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Research Article

Influence of feed moisture content and die temperature on the allergenic potency of extruded soy proteins

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Abstract

The influence of feed moisture content (FMC) on soy allergenicity was examined across different levels of die temperature (DT). A 3^2 Factorial Design was used, which required 9 experiments for the two factors. FMC (w: v) was set at; 20 %, 40% and 60%, while DT was varied at 80, 100 and 120°C. The molecular properties of soy proteins were studied using Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis (SDS-PAGE) and immunoreactivity estimated by immunoblotting and Enzyme Linked Immunosorbent Assay (ELISA) using soy total antibodies. SDS-PAGE showed that protein degradation occurred in samples extruded at DTs - (100°C and 120°C), across all levels of FMC. However, the best treatment combination was at DT-120°C and FMC-60%, with complete band degradation, no membrane staining and the highest ELISA IC_{50} value. The degraded bands were found to correspond mainly to β-conglycinin and to some sub-units of glycinin and P34. According to the IC_{50} values, there was a significant difference ($P < 0.05$) in immunoreactivity between treatments at FMC – (20% and 60%) and (40% and 60%) and no significant difference between treatments at FMC (20% and 40%). For the DTs, there was a significant difference between treatments at DT – (80°C and 120°C) and (100°C and 120°C) and no significant difference between treatments at DT – (80°C and 100°C). While FMC had a greater effect on IC_{50} values than DT, increasing FMC alone did not lead to significant differences in IC_{50} among treatments. It was concluded that DTs above 100°C lead to reduction of soy allergens and increasing FMC contributes to overall reduction in soy allergenicity.

Keywords: soybean, protein denaturation, SDS-PAGE, ELISA, textured soy protein immunoreactivity, Uganda, China.

Introduction

Soybean is an affordable, high quality protein source available for human consumption [1]. The soybean plant (*Glycine max*) originated in China, and was introduced to the American colonies in 1765 [2]. Soy extrusion is a process widely applied to soy protein owing to its unique characteristics that allow it to be processed to produce a variety of products of different form, texture and organoleptic properties, tailored to market demand [3, 4]. Extrusion cooking is a high-temperature, short-time process in which moistened, starchy and/or proteinaceous food materials are plasticized and cooked in a tube under pressure and mechanical shear resulting in molecular transformations and chemical reactions [5]. Owing to its unique nutritional and health benefits, as well as functional properties, there is high chance of soy being a hidden ingredient in food products and thus its consumption may be unavoidable.

The major safety concern with soy is its allergenicity [6, 7]. Studies have shown that there are 8 major food products that account for 90% of all immunoglobulin E (IgE)-mediated food allergies. These products include; cow's milk, eggs, fish, crustaceans, peanuts, soybeans, tree nuts and wheat [8]. The prevalence of food allergy has increased significantly among children in the last 10–15 years, particularly in developed countries such as the United States [9]. Soy allergy is not a major problem in Asia [10], however, the increased demand of Asian food products on the global market makes it necessary to ensure all measures are taken to produce hypoallergenic soy products to meet the standards of importing countries. Also, because soy protein is increasingly being used in a variety of food products, [11], it can be predicted that this increased exposure may result in increased hypersensitivity in the general population. Soybean allergens comprise proteins with molecular masses from 7.0 to 71 kDa, although only a few of these proteins are responsible for a majority ($\sim$90%) of the allergenic responses, making it important to identify these key proteins [8]. Soy storage proteins glycinin (11S) and β-conglycinin (7S) and immuno-dominant protein P34 (Glym Bd 30k) are recognized as the major allergenic proteins in soybean [8]. Extrusion has been investigated and found to play a role in attenuation of soy allergenicity [6, 7], thus making it a fundamental processing technique.

According to Chen *et al.*, [12] parameters that are of importance in the extrusion process can be categorized into three major groups, namely, process parameters (including screw speed, moisture content, barrel temperature, screw configuration, die dimension, raw material characteristics, etc.), system parameters (including energy input, residence time, etc.) and product properties (including colour, nutrition, texture, taste, etc.). All these parameters have been studied either individually or in combination for their effect on functional and nutritional properties of soy [12, 13]. However, apart from the DT and screw shape, there is apparently no available research that has investigated the effect of FMC in combination with DT on the allergenicity of soy protein. Therefore, this research sought to expand the earlier studies by investigating the influence of FMC across different DTs, on the allergenic potency of soy flour during twin-screw extrusion.

Materials and Methods

Raw materials

Biological materials

Defatted soybean meal containing about 48.45% crude protein and 7.17% ash was procured from Qinhuangdao Jinhai Food Industry Co., Ltd. A total of 3 newly weaned healthy male New Zealand white rabbits, each weighing approximately 2 kg were obtained from Zhenhu Experimental Animal Science and Technology Co. Ltd. of Suzhou. The rabbits had never been fed on soybean meal or soy protein diet. Goat anti-rabbit horseradish peroxidase (HRP) labeled antibody IgG was obtained from Sigma-Aldrich, St. Louis, MO, US.

Chemicals

Potassium dihydrogen phosphate, dodecahydrate, disodium hydrogen phosphate, sodium chloride, potassium chloride, Coomassie Brilliant Blue G-250, phosphoric acid, propylene amide (AR), N, N'-methylenebis propylene amide (AR), Tris, ammonium persulfate, TEMED, SDS, Tris-glycine electrophoresis buffer, mercaptoethanol, Tris-Hcl, dithothetol (DTT), glycerol and bromophenol blue were all obtained from Sinopharm Chemical Reagent Co., Ltd, China. Nitrocellulose (NC) membrane (pore size 0.45 μ m), Solarbio, Shanghai, China), 3,3' -0-diaminobenzindin tetrahydrochloride (DAB) substrate, Tween 20, Bovine Serum Albumin (BSA), and TMB substrate solution were obtained from the Swiss top Chemical Technology (Shanghai) Co.; the EL-TMB chromogenic reagent and Freund's complete adjuvant from Shanghai Sangon Co., Ltd. All reagents were of analytical grade.

Feed preparation

The defatted soybean flakes were ground into flour with a grinder mill (Model 6, Eka Technology, Hongkong) to pass through a 60-mesh sieve. The flour was dried in an open-air oven (DZF - 6030A, Shanghai, China) overnight at 60°C to attain constant moisture content. The initial moisture content was adjusted to the desired values of 20%, 40%, and 60% (v: w) using distilled water with a Kitchen Aid Mixer (Kitchenaid, KP26M1XER, USA). The samples were stored in sealed plastic bags for 16 h at 10°C prior to extrusion.

Extrusion operation

Extrusion was carried out in a pilot-scale twin-screw extruder (HAAKE PolyLab System), Model PTW 24/25D (USA, Thermo Electron Corporation), which had two co-rotating and intermeshing screws. The extruder had a screw length of 600 mm and a die head with circular dual-orifice holes of 3.0 mm diameter. The extruder barrel was segmented into three temperature-controlled zones, which are electrically heated and cooled with running water. Samples were fed to the extruder with a twin-screw volumetric feeding system, Model DDSR20N – (PRISM, Germany).

Experimental design

A 3^2 Factorial Design (Table 1) was used to investigate the effect of die temperature and feed moisture content. Based on studies by Chen *et al.* [14], the feed moisture contents were selected at 20% (low moisture), 40% (inter-mediate moisture) and 60% (high moisture) (w: v) and the cooking temperatures at the die zone of the extruder were set at 80, 100 and 120°C. The experimental design resulted in a total of 9 treatments, as shown in Table 2**.** The feed rate and screw speed were fixed at 20g/min and 120rpm, respectively. The temperatures of the other 2

zones were kept at 60°C and 80°C from feeding zone to die zone respectively. After extrusion, the extrudates were dried and ground to powder using 60-mesh sieve before further use.

Table 1. 3^2 Factorial experimental design for extrusion operation.

Factors	Levels
Feed moisture content (%)	20, 40, 60
Die temperature of the extruder (°C)	80, 100, 120

Protein extraction

To extract protein from both raw/untreated sample (R) and extruded samples, one gram of soy flour was homogenized with 10 ml phosphate-buffered saline (PBS) - (pH 7.4, 0.01 mol / L): potassium dihydrogen phosphate, 0.2 g; dodecahydrate, disodium hydrogen phosphate, 2.9 g; sodium chloride 8.0 g; potassium chloride 0.2g. The homogenate was extracted for 2 h at 37°C and then centrifuged at 4°C for 40 min at 600g (Eppendorf 5804R, Germany). Protein content of the supernatant from each sample was determined.

Total soluble protein quantification

Protein concentrations were determined relatively according to the method described by Bradford, [15] using bovine serum albumin as standard and Bradford reagent consisting of Coomassie Brilliant Blue G-250 and phosphoric acid. The absorbance was read at 595 nm. Total soluble protein concentration of samples was quantified based on the BSA standard curve ($y = 0.8184x+0.6183$, $r^2 = 0.994$) where y = Optical Density and x = Protein concentration. The concentration of protein in the extracts is shown in Table 2. The extracts of samples were stored at 18°C until further use.

Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis (SDS–PAGE)

The electrophoretic separation of proteins was performed in reducing conditions according to Saitoh *et al.*, [6] on 12% acrylamide gels using the BioRad Miniprotean II system (BioRad, Richmond, C.A.). Protein samples (20 µl) of equal concentrations were each mixed with 40 µl of loading buffer (250mM Tris-Hcl, Ph-6.8; 500mM dithothetol (DTT); 10% SDS; 50% glycerol and 0.5% bromophenol blue). Before electrophoresis, all samples were boiled for 5 min and vortexed for 3 min. A low molecular weight marker ranging from 97.4 -14.4 kDa (Sigma-Aldrich) was used. After running, the gels were stained with Coomassie Brilliant Blue G-250 (Sinopharm Chemical Reagent Co., Ltd, China).

Production of rabbit polyclonal antisera against native soy protein

The immunization schedule and methods of immunization via the multiple-injection technique were according to the method previously described by Morishita *et al.*, [16]. Briefly, two rabbits were each injected with native soy protein (2.0 mg mL^{-1}) in phosphate buffer (0.01 M, pH 7.5) containing 0.85% NaCl (PBS), emulsified with 2.0 mL of Freund's complete adjuvant. For booster injections, immunogen (1.0 mg mL^{-1}) in PBS and 2.0 mL of Freund's incomplete adjuvant were used for each rabbit. There was a 4 week interval between the first injection and the booster injection, followed by a 2 week interval for the subsequent booster injections for 8 weeks. The collected antisera were precipitated with $(NH_4)_2SO_4$ to a final saturation of 35% by mixing 2 mL of antisera with 1 mL of saturated $(NH_4)_2SO_4$ solution. The precipitates were dissolved in water and precipitated twice. Finally, the precipitates were reconstituted to half of the original volume

with distilled water, dialyzed against distilled water for 1 h and by phosphate buffer (0.01 M, pH 7.5) overnight. Dialysis was carried out at 4°C and the dialysate was stored at -20°C prior to further analysis.

Immunoblotting with polyclonal antibody

The NC membrane with the transferred proteins was blocked with 2% BSA-TBS advance blocking agent in TBS with 1% Tween-20 (TBST) for 16 h at 4°C, washed four times with TBST, and incubated with 1: 200 rabbit polyclonal antibody for 1 h at 37°C. The membrane was washed 4 times with TBST, incubated with 1:1000 goat anti-rabbit IgG HRP for 2 h, washed four times with TBST, and detected using 3,3' -0-diaminobenzindin tetrahydrochloride (DAB) (following manufacturer's instructions).

Indirect competitive ELISA procedure

Samples of raw or standard soy protein were diluted (1 μg mL^{-1}) in phosphate buffer (0.01 M, pH 7.5), an aliquot (100 μl) was added to the wells of the microtiter plates (Sigma-Aldrich) which were subsequently incubated at 4°C for 18 h. The residual free binding sites were blocked with 200 μl of 1% BSA in PBS per well for 2 h. Serial dilutions of samples of raw and extruded protein were diluted in PBS buffer starting with 5mg mL^{-1} to obtain eight dilutions (5:1, 5:10, 5:100, 5:1000, 5:10000, 5:100000, 5:1000000, 5:10000000) of which 50 μl were added to the wells. 50 μl of primary antibody (rabbit IgG) was also added to the wells however, some wells were only filled with 100 μl of primary antibody to obtain the non-competitor samples and maintained for 1 h at 37°C. After four washes with PBS-Tween 20, 100 μl of HRP-labelled goat anti-rabbit IgG was added to the plate and incubated for at 37°C for 1 h. After five washes, the bound IgG content was measured using TMB substrate of which 100 μl of substrate was added to wells and incubated in the dark for 15-30 min. The reaction was stopped by addition of 50 μl of 2 M sulphuric acid per well. The absorbance at 495 nm was determined on a microtiter automated ELISA plate reader (Thermo Multiskan MK3, USA). Each sample was analysed in duplicate. The results obtained were used to construct competitive curves for each sample.

Calculation of inhibition concentration (IC) values

$IC = (1-B/B_0) \times 100\%$

Where: B is the Sample Optical Density

B_0 is the Non - Competitor Optical Density

IC_{50} = IC at 50%.

Statistical analysis

Analysis of variance, significant differences among means, and correlation analysis were performed by use of IBM SPSS Statistics 19 (SPSS Inc., Chicago, USA). Duncan's multiple range tests was used to identify the significant difference of each treatment and the values were considered to be significantly different at $p < 0.05$.

Results and Discussion

Protein content of soy samples

Table 2. Extracted protein concentration and extrusion variables for soy samples.

Sample run/label	Die temperature (°C)	Feed moisture content (%)	Extracted protein (mg of protein per g of product)
R	*	*	300
1	80	20	280
2	100	20	58
3	120	20	95.7
4	80	40	280
5	100	40	46
6	120	40	280
7	80	60	280
8	100	60	260
9	120	60	130

* Not applicable. R represents untreated sample (Raw)

Table 2 presents the concentration of extracted protein and the various extrusion conditions of the 9 treatments. The extractable protein concentration of raw soybean was 300 mg of protein/g of soy flour, while that of the extruded products ranged from 46 to 280 mg of protein/g of extruded soy flour. The lowest extractable protein concentration for the extruded samples was 6 times lower than that of raw soy. This phenomenon may be explained by the report of MacDonald *et al*., [3] that increasing feed moisture content during extrusion was found to increase the interactions between disulfide bonds and hydrogen bonds and between disulfide bonds and hydrophobic interactions.

Protein separation by SDS-PAGE

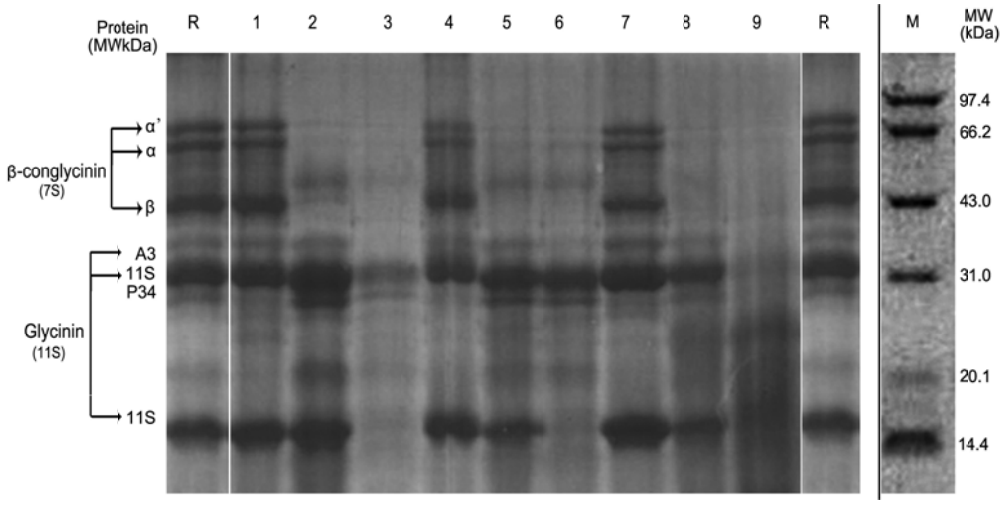

Figure 1. SDS-PAGE separations of extruded soy samples. Lane R represents the raw unextruded sample (control) while lanes 1 to 9 represent the different treatment combinations (die temperature and feed moisture content).

SDS-PAGE separations of the extracted soy proteins from both the raw and extruded soy flour samples are shown in Figure 1. Band degradation was clearly visible in several lanes across all levels of FMC, specifically in lanes corresponding to DT of 100°C and 120°C. This result indicates that regardless of the FMC, a high DT is important for degradation of protein bands during extrusion.

Figure 2 shows a comparison of the intensity in band degradation of treatments at the 3 levels of FMC (20%, 40% and 60%). There was visible band degradation at all levels of FMC with the exception of samples processed at the lowest DT of 80°C. In fact the protein bands of samples extruded at DT of 80°C across all FMC levels were similar to those of the raw sample. Bands with molecular weights of approximately 30kDa were degraded in lanes corresponding to DT 100°C and 120°C across all FMC levels, with the highest intensity in the sample treatment at DT-120°C. This result is supported by Ohishi *et al.*, [7], who reported that high molecular weight fractions, all exceeding 30kDa were reduced in proportion to the rise in die exit temperatures of 70-134°C. However, in this study, band degradation was only observable in samples extruded at 100°C and above. No band degradation was seen at 80°C across all levels of FMC. The discrepancy in these results could be due to the differences in other extrusion parameters such as feed particle size and screw shape, which also contribute to overall band degradation [6].

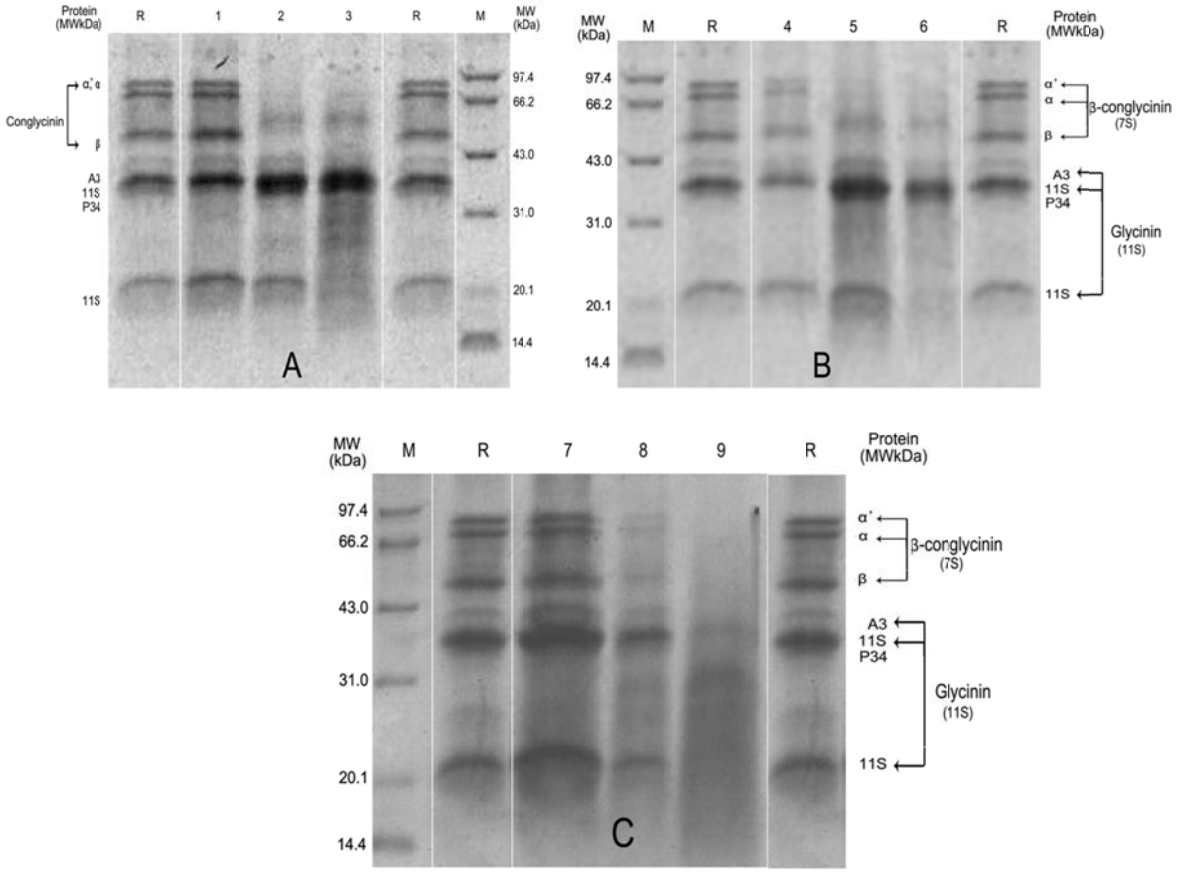

Figure 2. The intensity of soy protein band degradation across the different FMC levels (A: 20%, B: 40 %, and C: 60%), lane R represents the raw unextruded sample (control) while lanes 1 to 9 represent the different treatment combinations (die temperature and feed moisture content).

The degradation of bands in lanes 3 (DT - 120°C and FMC - 20%), 6 (DT - 120°C and FMC - 40%), and 9 (DT - 120°C and FMC - 60%) corresponds to the higher molecular weight allergens 7s (β-conglycinin): (50-76kDa), some sub units of 11s (glycinin): (12-45kDa) and P34: (31-34kDa). While there was some minimal band degradation in lanes corresponding to DT-100°C: (2, 5, 7) and DT-120°C: (3 and 6) across all FMC levels, there were some resistant bands, especially in the region corresponding to glycinin (12-45kDa) and P34 (31-34kDa). The disappearance of some bands with increasing DT could be attributed to the findings of Lin *et al.*, [13] that both 7S and 11S subunits of soy proteins, major components for texturization, start to unfold when heated above 100°C and became totally unfolded at 140°C. The degradation of P34 bands as a result of extrusion was reported by Wilson *et al.*, [8] who showed that P34 could be eliminated during the texturization of soy protein. The bands in lanes 2, 3, 5, 6, and 8 that remained resistant to extrusion treatment even at higher DT of 100°C and 120°C, correspond to molecular weights of approximately 34 – 44 kDa which represent the acidic polypeptides of glycinin, a finding supported by the work of Van de Lagemaat *et al* [17], who reported that the IgE epitopes in the 11S allergen seem to be mainly located in the acidic subunits of this protein.

On the other hand, the region of β-conglycinin (50-76) kDa exhibited the highest level of band degradation in all samples where band degradation was visible namely; (2, 5 and 7) and (3, 6 and 9), which correspond to DTs of 100 and 120°C respectively across all levels of FMC. Lane 9 of C (Figure 2), which corresponds to the highest DT (120°C) and highest FMC (60%), showed the most effective band degradation, with no visible bands for the proteins characterized here. This suggests that an increase in FMC in combination with an increase in DT leads to an increase in the degradation of soy allergens.

Immunoreactivity of samples

Figure 3 confirms the immunoreactivity of the bands represented by the SDS-PAGE images in Figures 1 and 2. The most reactive proteins were detected by the most prominent band staining, showing the degree of reaction with Immunoglobulin G (IgG). As expected, Lane R, corresponding to untreated soy was the most reactive to IgG, with visible staining in the regions of the major allergens namely; 11s (12-45kDa), 7s (50-76kDa) and P34 (31-34kD). Similarly, almost all the lanes (1-8) exhibited some amount of staining, with the exception of lane 9 that corresponds to DT-120°C and FMC-60%. From Figure 3 we may conclude that with the exception of sample 9 (DT - 120°C and FMC - 60%), none of the treatments were completely free of reactive proteins. The intensity of band staining and thus immunoreactivity decreased with increase in FMC from 40% - 60%.

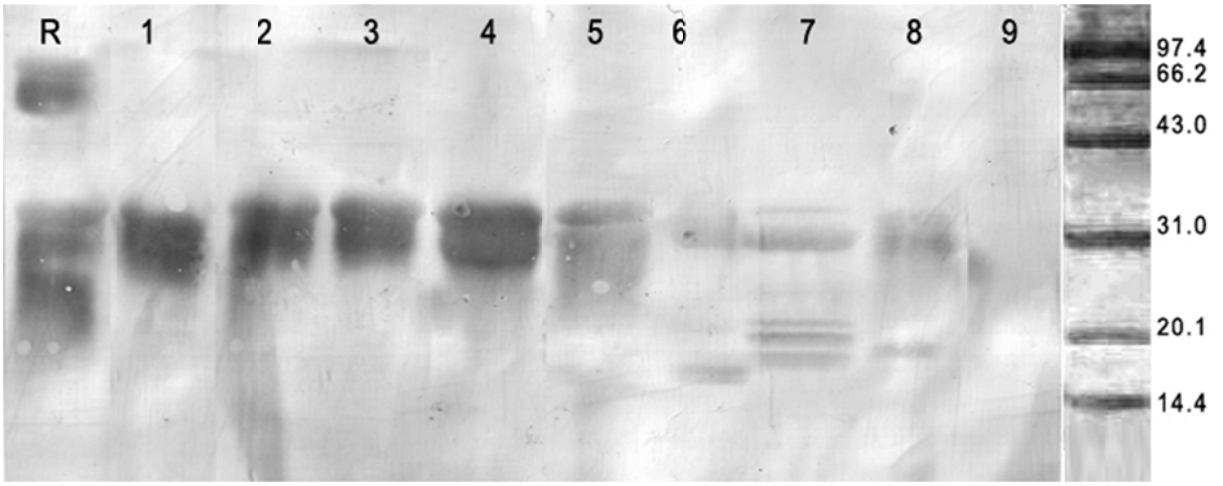

Figure 3. Immunoblot of extruded soy samples determined by using soybean total protein polyclonal antibodies. Lane R represents the raw unextruded sample (control) while lanes 1 to 9 represent the different treatment combinations (die temperature and feed moisture content).

The proteins presenting the highest reaction with IgG correspond to the region of the acidic polypeptides of glycinin (34–44 kDa) and partly to the imunodominant allergen P34 (31-34kDa). On the other hand, the reduction in immunoreactivity through degradation of bands in the β-conglycinin sub region (50-76kDa) in all samples extruded at 100°C and 120°C, across all FMC (Figure 1**)** is further confirmed by the absence of staining (Figure 3). This finding is in agreement with Saitoh *et al.*, [6], that the reduction of antigen protein during extrusion is closely related to the degradation of β-conglycinin. Although glycinin is not completely degraded across all treatments, some of the degraded glycinin bands and thus reduced immunoreactivity may be attributed to the findings of Lee *et al.*, [11] that glycinin is thermostable up to 95°C; and heating above 95°C causes large aggregates to form that retain secondary structure, which may contribute to the reduction of immunoreactivity.

Quantitation of the immunoreactivity of raw and extruded soybean proteins

The final goal of this study was to quantify the immunoreactivity of the samples. Therefore, IgG binding of the protein extracts obtained after extrusion was determined by indirect inhibition ELISA, using the raw sample (R) as the control.

The competitive curves of samples are shown in Figure 4 and the corresponding IC_{50} values are shown in Table 3. The competitive curves represent the 9 treated samples and the control (R) at various antigen dilutions.

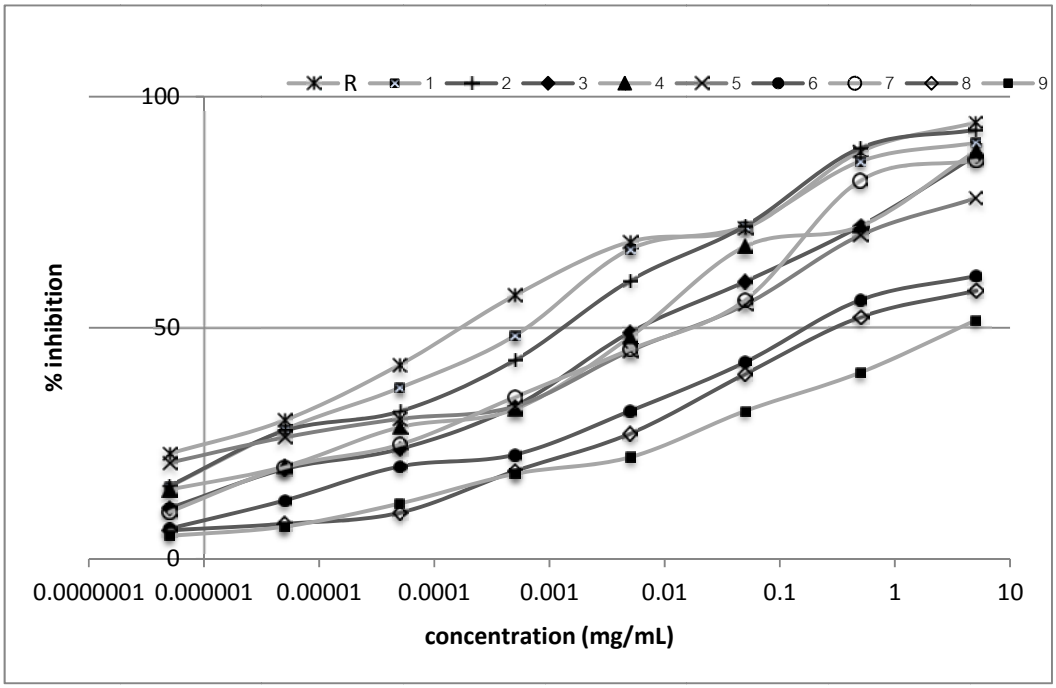

Figure 4: ELISA inhibition curves of reactivity to total soybean protein with the inhibitor of extruded soybean protein samples. Samples are R (raw soy), 1 (FMC-20%, DT-80°C), 2 (FMC-20%, DT-100°C), 3 (FMC-20%, DT-120°C) and 4 (FMC-40%, DT-80°C), 5 (FMC-40%, DT-80°C), 6 (FMC-40%, DT-120°C), 7 (FMC-60%, DT-80°C), 8 (FMC-60%, DT-100°C), 9 (FMC-60%, DT-120°C).

Table 3. IC50 values of Inhibition ELISA.

Samples	Extrusion conditions		IC_{50} value (mg mL^{-1})
	DT (°C)	FMC (%)	
R (raw untreated sample)	*	*	0.0002
1	80^a	20^a	0.0005
2	100^a	20^a	0.0008
3	120^b	20^a	0.0056
4	80^a	40^a	0.0034
5	100^a	40^a	0.0066
6	120^b	40^a	0.2971
7	80^a	60^b	0.0045
8	100^a	60^b	0.9600
9	120^b	60^b	15.6270

* - Not Applicable

IC_{50} – Refers to inhibition coeffecient.

The values having the same superscripts in the same column are not significantly different at $p<0.05$

The results of SDS-PAGE and immunoblot are underscored by the IC_{50} values of the samples. The most reactive samples are indicated by their low IC_{50} concentration values. The IC_{50} value represents the concentration of antigen that can inhibit an immuno-reaction by 50%. Therefore, from Figure 4, it is clear that sample 9 (DT - 120°C and FMC - 60%) was the best treatment because it reaches 50% inhibition much later compared to other treatments. Increasing DT across all levels of FMC significantly reduces immunoreactivity by increasing the IC_{50}, a result which is in agreement with Saitoh *et al.*, [6], who reported that temperature is a very important factor in the reduction of immunoreactivity during twin-screw extrusion. However, even sample 9 does not completely eliminate immunoreactivity because its IC_{50} is approximately 15.6270mg (Table 3), a figure which falls within the range reported in a previous study whereby soy clinical reactivity levels, also referred to as threshold dose, vary significantly and range from as low as 0.0013 to 500 mg of soy protein [18].

According to this study, the ANOVA test of IC_{50} values in Table 3 indicated that there were significant differences at ($p < 0.05$) in immunoreactivity of protein fractions among some of the sample treatments. There was significant difference between treatments at FMC – (20% and 60%) and (40% and 60%) and no significant difference between treatments at FMC (20% and 40%). For the DTs, there was a significant difference between treatments at DT – (80°C and 120°C) and (100°C and 120°C) and no significant difference between treatments at DT – (80°C and 100°C). While FMC had a greater effect on IC_{50} values than DT, increasing FMC alone did not lead to significant differences in IC_{50} among treatments. It was concluded that DT above 100°C leads to reduction of soy allergens and increasing FMC contributes to overall reduction in soy allergenicity.

For the protein extracts in sample 9, corresponding to the combination with the highest FMC- 60% and DT-120°C, the IC_{50} value was in a much higher microgram per millilitre range compared to other treatments. This could probably be supported by the fact that protein denaturation which is fundamental for degradation of allergens occurs at the die end of the extruder as explained by Van den Hout *et al*. [19] that during extrusion, as the materials reach the die, they are melted and proteins are denatured due to the high temperature, pressure and shearing action. Also, FMC was reported to influence the degree of aggregation and the difference in protein-protein interactions and protein subunit interactions within different zones of the extruder [3], which contributes greatly to protein denaturation. This phenomenon was investigated by Van den Hout *et al.*, [19], who found that breaking of the non-covalent interactions between groups in the protein molecule is sufficient to denature the protein. Thus the combined effect of a high DT and FMC on protein denaturation during extrusion probably contributes to the overall reduction in immunoreactivity of soy proteins through degradation of protein molecular structure as suggested by these results.

Conclusion

In this study it was shown that band degradation was mainly achieved in the β-conglycinin region (50-76kDa), although some sub units of 11s (glycinin): (12-45kDa) and P34: (31-34kDa) were also degraded in all samples extruded at DTs of (100°C and 120°C) across all levels of FMC. However, the best band degradation was realized in the combination treatment with the maximum levels of DT-120°C and FMC- 60%, which was the most effective in reducing the immunoreactivity of soy allergens as represented by the non-stained lane and highest IC_{50}.While FMC had a greater effect on IC_{50} values than DT, increasing FMC alone did not lead to significant

differences in immunoreactivity among the treatments. A comprehensive study combining the 3 parameters of extrusion including system, process and product parameters may give more comprehensive data in order to optimize the use of extrusion in producing hypoallergenic soy products for both food and animal feed.

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