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Influence of Extrusion on the Solubility of Defatted Soybean Flour in Enzymatic Hydrolysis

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Abstract Low-energy processing technology, which enhances the utility of defatted soybean flour (DSF), was developed using extrusion processing. DSF was extruded at different conditions using a twin screw extruder and then, dried at 40°C for 20 hr. The nitrogen solubility index (NSI), viscosity, water solubility index (WSI), and water absorption index (WAI) of DSF increased after extrusion processing. The density of DSF extrudates decreased with the decrease in water content from 53 to 33% and the increase in extrusion temperature from 110 to 160°C. The addition of NaOH from 1.2 to 1.8% and citric acid from 1 to 5% increased the total solubility (TS) of DSF due to the decrease of protein coiling and hydrophobic bonds formation during extrusion processing. When viscozyme was reacted first, TS, NSI, and soluble carbohydrate content of DSF hydrolysates increased about 12, 6, and 7%, respectively, compared to them reacted with protease first. The TS and NSI of DSF hydrolysates were increased about 15 and 10%, respectively, by extrusion processing at alkaline and acidic pH. Extrusion processing at alkaline and acidic pH contributed the increase of efficiency to hydrolyze DSF samples using enzyme.

Keywords: defatted soybean flour, extrusion, solubility, hydrolysis

Introduction

Soybeans have grown in popularity in recent years due to their many attributes and versatility. The most notable attributes of soybeans are their oil and protein contents. Soy foods are typically divided into 2 categories: non-fermented (soy flour, soybean curd, soymilk, and *okara*) and fermented (*tempeh*, *miso*, *cheonggukjang*, and soy sauce) (1, 2). Defatted soybean flour (DSF), a major soy protein product, is a highly concentrated protein by-product derived from white flakes made by dehulling, flaking, and defatting soybeans by hexane extraction. Commercial DSF typically contains approximately 50% protein, 30% carbohydrate, and considerably lower amounts of water, fat, fiber, and ash. Soybean meals intended for animal feeds typically are steamed in toasters to distill the residual solvent and have protein digestibility index (PDI) of between 10-50. However, the white flakes intended for food use are processed by flash solvent-removing systems and have protein digestibility index as high as 95 (3). Although high-PDI soy ingredients are more soluble, they have highly active enzymes and antinutritional factors, which should be deactivated by heat before the final product is consumed.

The low solubility of DSF limits its utilization in food stuffs such as beverage, dressing, and sauce, especially when the required functional properties depend on solubility, foaming, and emulsifying properties (4). Although the thermal denaturation of soybean proteins improves the aforementioned properties and water absorption capacity, it reduces water solubility of soybean proteins, especially at high concentration (5). Thermal treatment at acidic conditions (pH 2-3) is useful to increase the solubility through partial deamidation and mild hydrolysis of proteins (6). Acidic hydrolysis can destroy L-form amino acids, produce D-form amino acids, and can form toxic substances like lysino-alanine (7). Enzymatic deamidation of proteins offers several advantages over chemical methods, providing reaction selectivity and milder deamidation conditions by using a neutral pH and room temperature (8). Partial hydrolysis of some protein, especially soybean protein, produces a strong bitter taste. The mixture of endo-peptidase and exo-peptidases has been used for food protein hydrolysis in order to reduce the bitterness of hydrolysate as well as to increase the degree of hydrolysis (9, 10). However, enzymatic hydrolysis of DSF has relatively low water solubility and degree of hydrolysis compared to acid hydrolysis. Thus, in this study, extrusion was used as a preprocess in this study to increase the water solubility of DSF by enzymatic hydrolysis.

Twin-screw extruders can be used as effective continuous reactors (11-13). The extrusion process has been reported to increase the production yield of levulinic acid from starch (14). Mechanical and thermal energy inputs during extrusion induce change in the physical properties of an extruded substance, which affect functionality such as water solubility. Some hydrolysis of starch has been reported to occur during the extrusion of cereal starches and grits (15). Under high-temperature extrusion, these protein bodies are not dissolved but melt and fuse together by protein-protein reactions. The examination of DSF extrudates using a differential staining light microscopy revealed carbohydrate inclusions and steam-generated voids enclosed within a protein-rich matrix (16).

This study was conducted to develop an efficient alternative process to the traditional DSF hydrolysis process of soy sauce production by using extrusion. The

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objectives of this study were to: (1) study the influence of extrusion conditions on the physicochemical properties of DSF and (2) investigate the hydrolysis and solubility of DSF by extrusion and enzyme treatments.

Materials and Methods

Materials The DSF containing 58.9% protein, 28.9% starch, 6.2% fiber, 1.0% fat, and 5.0% ash was purchased from Yoosung Foods Inc. (Seoul, Korea). A commercial endo-protease (Alcalase 2.4 L, activity: 2.4 AU/g), exo-protease (Flavourzyme 1,000 L, activity: 1,000 LAPU/g), and carbohydrase (Viscozyme-L, activity: 100 FBG/g) were purchased from Novo Nordisk (Bagsvaerd, Denmark). Alcalase 2.4 L (AL) is a serine protease produced by submerged fermentation of *Bacillus licheniformis*. The optimum conditions for AL are a temperature from 55 to 70°C and pH 6.5 to 7.5. Flavourzyme 1,000L (FL) is a fungal protease and peptidase complex produced by submerged fermentation of *Aspergillus oryzae* and contains both endoprotease and exopeptidase activities. The optimum conditions for FL are a temperature of around 50°C and pH 5 to 7. Viscozyme-L (VL) is a multi-enzyme complex containing a wide range of carbohydrases, including arabanase, cellulase, β-glucanase, hemi-cellulase, and xylanase, and produced from *Aspergillus* group. The optimum conditions for VL are a temperature from 40 to 50°C and pH 3.5 to 5.5. NaOH, HCl, NaCl, and citric acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Extrusion processing The DSF was extruded at different barrel temperatures using a co-rotating and intermeshing twin-screw extruder (DNDL-44; Buhler Brothers Co., Uzwil, Switzerland), with a 44-mm barrel diameter and 20 length to diameter ratio. The die used for this study had a single circular orifice with a 3-mm diameter opening. The barrel temperature of each zone, from feed end (zone 1) to melt end (zone 4) of the extruder and the temperature at the die exit are listed in Table 1. The barrel of feed end (zone 1) was cooled for feeding of DSF using cold water. The screw configuration used in this study was: 3 × twin lead screws (TLS, 66-mm pitch), 2 × TLS (44-mm pitch), 3 × kneading disks (KD, F/R/F), 3 × TLS (44-mm pitch), 1 × KD (R), 3 × TLS (44-mm pitch), 6 × TLS (14.7-mm pitch, F/R/F/R/F/R), 8 × TLS (14.7-mm pitch), 1 × KD (F), 2 × TLS (14.7-mm pitch); F=forward, R=reverse, 1 KD=20 mm. This screw configuration used in this study was determined to make a high shear between screw and DSF. The feed rate of DSF, water, and chemical solutions (NaOH, HCl, NaCl, and citric acid) are listed in Table 2. The screw speed was set at 360 rpm. The DSF was extruded at different conditions such as screw configuration, DSF feed rate from 7 to 12 kg/hr, water feed rate from 3.0 to 11.0 kg/hr, temperature from 110 to 160°C, and screw speed from 320 to 420 rpm to determine optimum condition for the increase of its solubility. The DSF samples were each extruded in duplicate for each extrusion condition studied. After extrusion, extrudates were dried at 40°C overnight using a forced-air oven. The dried samples were ground into powder using a cyclone sample mill (Udy Corporation, Fort Collins, CO, USA) equipped with 0.5-mm mesh screens.

Table 1. The barrel temperatures of each zone from feed end to melt end (zone 1-4) and die exit temperature

Trial	Extruder barrel temperature (°C)				Die exit temperature (°C)
	Zone 1	Zone 2	Zone 3	Zone 4	
1	30	100	105	110	110
2	30	120	132	141	140
3	30	135	145	151	150
4	30	143	150	159	160

Enzymatic hydrolysis A 20 g sample of ground product was suspended in 300 mL of water, and the slurry was

Table 2. Extrusion conditions of defatted soybean flour samples

Sample	Die exit temperature (°C)	Feed rate (kg/hr)					
		DSF	Water	2 N NaOH	2 N HCl	NaCl	Citric acid
A	110	10.0	10.5				
B	140	10.0	11.0				
C	150	9.0	4.3				
D	160	9.0	2.5	2.05			
E	160	9.0	1.6	2.95			
F	160	9.0	2.5		2.05		
G	160	9.0	1.6		2.95		
H	160	8.9	4.3			0.1	
I	160	8.5	4.3			0.5	
J	160	8.9	4.3				0.1
K	160	8.5	4.3				0.5

mixed at 8,000 rpm for 3 min using a homogenizer (IKA-Labortechnik, German). The slurry was autoclaved at 121 °C for 25 min for thermal sterilization and then adjusted to pH 5 by dropwise addition of hydrochloric acid (HCl, Sigma). VL was added to the DSF slurry to hydrolyze carbohydrate. Then the slurry was continuously stirred at 50°C for 2 hr using a stand stirrer (MS3040: Tops Co., Korea). After 2 hr enzyme treatment, the slurry was readjusted to pH 7 by dropwise addition of 1 N sodium hydroxide solution (NaOH, Sigma). A series of two-step enzymatic reactions were performed to hydrolyze protein. The first enzymatic hydrolysis of DSF protein was performed by adding AL at 55°C for 3 hr. The extent of protein hydrolysis was controlled quantitatively using the pH-stat method of Adler-Nissen (17). After 3 hr enzyme treatment, the AL treated sample was further hydrolyzed by the mixtures of endo- and exo-enzymes (AL+FL) at 55 °C for 24 hr. During enzymatic reaction, sodium azide (0.01%, w/w of total DSF slurry) was added to prevent the putrefaction of DSF slurry. The enzyme solutions were passed through a membrane filter (d=0.45 μm) to remove microbial cells.

The concentration of each enzyme used was 0.1%(w/w) and 1.0%(w/w) of DSF samples. The order of enzyme treatments with 0.1%(w/w) enzyme concentration was changed to determine the enzymatic hydrolysis technology for more solubility of DSF samples. The hydrolysates were centrifuged at 6,000×g for 20 min, and nitrogen and α-amino nitrogen content of the supernatants was measured.

Analysis The apparent viscosity of the DSF slurry was measured using a Brookfield cylindrical viscometer (model DV-II+; Brookfield Engineering Lab., Inc., Stoughton, MA, USA) at 25°C, and a cylinder speed of 30 rpm using a S61 spindle after calibration of the viscometer by pure water. A gas multipycnometer (model UPY-14; Quantachrome Corp., Fairfield, NJ, USA) was used to measure the volume of the DSF extrudates. Helium, as an ideal gas, which can penetrate the finest pores to assure maximum accuracy, was used as the displacing fluid. The density was calculated by the volume and mass of the sample.

Moisture, nitrogen, starch, fat, and ash contents of the ground samples were determined by AACC methods 44-19, 46-13, 76-13, 30-25, and 08-03, respectively (18). Protein content was estimated using the N conversion factor of 6.25. Fiber content was obtained by subtraction of the other contents mentioned from 100%. The nitrogen solubility index (NSI) was calculated from the amount of nitrogen in the supernatants to the total nitrogen determined by micro-Kjeldahl method before and after enzyme treatments. The degree of protein hydrolysis was measured by AN/TN ratio, the amount of α-amino nitrogen present (AN) in the hydrolysate relative to the total amount of nitrogen present (TN) in the substrate. α-Amino nitrogen content was measured by Formol titration method using 0.02 N NaOH solution (18).

Water solubility index (WSI) and water absorption index (WAI) of ground samples were determined as described by Mason and Hoseney (19). A 2.5 g sample of ground product was suspended in 30 mL of water at 30°C. The sample was first shaken for 30 min with the aid of a vortex mixer (Scientific Products, McGraw Park, IL, USA) and then centrifuged at 1,000×g for 10 min. The supernatant was dried, and the amount of solubles was expressed as a percent of the original dry sample weight for WSI. The ratio between the total weight of the pellet and the weight of the solids in the pellet is the calculated WAI. All samples were run in duplicate.

Statistical analysis All statistical analyses were performed with a statistical analysis system (SAS Institute, Cary, NC, USA) using the general linear model procedure. One-way analysis of variance (ANOVA) was performed. Means were compared by the least significant difference (LSD) test at the α=0.05 level.

Results and Discussion

Effect of extrusion on the physicochemical properties of DSF The DSF was clogged in the barrel at below 30 % water content and 350 rpm screw speed during extrusion and did not expanded at over 400 rpm screw speed. The density of DSF extrudate decreased with the decrease in water feed rate from 11 to 4.3 kg/hr and the increase in die exit temperature from 110 to 160°C, while WSI increased. Thus, DSF feed rate, water feed rate, screw speed, and die exit temperature were determined 9 kg/hr, 4.3 kg/hr, 360 rpm, and 160°C, respectively.

The effects of water feed rate and extrusion temperature on physicochemical properties such as protein and soluble protein contents, viscosity, density, NSI, WSI, and WAI were examined using samples extruded at 11.0, 10.5, and 4.3 kg/hr water feed rate and 110, 140, and 150°C die exit temperature. The protein content of DSF was not changed significantly after extrusion processing; however, soluble protein content, viscosity, and WAI increased significantly (p<0.05, Table 3). Thermal treatment of proteins might result in structural changes such as hydrolysis of peptide bonds, modification of amino side chains, and the formation of new covalent isopeptide cross-link; in addition, proteins are more resistant to heat at lower water concentration (20). Stanley (21) reported that heat treatment of soy protein reduced protein solubility in extrusion. These results are in disagreement with those found in this study. This result might be caused that the DSF used in this study was already heated to remove hexane solvent. It was also reported that the increase of protein solubility can be achieved by the use of reagents that break ionic, hydrogen, hydrophobic, and disulfide bonds (21). Thus, the increase of soluble protein content in this study was probably caused that the remaining hexane solvent of DSF broke the ionic, hydrogen, and disulfide. The density of DSF decreased significantly after extrusion due to expansion processing (p<0.05). The increase of WAI and viscosity after extrusion processing may cause depolymerization of starch granules in DSF. The WAI correlated well with cold-paste viscosity because only depolymerized starch granules absorbed water at room temperature and swelled, creating increased viscosity (22).

Extrusion temperature and water feed rate did not have statistically significant effects on the protein and soluble protein contents, viscosity, and WAI (p<0.05, Table 3). Thermal energy input during extrusion might not affect

Table 3. Physicochemical properties of defatted soybean flour (DSF) and DSF samples extruded at different conditions[1)]

Sample	Moisture (%, wet basis)	Protein (%, dry basis)	Soluble protein (%, d.b.)	Viscosity (cp)	Density (kg/m^3)	WAI[2)]
DSF	1.70	58.92a	4.72a	2.2a	644a	2.54a
A	13.33	58.44a	5.35b	2.8b	639a	3.37b
B	8.27	58.34a	5.53b	2.6b	620b	3.43b
C	5.82	58.23a	5.57b	2.6b	605c	3.26b
D	9.99	56.33a	5.88c	3.6c	601c	4.69c
E	9.36	56.53a	6.20c	9.4d	593d	4.75c
F	5.08	55.64ab	6.12c	2.5b	606c	3.02b
G	4.72	55.80ab	5.56b	2.5b	609c	2.90b
H	7.92	56.15ab	5.19ab	3.0bc	605c	3.16b
I	5.52	54.50b	5.06ab	3.2bc	610c	3.10b
J	5.14	55.83ab	4.73a	3.0bc	608c	2.85b
K	6.01	56.26a	3.67d	3.2bc	607c	2.54a

[1)]Values followed by the same letter in the same column are not significantly different ($p<0.05$).
[2)]Water absorption index.

these functional properties of DSF because it was affected thermally by heat treatment for removing solvent. The density of DSF extrudates decreased significantly as extrusion temperature increased and water feed rate decreased (Table 3). Mechanical and thermal energy inputs during extrusion induce change in the physical properties of an extruded substance, which affect functionality such as density. Halek and Chang (23) reported that barrel temperature and feed moisture had an important influence on solid density, measured using a gas pycnometer, of extrudates from corn meal. The solid density of the extrudates from corn meal significantly decreased, like DSF extrudtes in this study, as extrusion temperature increased and feed moisture decreased.

The WSI, WAI, viscosity, and NSI of DSF extrudates increased significantly with the addition of NaOH (Table 3 and Fig. 1). The pH of DSF slurry, which contained DSF sample of 20 g and water of 300 mL, was 6.3 and increased from 8.8 to 10.0 as the feed rate of 2 N NaOH solution increased from 2.05 to 2.95 kg/hr. Dahl and Villota (24) reported that the NSI, viscosity, and water holding capacity (WHC) of DSF slurry had linear intercorrelations and increased as pH increased from 4.5, the isoelectric region, to 11.0. These findings are in good agreement with physicochemical tests performed in this study. The proteins of DSF are disassembled by extrusion and then reconnected into a fibrous, oriented structure with a characteristic texture (21). Noguchi (25) reported that the disulfide bonds in DSF might be cleaved at alkaline and acidic pH by the combination of heat and shearing effects during extrusion processing before the bonds were reformed to create the altered structure. This cleavage of disulfide bonds may probably cause the increase of solubility. The NSI of DSF extrudates decreased by the addition of HCl or citric acid, which decreased the pH of DSF slurry from 6.3 to 5.5; however, their WSI increased due to acid hydrolysis of starch during extrusion processing in the presence of an acid (Fig. 1).

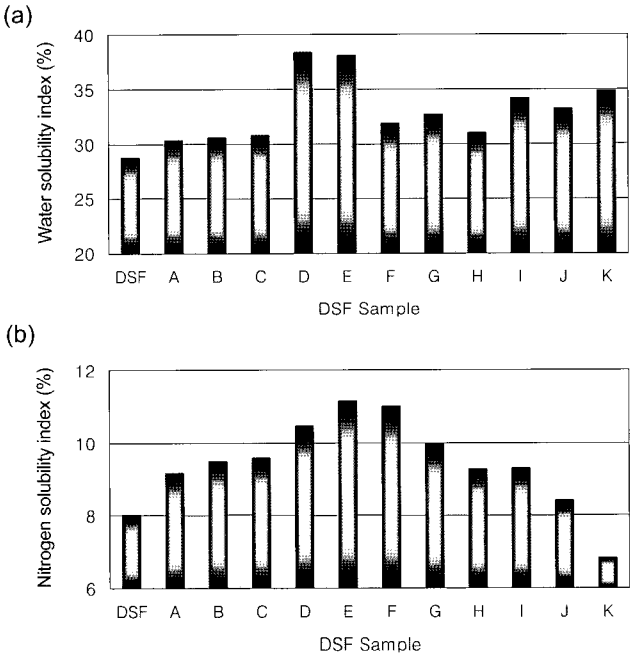

Fig. 1. (a) Water solubility index (WSI) and (b) nitrogen solubility index (NSI) of defatted soybean flour (DSF) and DSF samples extruded at different conditions.

Enzymatic hydrolysis of DSF extrudates Total solubility (TS), NSI, and soluble carbohydrate content of DSF and DSF extrudates after enzymatic reaction are listed in Table 4. The TS, NSI, and soluble carbohydrate content of DSF hydrolysates were increased by extrusion processing regardless of the order of enzyme treatments. They increased as extrusion temperature increased and water feed content decreased. When protease was used after viscozyme treatment, the TS, NSI, and soluble carbohydrate

Table 4. Total solubility, nitrogen solubility index (NSI), and soluble carbohydrate content of defatted soybean flour (DSF) and extruded DSF samples depending on enzyme treatment procedure with 0.1%(w/w) enzyme concentration[1)]

Enzyme treatment	Sample	Total solubility (%)	NSI (%)	Soluble carbohydrate (%)
	DSF	35.62a	26.24a	4.59a
Viscozyme	A	40.22b	31.66b	7.19b
First[2)]	B	45.78c	31.04b	17.84c
	C	48.80c	37.68c	18.05c
	DSF	23.17d	20.45d	3.47a
Protease	A	26.24d	28.91ab	4.60a
First[3)]	B	31.54a	28.89ab	10.02b
	C	32.13a	30.48b	10.00b

[1)]Values followed by the same letter in the same column are not significantly different ($p<0.05$).
[2)]Viscozyme was treated prior to protease treatment.
[3)]Proteases (AL and FL) were treated prior to viscozyme treatment.

Table 5. Total solubility, nitrogen solubility index (NSI), and degree of protein hydrolysis (DPH) of defatted soybean flour (DSF) and extruded DSF samples after enzyme treatments with 0.1%(w/w) enzyme concentration[1)]

Sample	Total solubility (%)	NSI (%)	DPH (%)
DSF	35.62a	26.24a	8.89a
A	40.22b	31.66b	10.26b
B	45.78c	31.04b	10.07b
C	48.80c	37.68c	11.17c
D	57.84d	53.40d	14.50d
E	58.53d	55.98d	14.49d

[1)]Values followed by the same letter in the same column are not significantly different ($p<0.05$).

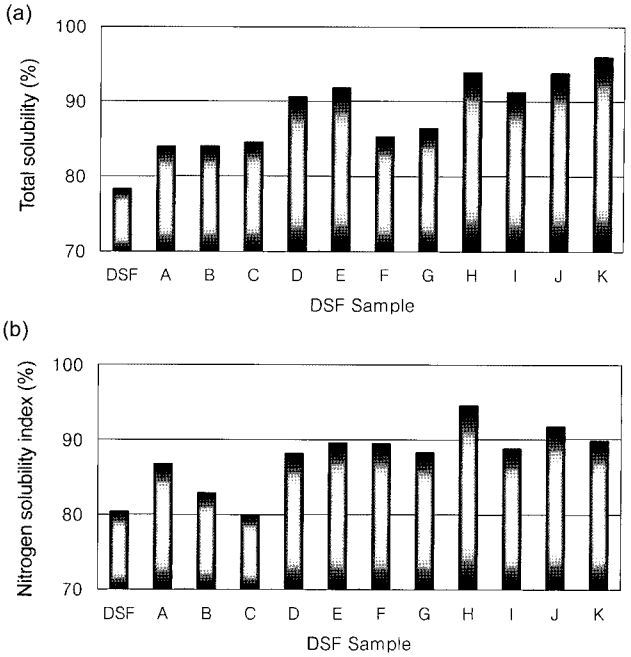

Fig. 2. (a) Water solubility index (WSI) and (b) nitrogen solubility index (NSI) of defatted soybean flour (DSF) and extruded DSF samples after enzyme treatments with 1% enzyme concentration.

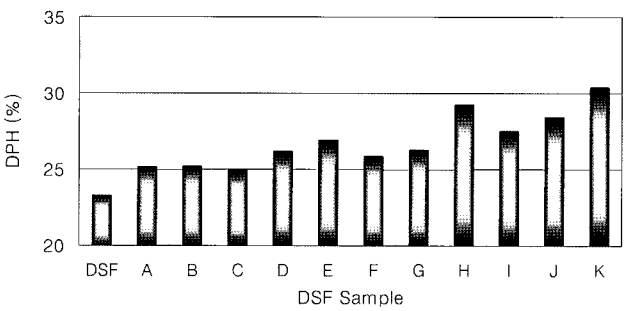

Fig. 3. Degree of protein hydrolysis (DPH) of defatted soybean flour (DSF) and extruded DSF samples after enzyme treatments with 1% enzyme concentration.

content of DSF samples were higher about 15, 5, and 6%, respectively, than those when viscozyme was used after protease treatment. This result indicates that the hydrolysis of carbohydrate prior to protein hydrolyzation increased the efficiency to hydrolyze DSF samples.

The TS, NSI, and degree of protein hydrolysis (DPH) of DSF hydrolysates reacted with 0.1%(w/w) enzyme concentration of DSF samples are listed in Table 5. The TS, NSI, and DPH of DSF after enzymatic hydrolysis were 35.62, 26.24, and 8.89%, respectively, and increased significantly by extrusion processing ($p<0.05$, Table 5). The TS, NSI, and DPH of DSF extrudates after enzyme treatments were significantly increased by the addition of NaOH at a given extrusion condition ($p<0.05$). This result might be caused by the cleavage of disulfide bonds during extrusion processing at alkaline pH.

Enzyme concentration increased from 0.1 to 1%(w/w) of DSF substrate to study its effect on the TS, NSI, and DPH of DSF samples after enzyme treatments. The TS of non-extruded DSF increased from 35.62 to 78.26% as the enzyme concentration (w/w) to DSF substrate increased from 0.1 to 1.0%. The NSI and DPH of non-extruded DSF after enzymatic treatments increased from 26.24 to 80.38% and from 8.89 to 23.27%, respectively, as the enzyme concentration increased from 0.1 to 1.0%. Figure 2 and 3 show the TS, NSI, and DPH of DSF samples reacted with 1% enzyme concentration. The TS, NSI, and DPH of DSF samples after enzymatic hydrolysis were increased by extrusion processing (Fig. 2). The TS and NSI of DSF extrudates were increased 7 and 10% by the addition of NaOH, respectively. The TS and NSI of DSF extrudates were increased 10 and 11% by the addition of citric acid, respectively. The TS and DPH of DSF extrudates with citric acid were the highest among DSF htdrolysates after enzyme treatments. Lee *et al.* (10) reported that the acid treatments of DSF increased solubility of DSF hydrolysates after enzyme treatment and accelerated enzyme hydrolysis. This result agreed with the

increase in NSI and DPH by the addition of citric acid during extrusion processing.

The NSI, viscosity, WSI, and WAI of DSF were increased by extrusion processing, while its density decreased. The addition of NaOH during extrusion processing increased the NSI, viscosity, WSI, and WAI of DSF extruded at a given condition. The TS and NSI of DSF hydrolysates were higher when viscozyme was used prior to protease treatments. Extrusion processing at alkaline and acidic pH contributed the increase of efficiency to hydrolyze DSF samples using enzyme. Most parts of DSF extrudates except insoluble parts such as ash and lipid were soluble in water by enzymatic treatments. Extrusion processing appears to be useful to develop various food ingredients from DSF by enhancing its enzymatic hydrolysis.

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