Texture and Chemical Characteristics of Soy Protein Meat Analog Extruded at High Moisture

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ABSTRACT: The relationships among extruder responses, texture, and protein solubility of soy protein meat analogs were studied. Soy protein isolate and wheat starch at 9:1 ratio were extruded at 60%, 65%, and 70% moisture contents and 137.8, 148.9, and 160 °C cooking temperatures. The results showed that moisture content was a more important factor on the overall product texture than cooking temperature. Lower moisture content resulted in higher die pressure, harder texture, and lower total protein solubility. At a fixed moisture content, a higher cooking temperature resulted in a softer and less chewy product but only slightly changed the protein solubility. According to partial least square regression, the data from Texture Profile Analysis, protein solubility, and extruder responses correlated well and could be used to predict each other.

Key Words: soy protein, extrusion, texture, protein solubility

Introduction

SOY PROTEIN HAS BEEN UTILIZED IN THE FOOD INDUSTRY TO serve as a partial or complete replacement of animal protein; uses include meat binders, meat analogs, soy beverages, and some ethnic foods in Asia. The protein value of soy protein is comparable to that of meat protein (deMan 1990). It has been shown that by consuming vegetable protein, the risk of heart disease can be reduced (Robbins 1987). Traditional extruded meat analogs are produced at a lower (about 30%, wet basis) moisture content level and with a expanded structure. When soy protein was extruded at a moisture content greater than 50% with a cooling die, fibrous and non-expanded products were produced (Cheftel and others 1992; Noguchi 1989). Studies in this field have focused on the chemical reactions of protein. This information on the chemical bonds in meat analogs is useful in understanding how these reactions are related to texture. However, different results have been reported (Ning 1993; Burgess and Stanley 1976; Jeunink and Cheftel 1979). Most of the studies on the effect of processing parameters on the chemical bonds formation were done under low-to-moderate moisture content, which might have different effects on protein structure than under high moisture content. In addition, the study of the relationship between texture and chemical properties of meat analogs was very limited. Thus, our objective was to determine, at high moisture extrusion, the relationships among extruder responses, textural profile analysis (TPA), and protein solubility of the soy protein meat analogs.

Results and Discussion

Extruder responses

The results of analysis of variance (ANOVA) (not shown) indicated that the extrusion moisture content was significant to all 3 extruder responses, while the cooking temperature was only significant to the product temperature ($p < 0.001$). Table 1 and Fig. 1 show that as the moisture content increased, the percent torque, die pressure, and product temperature decreased. The decrease in product temperature as the moisture content increased was due to the reduced shear force and mechanical energy input since water served as a lubricant (Lin and others 1997; Hayashi and others 1993). Many researchers (Baird and Reed 1989; Chen and others 1990) reported that the viscosity of dough was affected by both dough temperature and dough moisture content. At a fixed moisture content, dough viscosity decreased with increasing dough temperature; at a fixed dough temperature, dough viscosity decreased with increasing moisture. As dough viscosity decreases, the die pressure also decreases (Yacu 1985). Thus, the decreases in die pressure and percent torque with increasing moisture or increasing temperature were most likely due to the reduction in viscosity.

Texture Profile Analysis (TPA)

The TPA using the TA.XT2 analyzer recorded 5 textural attributes: springiness, cohesiveness, gumminess, hardness, and chewiness. According to the results of the multivariate ANOVA (MANOVA, not shown), both the moisture content and cooking temperature were significant factors affecting the texture of the product (Wilks' Lambda, $p < 0.0001$). The ANOVA results (not shown) indicated that both cooking temperature and moisture content were significant to gumminess, hardness, and chewiness ($p < 0.05$), but not cohesiveness and springiness. Table 2 shows that, as the moisture content decreased, the hardness, chewi-

Table 1—The effect of moisture content and cooking temperature on percent torque, die pressure, and product temperature

Treatment	Cooking temperature (°C)	Moisture content (%)	Torque (%)	Die pressure (kPa)	Product temperature (°C)
1	137.8	70	7.8^{bc*}	571.9^{d}	120.2^{f}
2	148.9	70	7.4^{c}	485.7^{de}	125.2^{e}
3	160	70	7.1^{c}	418.2^{e}	129.1^{d}
4	160	65	9.0^{b}	837.8^{c}	135.7^{b}
5	148.9	65	9.0^{b}	850.9^{c}	129.6^{cd}
6	137.8	65	9.1^{b}	880.5^{c}	125.2^{e}
7	137.8	60	12.0^{a}	1727.3^{a}	130.3^{c}
8	148.9	60	11.4^{a}	1601.2^{b}	136.2^{b}
9	160	60	11.1^{a}	1482.7^{b}	141.1

[a]*Within a column, values with the same letters are not significantly different at $p < 0.05$.

Table 2—Least Square Difference in gumminess, chewiness, and hardness for products from different moisture contents and cooking temperatures

Treatment	Cooking temperature (°C)	Moisture content (%)	Gumminess	Hardness (g)	Chewiness
1	137.8	70	2337^{e*}	3869^{e}	2078^{e}
2	148.9	70	2227^{e}	3649^{e}	1893^{e}
3	160.0	70	2129^{e}	3514^{e}	1805^{e}
4	160.0	65	2860de	4896de	2457de
5	148.9	65	4098^{cd}	6674^{cd}	3400^{cd}
6	137.8	65	5046^{bc}	8250^{bc}	4244^{bc}
7	137.8	60	6788^{a}	10976^{a}	5760^{a}
8	148.9	60	5444^{b}	8841^{b}	4673^{b}
9	160.0	60	4362^{bc}	7012^{bc}	3758^{bc}

*Within a column, values with the same letter are not significantly different ($p < 0.05$).

ness, gumminess, and cohesiveness increased ($p < 0.05$).

Figure 2 shows that the effect of cooking temperature was significant to the TPA attributes at lower moisture contents, but not at higher moisture contents. Figure 1A shows that the product temperature increased from 120.2 °C to 129.1 °C at 70% moisture when increasing the cooking temperature from 138° to 160 °C, while the product temperature increased from 130.3° to 141.1 °C at 60% moisture. Thus, the cooking temperature caused a slightly greater increase in product temperatures, and product temperature ranges were also higher when the moisture was lower. Both higher cooking temperatures and product temperatures have contributed to a greater effect on the TPA attributes. Kitabatake and others (1985) found that when extruding soy protein isolate at greater than 70% moisture, the cooking temperature had little effect on the extruder responses and product texture. The results from LSD (Table 2) and Fig. 2 show that samples extruded at 70% moisture had the lowest hardness, chewiness, and gumminess, which were likely due to more water contained within the samples. Also, the higher the moisture content, the lower the viscosity, elasticity, and dough temperature (Lin and others 1997), which might have resulted in an incomplete texturization process (Kitabatake and others 1985) and a softer texture (Noguchi 1989). Gumminess and chewiness all followed the same trend as hardness, because they were all hardness-related, according to the definition of chewiness and gumminess (Bourne 1982). At a lower moisture content, product hardness reduced with increasing cooking temperature. The extrudate tended to puff slightly when exiting from the cooling die. This could have weakened the structure and resulted in a lower hardness.

Noguchi (1989) found that soy flour at 60% moisture melted at 130 °C when measured with a differential scanning calorimeter. This temperature was recognized to be the lowest temperature for extrusion texturization of soy flour in his experiment. However, all samples showed some fibrous structure in our results even though the product temperature of some samples did not reach 130 °C before entering the cooling die, and the moisture content was higher than 60%. This could be due to differences in the protein content of the raw materials. In this study, SPI with about 85% to 90% protein content was used. The higher the protein content the greater chance for the protein to form the chemical bonds that were required for texturization. Thus, Petruccelli and Anon (1995) reported that increasing soy protein content increased thermal aggregation. However, the product extruded at a moisture content of 70% appeared less fibrous than others, which may be due to incomplete texturization. Kitabatake and others (1985) also found that at 80% moisture, the protein denaturation appeared to be incomplete.

According to Soeda (1994), both 7S and 11S subunits of soy protein and major components for texturization, start to unfold

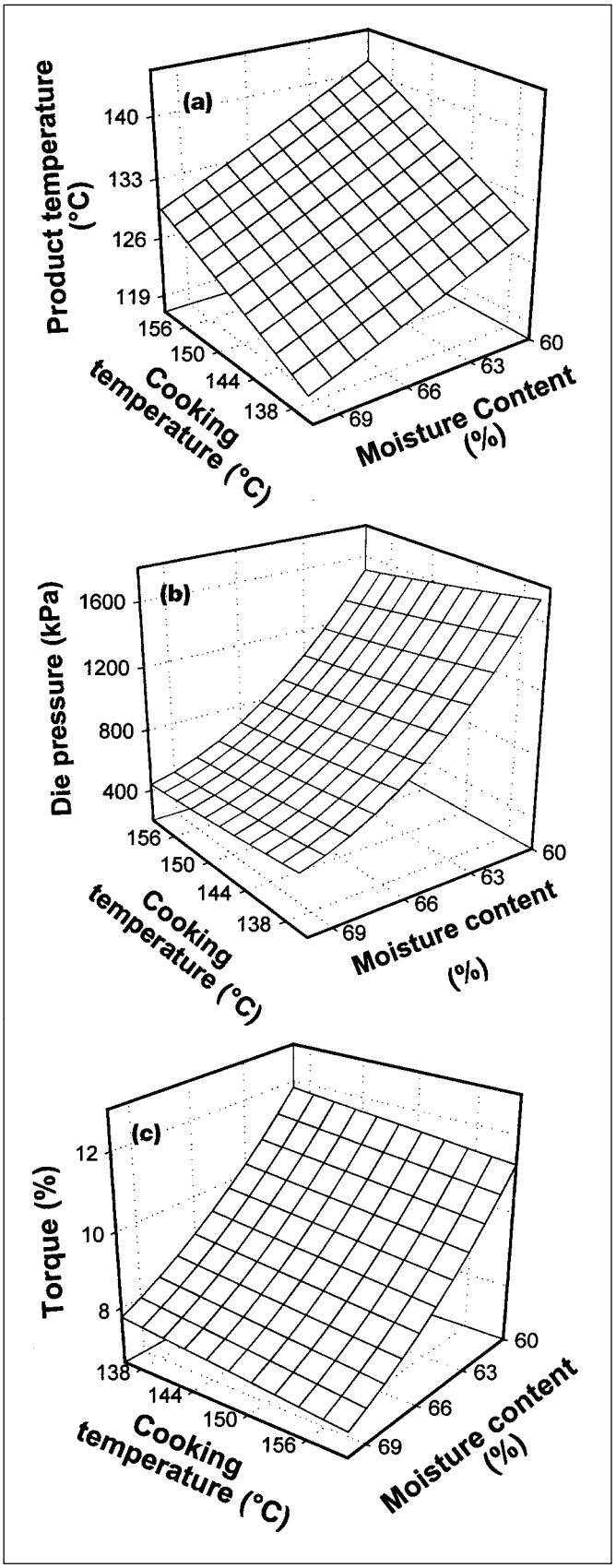

Fig. 1—The effect of moisture content and cooking temperature on (a) product temperature, (b) die pressure, and (c) percent torque.

Table 3—The percentage of proteins extracted by different solvents

Treatment	P[a]	P+2-Me[b]	P+Urea[c]	P+SDS[d]	P+U+2-Me[e]	P+U+SDS[f]
Control	7.5 ± 0.5	30.2 ± 2.7	33.1 ± 2.4	20.5 ± 2.3	83.9 ± 3.9	24.4 ± 2.6
1	2.1 ± 0.5	14.5 ± 2.5	26.7 ± 4.5	7.2 ± 0.6	74.1 ± 4.4	18.0 ± 3.8
2	2.7 ± 0.8	16.6 ± 2.2	27.5 ± 0.5	10.1 ± 1.0	71.5 ± 1.9	20.1 ± 1.3
3	1.4 ± 0.3	17.2 ± 2.0	27.4 ± 2.0	13.8 ± 0.5	71.1 ± 4.3	18.1 ± 1.1
4	2.9 ± 0.6	12.4 ± 1.4	20.2 ± 2.4	5.2 ± 1.3	61.8 ± 4.3	13.2 ± 0.9
5	2.0 ± 0.7	10.8 ± 1.5	21.5 ± 2.1	7.2 ± 0.9	62.9 ± 3.7	8.8 ± 1.3
6	0.8 ± 0.1	11.7 ± 1.0	19.9 ± 1.4	7.2 ± 0.2	63.9 ± 3.7	9.6 ± 1.2
7	2.2 ± 0.1	12.6 ± 1.2	18.7 ± 1.3	7.4 ± 1.0	63.3 ± 2.7	12.7 ± 1.1
8	1.0 ± 0.1	12.0 ± 0.6	21.6 ± 3.3	7.6 ± 1.8	56.6 ± 2.1	7.8 ± 0.4
9	1.8 ± 0.3	11.7 ± 0.5	25.3 ± 2.6	7.7 ± 1.1	59.4 ± 2.7	11.3 ± 0.9

[a]Phosphate buffer (pH 7.6)
[b]phosphate buffer+2% 2-mercaptoethanol
[c]phosphate buffer+8M urea
[d]phosphate buffer+1.5% sodium dodecyl sulfate
[e]phosphate buffer+8M urea+buffer+2% 2-mercaptoethanol
[f]phosphate buffer+8M urea+1.5% sodium dodecyl sulfate.

when heated above 100 °C and become totally unfolded at 140 °C. This probably explains why the fibrous structure was formed even when the product temperature did not reach 130 °C. Nagano and others (1995) found that at a temperature as low as 65 °C, the b-conglycinin in 11S of soy protein started to denature. With the help of shear and pressure inside the extruder, the protein could be more easily denatured and texturized. On the other hand, as the moisture content increased, the product inside the cooling die became less viscous; therefore, the shear and friction (Noguchi 1989) or the strain inside the die that were critical for texturization were reduced, and there was less fiber formation at a higher moisture content.

Protein solubility

The protein solubility test was performed to investigate the forces that were responsible for stabilizing the extrudate during the extrusion. The moisture content of each sample was determined before the protein extraction. Table 3 shows the percentage of protein extracted by each solvent from all treatments. As shown, the extractable protein decreased in all solvents after extrusion (samples 1 to 9 vs control). This phenomenon was probably due to the formation of some protein with new chemical linkages, such as non-disulfide covalent bonds that were not disrupted by the solvents used or the formation of some polymers with very high molecular weight as reported by Li and Lee (1996).

The amount of protein extracted by the phosphate buffer in both raw material and extrudates was significantly less than the amounts extracted by other solvents (Table 3). Horvath and Czukor (1993) reported that the phosphate buffer alone only dissolved protein molecules that were in their native states. Furthermore, the raw material (SPI) had been subjected to alkaline- and then acid-processing and further denatured by heat. Thus, most of its water soluble or low molecular weight proteins were removed (Berk 1992). When a 2nd solvent was combined with the phosphate buffer, protein solubilities increased, which suggested that the protein aggregated with more than 1 type of chemical bond. Several researchers (Rhee and others 1981; Neumann and others 1984) had suggested that disulfide bonds were the major force that stabilized the proteinaceous extrudate. However, Hayakawa and others (1988) found that the disulfide bonds were not important in forming the protein matrix in the extrudate of full fat soy flour. The protein extracted by phosphate buffer + 2-Me was below 18% (Table 3). Thus, it appeared that the disulfide bonds were not the sole or major force in stabilizing the protein structure.

The amount of protein extracted by phosphate buffer + urea was the 2nd highest among all extracting solvents (Table 3), indicating a significant portion of protein was linked with the hydro-

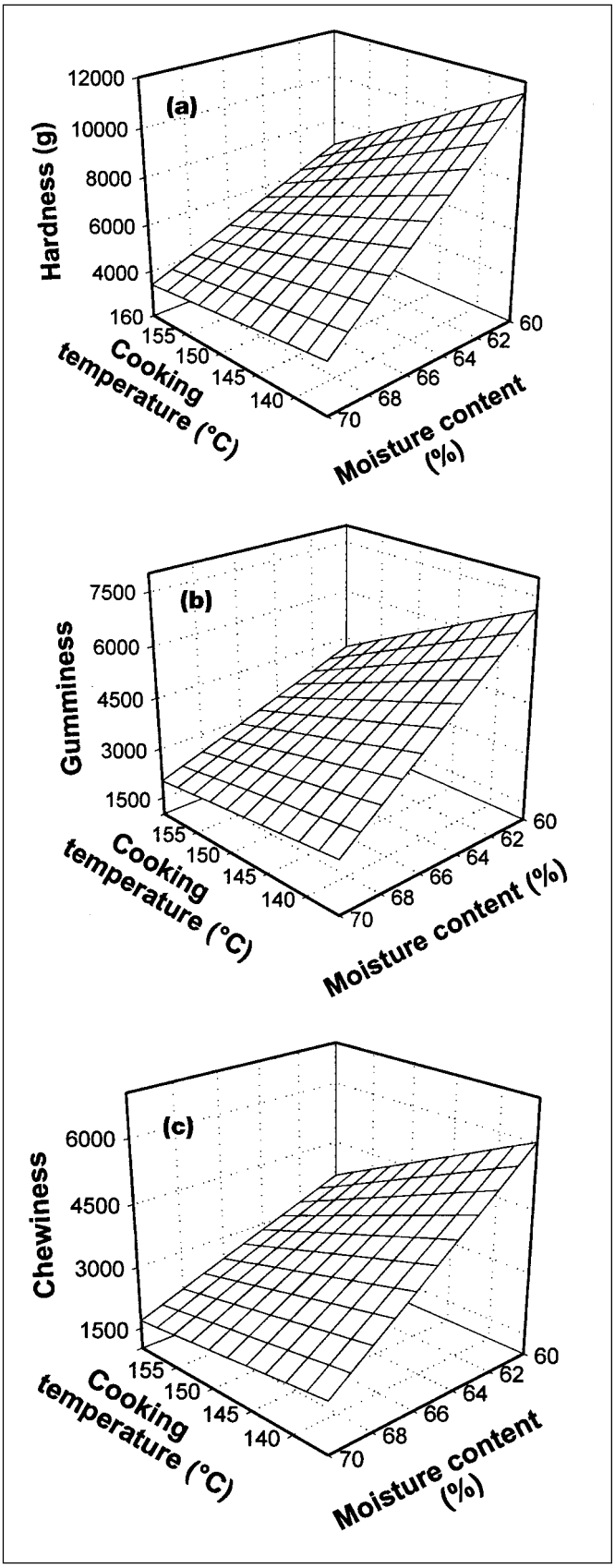

Fig. 2—The effect of cooking temperature and moisture content on the Texture Profile Analysis attributes: (a) hardness, (b) gumminess, and (c) chewiness.

gen bonds. On the other hand, the low amount of protein extracted by phosphate buffer + SDS (Table 3) showed that the hydrophobic interactions were not very important. These results were different from those under low-medium moisture (30% to 40%) extrusion. Under low-medium moisture (30% to 40%) extrusion conditions, both hydrophobic bonds and disulfide bonds were found to be important in stabilizing the protein matrix (Alfredo and Areas 1986, Li and Lee 1996, Mitchell and Areas 1992).

The combination of noncovalent forces (hydrogen bond and hydrophobic interactions) alone was not very important in supporting the protein structure according to the small amount of protein extracted by phosphate buffer + urea + SDS (Table 3). On the other hand, with the combination of phosphate buffer, urea, and 2-Me, the amount of protein extracted was more than 60% in most cases (Table 3), indicating the extrudate protein structures were supported by hydrogen bonds, hydrophobic bonds, disulfide bonds, and their combinations. Stanley (1989) and Li and Lee (1996) also reported that the combination of disulfide bonds and noncovalent bonds were part of the force that stabilized the proteinaceous extrudate. Protein that could not be extracted by any one of the 6 solvent systems in this study had nondisulfide covalent bonds (Stanley 1989). Ning (1993) concluded that a high extrudate temperature, resulting from a high cooking temperature, was necessary for a significant effect on the protein reactions under high moisture extrusion conditions.

The MANOVA (not shown) indicated that only moisture content had a significant effect on protein solubility (Wilks' Lambda $p < 0.01$). The cooking temperature, as well as the product temperature, did not affect the amount of extractable protein. This differed from the results reported by Horvath and Czukor (1993) using lower than 20% extrusion moisture. In their study, the cooking temperature significantly affected the amount of protein extracted. Figure 2 also shows that the cooking temperature did not affect the TPA attributes at high extrusion moisture contents, but the effect became noticeable as the extrusion moisture was reduced. This was because the cooking temperature caused a greater increase in product temperature and product temperature was also higher when the extrusion moisture was lower (Fig. 1B). Both higher product temperatures and greater temperature increase would have contributed to a greater effect on the protein solubility, as well as the TPA attributes as discussed in previously. Therefore, at low moisture conditions, the extrusion temperature was a more important factor than the extrusion moisture on the extrudate protein solubility.

The ANOVA (not shown) indicated that moisture content was significant for protein solubility in all solvents except the phosphate buffer ($p < 0.05$). This was because most of the phosphate buffer soluble proteins were removed during the production of SPI (Berk 1992). The data in Table 4 shows that as the extrusion moisture decreased, the amount of extractable protein in each solvent decreased (and the amount of insoluble protein increased). In samples 1 to 3, protein was not treated as severely as those in samples 4 to 9 because moisture content was higher which served as a lubricant and because the product temperature was lower before the die (Table 1); thus, intramolecular bonding was less and the texturization reaction might have not been as complete (Kitabatake and others 1985). Therefore, these samples had a higher amount of extractable protein. When the extrusion moisture decreased to 65% and 60%, the amount of extractable protein in each solvent was not significantly different (Table 4), thus indicating these 2 moisture contents probably provided similar conditions for texturization.

Tables 5 and 6 show the relationships between TPA and protein solubility of samples. The results suggested that when moisture content was below 70%, the physical properties of extrudate were not related to most of the chemical bonds in the protein that was extractable by solvent. Thus, the texture differences in samples extruded from 65% and 60% moisture content were probably caused by the product moisture. Shimada and Cheftel (1988) found that the firmness of the gel increased as the protein solubility decreased in the heat induced soy protein isolate gel. The negative relationships between soluble protein and texture properties (Tables 5 and 6) could be explained as the positive relationships between nonextractable protein and texture properties. As the nonextractable protein content increased and the moisture decreased from 70% to 65% and 60%, the hardness, gumminess, and chewiness of the product increased.

Table 4—The Least Significant Difference (LSD) results of protein solubility (%) at different moisture contents

Moisture (%)	P[a]	P+2-Me[b]	P+Urea[c]	P+SDS[d]	P+U+2-Me[e]	P+U+SDS[f]
70	2.05[a*]	16.08[a]	27.19[a]	10.38[a]	72.24[a]	18.74[a]
65	1.87[a]	11.64[b]	20.51[b]	6.51[b]	62.88[b]	10.50[b]
60	1.67[a]	12.07[b]	21.86[b]	7.53[b]	59.75[b]	10.36[b]

*Within each column, values with the same superscript are not significantly different at $p < 0.05$
[a]Phosphate buffer (pH 7.6)
[b]phosphate buffer+2% 2-mercaptoethanol
[c]phosphate buffer+8M urea
[d]phosphate buffer + 1.5% sodium dodecyl sulfate
[e]phosphate buffer + 8M urea + buffer + 2% 2-mercaptoethanol
[f]phophate buffer + 8M urea + 1.5% sodium dodecyl sulfate.

Table 5—The correlation coefficients of Texture Profile Analysis (TPA) and protein solubility of all samples

	P[a]	P+2-Me[b]	P+Urea[c]	P+SDS[d]	P+U+2-Me[e]	P+U+SDS[f]
Gumminess	–0.43	–0.78**	–0.86***	–0.55	–0.79**	–0.79**
Hardness	–0.19	–0.60*	–0.77**	–0.43	–0.71**	–0.65*
Chewiness	–0.43	–0.78**	–0.86***	–0.55	–0.79**	–0.78

*Signficant at $p < 0.05$ level
**significant at $p < 0.01$ level
***Significant at $p < 0.05$ level
[a]phosphate buffer (pH 7.6)
[b]phosphate buffer + 2% 2-mercaptoethanol
[c]phosphate buffer + 8M urea
[d]phosphate buffer + 1.5% sodium dodecyl sulfate
[e]phosphate buffer + 8M urea + buffer + 2% 2-mercaptoethanol
[f]phosphate buffer + 8M urea + 1.5% sodium dodecyl sulfate.

Table 6—The correlation coefficients of Texture Profile Analysis (TPA) and protein solubility of samples extruded at 65% and 60% moisture contents

	P[a]	P+2-Me[b]	P+Urea[c]	P+SDS[d]	P+U+2-Me[e]	P+U+SDS[f]
Gumminess	-0.47	-0.47	-0.81*	0.66	-0.34	-0.35
Hardness	-0.44	-0.44	-0.82*	0.64	-0.35	-0.33
Chewiness	-0.47	-0.46	-0.83*	0.66	-0.37	-0.35

*Significant at $p < 0.05$ level.
[a]phosphate buffer (pH 7.6)
[b]phosphate buffer + 2% 2-mercaptoethanol
[c]phosphate buffer + 8M urea
[d]phosphate buffer + 1.5% sodium dodecyl sulfate
[e]phosphate buffer + 8M urea + buffer + 2% 2-mercaptoethanol
[f]phosphate buffer + 8M urea + 1.5% sodium dodecyl sulfate.

Partial least square (PLS) regression study on protein solubility, extruder responses, and TPA

PLS is one of the multivariate analysis methods dealing with multicollinearity. This method is useful in predicting the responses and finding the relationship between 2 data sets. The reason PLS was applicable in this study was that many of the attributes were highly correlated to each other, such as chewiness and hardness in TPA. In addition, the die pressure correlated to the torque of the extruder and moisture content correlated to the product temperature. The results from the PLS regression illustrated the percentage of the total variance that was explained by the latent factors extracted from these data sets.

From Table 7, it can be seen that when using the data of hardness, chewiness, and gumminess from TPA to predict protein solubility data, the total variance explained by the 5 latent factors extracted by PLS was 61.1%. This indicates that these 2 data sets

Table 7—The Partial Least Square (PLS) regression of protein solubility, extruder responses and Texture Profile Analysis (TPA)

Number of latent factor	Protein solubility	Extruder responses	Texture profile analysis
5	61.1%*	—	100%
4	64.3%	100%	—

*The percentage for each analysis represents the percentage of total variance explained by the latent factors.

could partially represent each other. When using extruder response data to predict protein solubility data, the total variance explained by the 4 latent factors extracted by PLS was 64.3%. The insignificant difference in the amount of protein extracted from the samples extruded from 60% and 65% moisture contents lowered the percentages of total variance, because in both TPA and extruder responses, the effect of moisture content was significant. Although the results of protein solubility analysis were not exactly the same as the other 2 analyses (TPA data and extruder response data), the results from PLS regression were still satisfactory.

In summary, the extruder responses showed changes in percent torque, die pressure, and product temperature. The die pressures were in the range of 418.2 kPa and 1727.3 kPa and the product temperatures, 120.2 °C and 141.1 °C. As the moisture content decreased or the cooking temperature increased, the product temperature increased. The increase in product temperature might have reduced the extrudate's viscosity, which, in turn, resulted in a lower die pressure and a lower percent torque. Both cooking temperature and extrusion moisture content were significant for the TPA attributes of hardness, gumminess, and chewiness. The lower the moisture content, the harder and chewier the product. At the same moisture content, the higher the cooking temperature, the softer and less chewy the product. Protein solubility provided the information on the amount of protein that was supported by the combinations of disulfide bonds, hydrogen bonds, and hydrophobic bonds. According to the TPA results, products that were high in hardness or chewiness had more protein that was not extractable. Extractable protein decreased as extrusion moisture content decreased, indicating the formation of higher molecular weight polymers or intramolecular bonding as the extrusion became more severe. The texture differences in products extruded at 65% and 60% moisture contents were probably due to their moisture content. PLS regression showed that, in general, the data from TPA, protein solubility, and extruder responses correlated well and that these 3 data sets could partially represent each other.

Conclusion

SOY PROTEIN MEAT ANALOG EXTRUDED AT HIGH MOISTURE WAS A simple system to study the relationships among extruder responses, texture and protein solubility. The texture of meat analog could be tailored by controlling the moisture content and cooking temperature during extrusion. Further studies are needed to determine the sensory properties of this meat analog and the potential of using this twin-screw extruder with a cooling die to prepare meat analog with proteinaceous and starchy ingredients other than soy protein isolate and wheat starch.

Materials and Methods

Sample preparation

A 3 (moisture content) ∞ 3 (cooking temperature) ∞ 2 (replication) factorial experiment was conducted to determine the effects of cooking temperature and moisture content on the product properties. A blend of soy protein isolate (SPI) (500E, Protein Technologies Intl., St. Louis, Mo., U.S.A.) and unmodified wheat starch (Midsol 50, Midwest Grain Products, Atchison, Kans., U.S.A.) in a ratio of 9:1 (wet basis) was extruded at 137.8, 148.9, and 160 °C cooking temperatures (CT) and 60%, 65%, and 70% (wet basis) moisture contents using an MPF 50/25 twin-screw extruder (APV Baker, Grand Rapids, Mich., U.S.A.). A barrel length-to-diameter ratio (L/D) of 15:1 was used. The screw profile comprised of (from feed to exit): 225 mm, twin lead feed screw; 25-mm, 90° paddles; 75-mm, single-lead screw; 50-mm, 30° reversing paddles; 100-mm, single-lead screw; 50-mm , 30° forwarding paddles; 100-mm, single-lead screw; 75-mm, 30° reversing paddles; and 50-mm, single-lead screw. Temperature settings from the feeding zone to the cooking temperature were: 26.6, 60, 107.2, 129.4, and CT (°C). To prevent expansion at the die exit, a cooling die with dimensions W ∞ H ∞ L of 30 ∞ 10 ∞ 300 (mm) was used. Cold water (4.4 °C) was used as the cooling media for the cooling die. The dry-mix feed rate was fixed at 6.8 kg/h, and the screw speed was 150 rpm.

Extruder responses

Extruder responses, including die pressure, percent torque, and product temperature before the cooling die, were recorded on-line using Labtech Notebook software (Laboratory Technologies Corp., Wilmington, Mass., U.S.A.) and an RS-232 port.

Texture Profile Analysis (TPA)

The TPA of freshly extruded products was performed by using a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, N.Y., U.S.A.) immediately after the extrusion. A 25.4-mm-square piece with thickness ranging from 8 to 10 mm was compressed to 50% of its original thickness and the springiness, cohesiveness, hardness, and chewiness data were recorded. A cylindrical probe (25.4 mm in dia.) was used for the test. The data from 6 pieces of each treatment was recorded.

Protein solubility

Protein solubility was performed on both raw soy-wheat starch material and extruded products. The following solutions were prepared for measuring the protein solubility according to Burgess and Stanley (1976): (1) 0.035-M, pH 7.6 phosphate buffer solution, (2) 1.5% sodium dodecyl sulfate (SDS) (breaking noncovalent bond and hydrophobic interactions) in the buffer solution, (3) 8-M urea in the buffer solution (interrupting hydrogen bond), (4) 0.1-M 2-mercaptoethanol (2-ME) (breaking disulfide bond) + SDS in the buffer solution, (5) 8-M urea + 1.5% SDS in the buffer solution, (6) 8-M urea + 0.1 M 2-ME.

About 200-g samples were finely chopped with a blender for 1 min. The moisture content of each sample was measured before the extraction. The control sample was prepared by adjusting the moisture content of the SPI-wheat starch mixture to 70%. Two-gram sample from each treatment was extracted with 40 mL of solvent for 2.5 h in a 40 °C water bath with shaking (100 rpm). The suspension was taken and centrifuged at 20,000 g for

40 min (Beckman J2-21M/E centrifuge, Schaumburg, Ill., U.S.A.). The concentration of protein was determined by using a Micro Protein Determination Kit (Sigma Diagnostics, St. Louis, Mo., U.S.A.), measuring the absorbance at 595 nm with a Milton Roy Spectronic 601 spectrophotometer (Rochester, N.Y., U.S.A.). Three concentration standards (15 mg/dL, 30 mg/dL, and 50 mg/dL) were used to plot a standard curve.

Statistical analysis

The data of each treatment except the control sample was analyzed with multivariate analysis of variance (MANOVA), analysis of variance (ANOVA), least significant difference (LSD), and partial least square regression (PLS) in SAS® (1998) program. The main effects on ANOVA included moisture content of extrusion, cooking temperature, and their interactions.

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