-coated indirect ELISA

The soybean IgE immunoreactivity was determined using plasma from donors who are allergic to soy and corn using the enzyme-linked immunosorbent assay (ELISA). First, 0.1 mL of the protein extracts was diluted to different concentrations with phosphate-buffered saline (PBS), dropped onto an immunoassay plate, and stored at 4 °C for 12 h. The process was repeated three times. Next, 250 μ L of TBS solution containing 1% Tween-20 and 5% BSA was added to the unused wells

for 60 min at 20 °C. The plate was washed 4 times in pH 7.4 PBS using an automatic strip washer (Elx50, Biotek; VA, USA) before the next step. Next, 0.1 mL (1:5 in 1% BSA) of donated plasma with specific IgE (32.8 IU/mL of soybean allergenic IgE and 19.0 IU/mL of corn allergenic IgE) was added to each well of the immunoassay plate, which was incubated at 37 °C for 60 min and rinsed again; treatment with buffer only was used as a negative control. Next, 0.1 mL of goat anti-human IgE alkaline phosphatase diluted 1000-fold with 1% BSA in antibody buffer was added to each well, and then the plate was incubated for 60 min at 20 °C, followed by removal of the unbound antibody by rinsing. Thereafter, 0.1 mL of the chromogenic agent (*p*-nitrophenyl phosphate) was applied to each well, and the bound secondary antibody was detected at room temperature after 45 min. The chromogenic process was ceased after the addition of 3 N NaOH. The absorbance was measured at 405 nm using an ultra-micro automatic reader (Elx80810; Biotek, Winooski, VA, USA), and the data were analyzed using Microsoft Excel™. The values are presented as OD absorbance readings per gram of protein and OD values per gram of product.

2.10. Circular dichroism spectroscopy

Far ultraviolet circular dichroism (Far-UV CD) spectroscopy was performed using a JASCO (model J-715; Tokyo, Japan) spectropolarimeter (Welltech Enterprises, Inc.) with a water bath and temperature controller. The measurements were performed in a 20-mm quartz cuvette covered with Teflon at room temperature (20 °C). Soluble protein was extracted from the sample at a density of 1.5 mg/mL using pH 8.2 Tris-HCl buffer. The sample was scanned in the far-UV region from 190 to 260 nm an average of 50 times, while the buffer solution was used as a control. The specific parameters were as follows: scanning speed: 200 nm/min; bandwidth: 5.0 nm; sensitivity: 50 mdeg; response: 0.25 s; step resolution: 0.5 nm. The protein secondary structure estimation was conducted as previously described by Tang (Tang & Wang, 2010).

2.11. Physical properties

The expansion ratio was obtained by gauging the ratio of the diameter of the snack to that of the die (2 mm) (Rong-Fei et al., 2008). The diameter of each sample was measured using a Vernier caliper (Mecanic 6911; Bel-Art Products, Pequannock, N.J., U.S.A.) for an average of 30 measurements.

The specific volume (SV) of the snack was measured by the rapeseed replacement method according to the method 10-05 published by the American Association of Cereal Chemists (Quinton & Kennedy, 2000). A container with a volume (V_1) of 320 mL was filled with rapeseeds, and the rapeseed density was determined. The extrudate was placed in the empty container, and the weight of the container and extrudate was recorded. The free volume of the container (V_2) was filled with rapeseeds, and the weight was recorded. The above process was performed in duplicate. The specific volume was calculated using Eq. (1):

$$SV = \frac{V_1 - V_2}{W} \quad (1)$$

The measurement of the water hydration capacity (WHC) was based on the method by Quinn and Paton (1979) with modifications. The samples were first ground in a mill (3383L10; Wiley® Mini Mill, Thomas Scientific, Swedesboro, NJ) and passed through a 40-mesh filter. Five grams of the sample was ground and placed in a 50-mL centrifuge tube, and the weight (W_1) was recorded. Next, 25 mL of distilled water was added to the centrifuge tube, hydrated for 60 min and stirred every 10 min. The sample was then centrifuged at 5000g for 20 min at 4 °C (J2-HS; Beckman Coulter, Inc., Schaumburg, IL, U.S.A.). After removing the supernatant, the tube weight (W_2) was recorded with the precipitate. The above process was performed in duplicate. WHC was calculated using Eq. (2):

$$WHC(\%) = \frac{W_2 - W_1}{5.0} \times 100\% \quad (2)$$

The sample texture was analyzed using a texture analyzer (TA-XT2i; Texture Technologies Corp., Scarsdale, USA) with a blade probe. Compressive force was gauged using a piece of the sample at a probe speed of 2.0 mm/s with 90% compression. The energy required to cut the sample was obtained by calculating the peak area of the force-time curve. Measurements were based on 10 replications.

Color was determined using a Labscan 6000 colorimeter (Hunter Associates Laboratory, Inc., Reston, Va., USA). Ground samples were placed in a Petri dish and evaluated using the CIELAB 10°/D65 setting. Three parameters were measured: L* (100 = white; 0 = black), a* (+ = red; − = green), and b* (+ = yellow; − = blue). This step was repeated three times for each sample.

2.12. Statistical analysis

Statistical analysis was performed using the SAS 9.2 system to determine significant impacts and major interactions (SAS Institute, Inc.). Significance was judged according to $P < 0.05$.

3. Results and discussion

3.1. Soluble protein concentration

The content of soluble protein in the SPI-corn and SF-corn initial materials and extrudates are presented in Table 1. Extrusion processing significantly decreased the protein solubility of both SPI-corn (12.5%–22.4%) and SF-corn (11.5%–19.1%) extrudates compared with that of the SPI-corn (54.2%) and SF-corn (56.7%) initial materials. Thus, the protein solubility decreased after extrusion by > 58.7% in SPI-corn and by > 66.3% in SF-corn extrudates compared with the initial materials. Temperature had a marked impact on protein solubility ($P < 0.05$); a higher extrusion temperature resulted in higher extracted soluble protein concentration for both SPI-corn ($P = 0.048$) and SF-corn ($P = 0.002$) products. Moisture also had a significant effect on protein solubility, but the effect was different for SPI-corn and SF-corn products. Soluble protein in SPI-corn products obtained with 40% moisture was lower than with 20% moisture ($P = 0.0001$). For the SF-corn products, the soluble protein obtained with 40% moisture was higher ($P = 0.0004$). For the SPI-corn products, the screw speed showed no effect on the protein solubility of the products ($P = 0.078$). For the SF-corn products, soluble protein obtained under 400 rpm was 16.0%, higher than 13.8% obtained under 200 rpm ($P = 0.011$). Higher screw speeds shorten the extrusion time of the sample in the mold, resulting in higher pressure; thus, the results from the screw speed may illustrate that higher pressure dramatically decreases protein solubility in SF-corn products. Processing can influence protein solubility, such as high pressure and thermal processing, due to the denaturation of proteins, resulting in protein aggregation that will decrease protein solubility (Jiang, Xiong, & Chen, 2010; Kinsella, 1979; Moure, Sineiro, Domínguez, & Parajó, 2006; Petruccielli & Añón, 1996). Both hydrogen and disulfide bond formation play an important role in the aggregation of proteins during heat treatment. This aggregation increased the molecular mass and decreased solubility (Rangavajhyala, Ghorpade, & Hanna, 1997). No significant interaction effects occurred among temperature, moisture and screw speed on the protein solubility of SPI-corn and SF-corn products.

3.2. Immunoreactivity determined by indirect ELISA and Western blotting

The immunoreactivity of SPI-corn and SF-corn extrudates extracted with Tris-HCl pH 8.2 buffer and determined by indirect ELISA with human plasma obtained from the subject allergenic to both soybean and corn are shown in Table 2. The immunoreactive proteins were

Table 1

Effects of extrusion parameters on soluble protein concentration of SPI-corn and SF-corn extrudates.

Soluble protein (%) ¹		
Sample code	SPI-corn	SF-corn
Initial materials	54.2 ± 5.3	56.7 ± 4.7
1	20.0 ± 2.5	11.5 ± 2.3
2	22.4 ± 3.6	16.4 ± 2.5
3	14.9 ± 2.9	18.0 ± 2.8
4	15.5 ± 3.2	19.1 ± 3.4
5	18.8 ± 2.7	11.6 ± 1.8
6	21.8 ± 4.5	13.6 ± 4.3
7	12.5 ± 2.3	14.0 ± 4.6
8	12.6 ± 3.7	14.9 ± 2.5
Parameters effects		
Parameter	SPI-corn	SF-corn
T (°C) ²		
110	16.4 ± 5.2b ³	13.4 ± 3.5b
140	18.2 ± 4.3a	16.3 ± 3.9a
MC (%)		
20	20.7 ± 3.5a	13.3 ± 3.4b
40	13.9 ± 3.2b	16.5 ± 3.9a
SS (rpm)		
200	16.5 ± 3.9a	13.8 ± 3.9b
400	18.1 ± 5.5a	16.0 ± 3.7a
Parameters and interaction (P values)		
Parameter	SPI-corn	SF-corn
T	0.0476 ⁴	0.0022
MC	< 0.0001	0.0004
SS	0.0779	0.0108
T*MC	0.3046	0.1189
T*SS	0.9867	0.4465
MC*SS	0.1914	0.1995
T*MC*SS	0.5641	0.1981

¹ Values are composed of means and standard error of a triplicate from seven independent experiments;

² The temperature 110 °C represents the temperature of five zones along the barrel from inlet to die is 30, 75, 105, 105 and 110 °C respectively, and the temperature 140 °C represents the temperature of five zones along the barrel from inlet to die is 30, 75, 120, 130 and 140 °C respectively;

³ The different letters in a column, within each main effect, were significantly different ($P < 0.05$). Temperature (T); moisture content (MC), and screw speed (SS).

⁴ Bold numbers indicate statistically different.

expressed as OD absorbance values per kg of protein and OD absorbance values per kg of product. ELISA demonstrated that extrusion dramatically decreased the immunoreactivity of SPI-corn and SF-corn products. The immunoreactivity of SPI-corn and SF-corn products, when expressed per kg of product, was decreased between 53%–68% and 79%–86%, respectively. Moisture had a significant influence on the protein immunoreactivity of SPI-corn extrudates expressed as OD absorbance values per kg of product, while temperature had a significant influence on the protein immunoreactivity of SF-corn extrudates expressed as OD absorbance values per kg of protein. The other extrusion conditions showed no significant influence on both SPI-corn extrudates and SF-corn extrudates. MC-SS and T-MC-SS showed a noteworthy interaction influence on the protein immunoreactivity for SF-corn products when expressed as OD absorbance values per kg of protein. However, no significant parameter interaction effects were observed for SPI-corn products. The protein immunoreactivity of SPI-corn products was lower than that of SF-corn products for the same protein content.

Table 2

Immunoreactivity (OD) Determined by Indirect ELISA of Different Soybean Protein extrudates Extracted with Tris-HCl pH 8.2.

SPI-corn				SF-corn		
ELISA data						
Sample code	OD/kg protein	OD/kg product	Reduction ² (%)	OD/kg protein	OD/kg product	Reduction (%)
Initial material	3.57 ± 0.19 ¹	0.038 ± 0.002		2.60 ± 0.17	0.071 ± 0.006	
1	0.19 ± 0.04	0.016 ± 0.004	58	0.24 ± 0.08	0.010 ± 0.004	86
2	0.18 ± 0.04	0.016 ± 0.004	58	0.38 ± 0.12	0.015 ± 0.005	79
3	0.19 ± 0.08	0.012 ± 0.005	68	0.34 ± 0.11	0.015 ± 0.005	79
4	0.20 ± 0.10	0.013 ± 0.005	66	0.28 ± 0.08	0.013 ± 0.005	82
5	0.21 ± 0.06	0.018 ± 0.006	53	0.25 ± 0.09	0.012 ± 0.005	83
6	0.17 ± 0.04	0.017 ± 0.004	55	0.29 ± 0.08	0.013 ± 0.005	82
7	0.19 ± 0.06	0.015 ± 0.006	61	0.25 ± 0.09	0.014 ± 0.007	80
8	0.23 ± 0.08	0.015 ± 0.006	61	0.25 ± 0.06	0.014 ± 0.006	80
Parameter	OD/kg protein	OD/kg product	Parameter	OD/kg protein	OD/kg product	
T (°C)			T(°C)			
110	0.19 ± 0.07a ³	0.014 ± 0.005a	110	0.31 ± 0.11a	0.013 ± 0.005a	
140	0.20 ± 0.06a	0.014 ± 0.005a	140	0.26 ± 0.08b	0.013 ± 0.005a	
MC (%)			MC (%)			
20	0.19 ± 0.05a	0.017 ± 0.004a	20	0.29 ± 0.11a	0.012 ± 0.005a	
40	0.20 ± 0.08a	0.014 ± 0.005b	40	0.28 ± 0.09a	0.014 ± 0.005a	
SS (rpm)			SS (rpm)			
200	0.20 ± 0.06a	0.015 ± 0.005a	200	0.27 ± 0.10a	0.013 ± 0.005a	
400	0.20 ± 0.07a	0.015 ± 0.005a	400	0.30 ± 0.10a	0.014 ± 0.005a	
Parameter	OD/kg protein	OD/kg product	Parameter	OD/kg protein	OD/kg product	
T	0.563	0.105	T	0.031	0.979	
MC	0.339	0.013	MC	0.824	0.193	
T*MC	0.870	0.676	T*MC	0.732	0.914	
T*SS	0.739	0.799	T*SS	0.720	0.795	
MC*SS	0.150	0.758	MC*SS	0.019	0.106	
T*MC*SS	0.560	0.983	T*MC*SS	0.049	0.446	

¹ Values are composed of means and standard error of a triplicate from four independent experiments.² The reduction percentage means the decrease percentage of immunoreactivity per kg product.³ Different letters in a column, within each main effect, were significantly different ($P < 0.05$). Temperature (T); moisture content (MC), and screw speed (SS). OD indicates optical density that correlates directly with immunoreactivity. Bold numbers indicate statistically different.

These results may be useful for industry when determining the feasibility of raw materials for snacks.

Fig. 1A and B shows the comparison of SDS-PAGE and western blotting of different products after extrusion. Additionally, the products extruded under different conditions were compared using pure corn flour, glycinin and β -conglycinin as references. The 11S fraction of soy protein glycinin has been implicated as a major allergen. Glycine comprises six subunits containing disulfide-linked acidic and basic polypeptide chains (L'Hocine & Boye, 2007). As shown in Fig. 1, the acid chain of glycinin comprises Gly 1–5, which correspond to 35, 32, 38, 30 and 10 kDa, respectively, while the other chain corresponds to 18–19 kDa. The 7S protein β -conglycinin has been identified as another major soy protein allergen. The results showed that β -conglycinin comprises α , α' and β subunits of 75, 67 and 50 kDa, respectively. Another 7S allergenic protein, the 22-kDa Kunitz trypsin inhibitor (KTI), and 17 kDa 2S globulin were identified as allergenic proteins in SF and SPI from human plasma. Another allergen, lipoxygenase (102 kDa), was identified in SF but at a lower amount in SPI. In corn flour, Zea profilin, Zea m 1, γ -zein and Zea m 14 with molecular weights of 60, 35, 27, 9 kDa are sensitizing (Krishnan et al., 2010; Krishnan et al., 2011; Sung-Ho et al., 2005; Weichel et al., 2010). The results showed that the extruded materials, whether soybean or corn, were significantly reduced allergens compared with the raw materials. Additionally, western blotting indicated that squeezed SPI-corn and SPI1 have almost no allergic bands, while only a low intensity of the fraction bands of the β -conglycinin α' and α subunits, Gly2 and 2S-globulin were present in the SF-corn products.

The extruded materials, in general, possessed lower immunoreactivity than the original materials. Compared with SPI8/SF8, the extrusion conditions used in SPI1/SF1 reduced the immunoreactivity more effectively.

Fig. 1C and D shows the SDS-PAGE and immune response profiles of SPI-corn before and after extrusion under different conditions. The results indicate that different extrusion conditions have different effects on immunoreactivity in SPI-corn. For example, the immunoreactivity of a sample with a moisture content of 20% (samples 1, 2, 5, and 6) after extrusion was significantly lower than that of a sample with a moisture content of 40% (samples 3, 4, 7, and 8). Additionally, the allergens of 60 kDa in maize were removed thoroughly after extrusion under the condition of low moisture content, while other conditions had little effect on them. Concerning zein and γ -zein, all the processing conditions contributed to their removal.

The effect of extrusion on allergenic proteins in SF-corn products by SDS-PAGE and western blotting are shown in Fig. 1E and F, respectively. The processing conditions used for samples 1 and 2 (high temperature and low moisture) compared with those for samples 3 and 4 (high temperature and high moisture) indicate the benefits of low moisture in the reduction of immunoreactive proteins. The cause may be that faster evaporation under lower moisture than high moisture results in the destruction of the specific IgE of the allergenic protein under a higher temperature of the steam.

SPI products showed lower immunoreactivity of all the allergens than SF products. Regardless of the conditions, glycinin was more difficult to eliminate than β -conglycinin. Additionally, low moisture

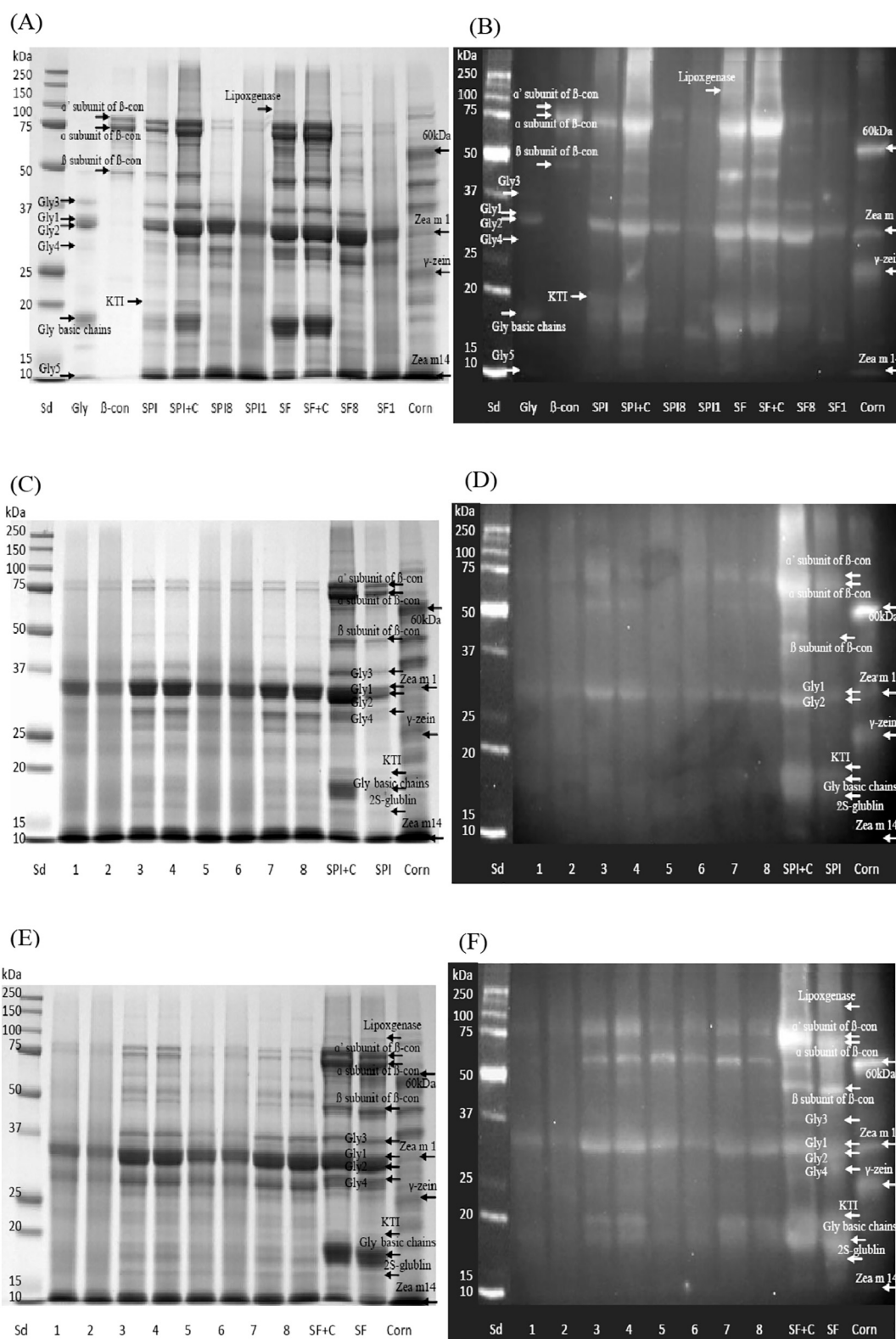


Fig. 1. SDS-PAGE electrophoretic and Western blot profiles of SPI, SF and corn and their extrudates. A, B. Comparison of the immunoreactivity of selected extrudates with materials extracted with pH 8.2 buffer. C, D. SPI-corn samples and control. E, F. SF-corn samples and control. SPI + C represent SPI-corn blends before extrusion; SF + C represent SF-corn blends before extrusion; SPI8 and SPI1 represent the SPI-corn extrudates, SF8 and SF1 represent the SF-corn extrudates, Numbers 1 and 8 represent the product number processed using the conditions indicated in [Materials and methods](#) section; Sd represents the precise standard of proteins; Gly represent glycinin; β-con represent β-conglycinin; KTI represent Kunitz tripsin inhibitor.

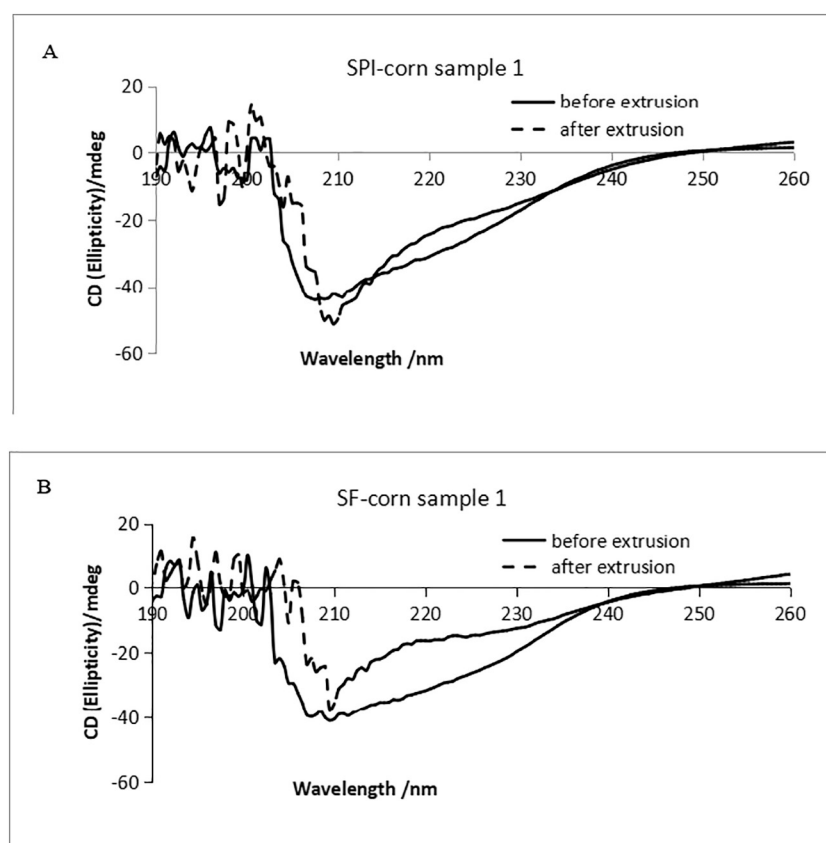


Fig. 2. CD spectra of SPI-corn sample 1 (A) and SF-corn sample 1 (B) obtained before and after extrusion. Protein concentration was 1.5 mg/mL. CD values are presented in ellipticity, mdeg. Read at 200 nm/min with 0.5 nm intervals, 5.0 nm bandwidth, 0.5 nm resolution, 50 mdeg sensitivity, 0.25 s response, and 1 accumulation. The inserted table presents the percentage of each secondary structure conformation obtained from CD spectra. SPI-corn sample 1 and SF-corn sample 1 were processed with the following parameters: the five temperature values represent the temperatures of five zones along the barrel from inlet to die: 30, 75, 120, 120, 140 °C, 20% moisture, 200 rpm screw speed. SPI-corn sample 8 and SF-corn sample 8 were processed with the following parameters: the five temperature values represent the temperatures of five zones along the barrel from inlet to die are 30, 75, 105, 105, 110 °C, 40% moisture, 400 rpm screw speed.

played a major role in the removal of KT1 and SS showed no effect. Therefore, the use of appropriate moisture and temperature levels in the production of soy foods has a significant impact on the sensitization of the final product. In this study, we observed the removal of allergens in the product by moisture and temperature.

3.3. CD spectra of the soluble protein fractions of samples before and after extrusion

Circular dichroism (CD) utilizes the high sensitivity of Far-UV CD spectra (below 240–250 nm) to different secondary structures in proteins and peptides and determines the structure of proteins based on the characteristic wavelengths and bands of different secondary structures. The CD spectra of different SPI-corn (Fig. 2A) and SF-corn (Fig. 2B) extrudates showed that the profile is significantly different before and after extrusion. The estimation of protein secondary structure indicated that the percentage of α -helix and β -strand protein structures in the soy initial materials used is distinctively different from that in the extrudates. For SPI-corn sample 1, after extrusion, the α -helix structure decreased from 9.5% to 2.2% while that of the β -strand increased from 36.6% to 46.6%. The same trend was also observed for SF-corn sample 1. After extrusion, the α -helix decreased from 10.5% to 2.5% while that of the β -strand increased from 32.4% to 45.0%. For both SPI-corn and SF-corn, the turn and random structure percentages of the extrudates did not change before and after extrusion.



Fig. 3. Pictures of final products of A. SPI-corn and B. SF-corn extrudates. Numbers 1 to 8 represent the product number processed using the conditions indicated in [Materials and methods](#) section.

Table 3

Effect of temperature (T), moisture content (MC), and screw speed (SS) on physical properties of SPI-corn and SF-corn extrudates.

	Parameter	Expansion ratio	Specific volume	WHC	Area-FT ²	Color		
			(mL/g)	(%)	(Ns)	L*	a*	b*
SPI-corn	T (°C)							
	110	5.86 ± 1.94a ¹	7.67 ± 5.54a	301.4 ± 43.60a	48.82 ± 51.25a	69.32 ± 1.11b	6.89 ± 0.57a	33.66 ± 2.76a
	140	5.47 ± 3.29b	7.77 ± 5.25a	284.5 ± 35.69b	36.71 ± 34.96b	69.98 ± 1.61a	6.76 ± 0.47a	31.92 ± 2.65b
	MC (%)							
	20	7.48 ± 2.80a	12.71 ± 2.21a	325.3 ± 30.29a	20.07 ± 9.50b	69.42 ± 1.40a	6.92 ± 0.45a	31.05 ± 2.32b
	40	3.85 ± 0.49b	2.72 ± 0.44b	260.6 ± 15.10b	65.46 ± 52.81a	69.87 ± 1.41a	6.73 ± 0.58a	34.53 ± 2.12a
	SS (rpm)							
	200	5.87 ± 3.38a	6.82 ± 4.64b	293.7 ± 41.60a	51.80 ± 53.92a	69.16 ± 1.42b	6.84 ± 0.55a	32.90 ± 2.07a
	400	5.46 ± 1.78b	8.62 ± 5.93a	292.2 ± 39.90a	33.74 ± 29.17b	70.13 ± 1.24a	6.82 ± 0.50a	32.68 ± 3.45a
	T (°C)							
SF-corn	110	3.65 ± 1.16a	4.08 ± 2.56b	201.7 ± 24.74b	54.80 ± 71.55a	72.55 ± 2.88a	6.23 ± 1.19b	31.52 ± 4.67a
	140	3.53 ± 0.59b	4.92 ± 2.38a	225.2 ± 15.17a	27.25 ± 16.52b	69.96 ± 2.57b	7.02 ± 1.31a	31.95 ± 2.99a
	MC (%)							
	20	4.30 ± 0.65a	6.77 ± 1.13a	224.0 ± 13.82a	26.75 ± 19.23b	69.48 ± 2.06b	7.42 ± 0.92a	31.49 ± 2.08a
	40	2.88 ± 0.53b	2.24 ± 0.62b	202.8 ± 26.54b	55.30 ± 70.67a	73.03 ± 2.76a	5.84 ± 1.15b	31.98 ± 5.14a
	SS (rpm)							
	200	3.70 ± 0.99a	3.98 ± 1.98b	211.1 ± 23.83a	49.84 ± 65.44a	72.01 ± 2.92a	6.37 ± 1.30a	31.04 ± 3.80a
	400	3.48 ± 0.84b	5.02 ± 2.85a	215.8 ± 23.46a	32.21 ± 36.54b	70.50 ± 2.95b	6.89 ± 1.27a	32.43 ± 3.92a

¹ Values are composed of means and standard error, and different letters in a column, within each main effect, were significantly different ($P < 0.05$). WHC= Water hydration capacity;

² FT = Force time (Ns = Newton second). Temperature (T); moisture content (MC), and screw speed (SS).

3.4. Physical properties

3.4.1. Expansion ratio

Fig. 3 represents all the extrudates obtained after processing. The products are more brittle and swellable under low moisture conditions (samples 1, 2, 5 and 6) than those under high moisture conditions (samples 3, 4, 7, and 8). Additionally, at higher moisture and lower temperatures, the product shrinks and the texture is very hard (samples 7 and 8). At the moment of squeeze out at a high temperature, the rapid evaporation of water makes the product expand and have good brittleness. The effects of T, MC, and SS on the physical characteristics of SPI-corn and SF-corn extrudates are shown in Tables 3 and 4; significant interactions were found between different conditions regarding the qualities of the extrudates ($P < 0.05$).

The numbers in bold indicate significantly different expansion ratios. Temperature, moisture and screw speed significantly affected the expansion ratio of the products ($P < 0.05$). After increasing the temperature, the humidity and screw speed led to a significant reduction in the rate of expansion for both SPI-corn and SF-corn extrudates, with moisture being the dominant parameter in the expansion ratio ($P < 0.0001$). The moisture increased from 20% to 40%, and the

expansion rate decreased by 48% from 7.48% to 3.85% for SPI-corn extrudates and by 33% from 4.30% to 2.88% for SF-corn extrudates. For SPI-corn extrudates, MC-SS and T-MC-SS showed an interaction with the decreased ratio of expansion. However, for SF-corn extrudates, T-SS and T-MC-SS showed a prominent interaction.

3.4.2. Specific volume (SV)

Moisture and SS were much more significant parameters regarding SV than temperature for both SPI-corn and SF-corn products. Temperature had no remarkable effect on the specific volume of SPI-corn products. However, for SF-corn extrudates, the increase in temperature caused a notable increase in the specific volume from 4.08 mL/g to 4.92 mL/g. For both SPI-corn and SF-corn extrudates, the MC and SS had a noteworthy effect on the specific volume ($P < 0.0001$). Increasing the moisture from 20% to 40% caused a 78% reduction in the specific volume from 12.71 mL/g to 2.72 mL/g for SPI-corn extrudates and a 67% decrease from 6.77 mL/g to 2.24 mL/g for SF-corn products. Increasing the screw speed from 200 rpm to 400 rpm resulted in a 26% increase in the specific volume from 6.82 mL/g to 8.62 mL/g for SPI-corn extrudates and a 28% increase from 3.98 mL/g to 5.02 mL/g for SF-corn extrudates. For both SPI-corn and SF-corn

Table 4Interaction results for temperature (T), moisture content (MC), and screw speed (SS) on physical properties of extruded extrudates (P values).

	Parameter	Expansion ratio	Specific volume	WHC	Area-FT	Color		
			(mL/g)	(%)	(Ns)	L*	a*	b*
SPI-corn	T	0.0319	0.7780	0.0175	0.0299	0.0413	0.2609	0.0012
	MC	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.1606	0.1013	< 0.0001
	SS	0.0243	< 0.0001	0.8234	0.0013	0.0036	0.8493	0.6571
	T*MC	0.7340	0.4639	0.6557	0.1159	0.0039	0.7504	0.7345
	T*SS	0.1141	0.6968	0.6324	0.4358	0.3100	0.0050	0.0412
	MC*SS	0.0091	0.0031	0.8547	0.0003	0.0445	0.0002	0.0043
	T*MC*SS	0.0044	0.0009	0.9183	0.0013	0.0011	< 0.0001	0.0008
	T	0.0090	< 0.0001	< 0.0001	0.0004	< 0.0001	0.0055	0.6592
SF-corn	MC	< 0.0001	< 0.0001	< 0.0001	0.0002	< 0.0001	< 0.0001	0.6237
	SS	< 0.0001	< 0.0001	0.1898	0.0212	0.0096	0.0584	0.1615
	T*MC	< 0.0001	0.3289	< 0.0001	0.0119	0.9940	0.6174	0.1536
	T*SS	0.0624	0.8295	0.7712	0.0314	0.5473	0.9688	0.6749
	MC*SS	0.2192	0.0005	0.2410	0.8576	0.6407	0.9376	0.0045
	T*MC*SS	< 0.0001	< 0.0001	< 0.0001	0.0081	0.3596	0.3742	0.0030

Bold numbers indicate statistically different.

extrudates, MC-SS and T-MC-SS affected the specific volume ($P < 0.05$).

3.4.3. Water hydration capacity (WHC)

The WHC of the SPI-corn and SF-corn extrudates was affected by both the temperature and moisture but not by the screw speed. Moisture was a dominant factor on WHC because the moisture changed from 20% to 40%, resulting in a significant decrease in the WHC from 325.3% to 260.6% for SPI-corn extrudates and from 224.0% to 202.8% for SF-corn extrudates ($P < 0.0001$). The SPI-corn extrudates were hydrated more than the SF-corn extrudates.

3.4.4. Texture (area-FT)

The energy required to cut the samples indicated that all the extrusion conditions (T, MC and SS) had a significant influence on the texture of the SPI-corn and SF-corn extrudates ($P < 0.05$). A higher temperature and screw speed and lower moisture caused obvious changes in the required energy to cut extrudates, a finding that is related to the crunchiness of the samples. For the SPI-corn extrudates, a significant interaction occurred between MC-SS ($P = 0.0003$) and T-MC-SS ($P = 0.0013$) on the texture was shown. For SF-corn extrudates, the effects of T-MC ($P = 0.0119$), T-SS ($P = 0.0314$), and T-MC-SS ($P = 0.0081$) on the texture were significant.

3.4.5. Color

For SPI-corn products, a higher screw speed and temperature showed a marked increase in whiteness (L^*), while moisture showed no significant effect. A higher temperature and screw speed increased the lightness of SPI-corn products. All the parameters showed a significant influence on the redness (a^*) ($P > 0.05$). A higher temperature and lower moisture showed a significant decrease in yellowness (b^*), while the screw speed showed no significant effect.

For SF-corn products, a higher temperature and screw speed and lower moisture showed a significant decrease in whiteness (L^*). A higher temperature and lower moisture showed a significant increase in redness (a^*), while the screw speed showed no notable effect. Additionally, three conditions did not affect the yellowness (b^*) ($P > 0.05$).

Many reactions in the extrusion process affect the color of the product, such as the Maillard reaction, caramelization, hydrolysis, and pigment degradation (Camire, Camire, & Krumhar, 1990). Thus, several reasons explain why the temperature and screw speed show a reverse influence on the whiteness of SPI-corn and SF-corn products. Color is a very important part of food, and it is closely related to the quality and acceptance. Thus, the measurement of the color of the extrudate is an important physical indicator (Patil & Fanta, 1995).

4. Conclusions

In this study, the extrusion process proved to be a good method of food processing to decrease the activities of allergenic proteins in both SPI-corn and SF-corn products. Moisture plays a major role in reducing the immunoreactivity of SF-corn products while that of SPI-corn products is determined by temperature. Additionally, under any condition, the removal of β -conglycinin is better than that of glycinin, and the 60-kDa allergen in corn can be completely removed. Extrusion processing decreased the α -helix structure and increased the β -strand structure in both SPI-corn and SF-corn products. T-MC-SS had a notable effect on the expansion rate, WHC and texture properties of the SPI-corn and SF-corn products ($P < 0.005$). The increase in moisture significantly decreased the expansion rate, specific volume and WHC, and increased the area-FT value for both SPI-corn and SF-corn products. Products 1 and 2 were more acceptable based on the physical properties and absence of allergenic proteins regardless of the starting raw materials.

Declaration of competing interest

"There are no conflicts to declare".

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Author statement

Huanyu Zheng: Methodology, Investigation, Resources, Writing-Review & Editing, Funding acquisition.

Guosen Yan: Investigation, Writing - Original Draft, Software.

Susan Marquez: Validation, Formal analysis.

Youngsoo Lee: Data Curation.

Christina Alcaraz: Resources.

Elvira Gonzalez de Mejia: Conceptualization, Methodology Resources.

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