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Hydrophobicity of Bitter Peptides from Soy Protein Hydrolysates

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Soy peptides were characterized for flavor, chemical properties, and hydrophobicity to investigate their relationships with bitterness. Five peptide fractions ranging in average molecular mass from 580 to 11300 Da were fractionated by ultrafiltration from two commercial soy protein hydrolysates. The bitterness of fractionated peptides was related to molecular mass, with maximum bitterness observed at approximately 4000 Da for one hydrolysate and 2000 Da for the other. The bitterness increased as the peptide M_w decreased to 3000 Da for the first hydrolysate and to 2000 Da for the second one and then decreased as the peptide M_w decreased below 1000 Da. The peptide fraction with molecular mass of <1000 Da showed the lowest bitterness for both. The hydrophobicity data based on *Q* values do not support Ney's *Q* rule as a predictor of bitterness for soy peptides.

KEYWORDS: Hydrophobicity; peptides; protein hydrolysates

INTRODUCTION

Proteolytic enzyme treatment of food proteins is carried out to improve the chemical, functional, and nutritional properties of proteins. The protein sources most commonly used in producing food protein hydrolyates are casein, whey, and soy proteins. Although the enzyme treatment of proteins provides targeted desirable characteristics, it also introduces undesirable attributes to the products. Among these, bitterness is one of the most objectionable characters. Bitterness has been the major limitation in utilizing protein hydrolysates in various applications, particularly in beverages.

Ney (*1*) hypothesized that the degree of hydrophobicity is the most important predicator of peptide bitterness and established the *Q* rule. Ney's *Q* value is the average free energy for the transfer of the amino acid side chains from ethanol to water ($Q = \Sigma\Delta f/n$). These free energy values were originally applied by Tanford (*2*) for assessing the relative hydrophobicity of amino acids, peptides, and proteins. Ney found that all bitter peptides identified had *Q* values >1400 cal/mol, whereas all nonbitter peptides found at that time had a *Q* value <1300 cal/mol. Between there was no correlation.

This principle is valid for molecular masses of up to ∼6000 Da. Above this limit, peptides with *Q* values >1400 cal/residue are not bitter (*3*). Ney's *Q* rule has been supported by other workers (*4*−*6*). On the basis of Tanford's original data, Bigelow (*7*) calculated the average hydrophobicity of more than 150 proteins. Bigelow and Channon (*8*) recalculated Bigelow's earlier data and compiled a list of 620 pure proteins with their hydrophobicity values. Adler-Nissen thoroughly examined Ney's *Q* values with other reference values including his own (*9*−*11*). Although Ney's Q rule can be applied to the majority of known isolated or synthetic peptides with defined amino acid composition, chain length, and flavor, there are exceptions. Lysine and proline have too high *Q* values for nonbitter peptides (*12*).

Matoba and Hata (*13*) also proposed that the bitterness was caused by peptides with high content of hydrophobic amino acids, regardless of their primary structure. The higher the content of hydrophobic amino acids in a certain protein, the more pronounced its tendency to form bitter peptides. Belitz et al. (*14*, *15*) confirmed that hydrophobicity was essential for the bitter taste and established a quantitative relationship between the bitterness of amino acids, di- and tripeptides, and their hydrophobicity.

There have been no published studies on hydrophobicity versus bitterness of soy peptides fractionated from commercially produced soy protein hydrolysates. Knowledge about the bitter peptides of soy protein hydrolysates may lead to development of new technologies for making nonbitter functional peptides. Good-tasting functional soy peptides can increase applications of soy protein hydrolysates. The objectives of this paper were to fractionate bitter peptides from commercially available soy protein hydrolysates and to investigate a relationship between the bitterness and hydrophobicity of the fractionated soy peptides.

MATERIALS AND METHODS

Materials. *Starting Material.* Commercially available soy protein hydrolysates, Supro 710 (lot E7M J0059) and FP 900 (lot C7M XGN9034) manufactured by Protein Technologies International (PTI, St. Louis, MO), were used as the sources of bitter peptides. Supro 710

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and FP 900 contain 91.6 and 94.2% protein on a dry basis (db, Kjeldahl N × 6.25), respectively. Supro 500E (lot E8HEN0098), also manufactured by PTI, was included as the nonbitter protein control for evaluation of bitterness, molecular weight, and hydrophobicity. Supro 500E containing 92.0% protein (db) is an intact soy protein isolate from which Supro 710 and FP 900 type products could be made.

Fractionation of Soy Peptides. (a) Preparation of Soluble Starting Material for Ultrafiltration. Supro 710 powder was dispersed at 10% in deionized water, heated to 80 °C, and held for 10 min to fully hydrate the protein as well for a pasteurizing effect. The heating condition used here would not cause further heat denatuation in Supro 710 and FP 900 proteins. The protein slurry was centrifuged at 9880*g* for 20 min using a Beckman JA-14 rotor. FP 900 was dispersed at 15% and clarified according to the same procedure as Supro 710. About 21190 g of the soluble fraction of Supro 710 (5.6% solids) and 9560 g of FP 900 (14.1% solids) were prepared. These soluble fractions of Supro 710 and FP 900 were used as the starting materials for fractionation by ultrafiltration. The soluble fraction of Supro 500E was prepared from a 5% slurry under the same centrifuging condition and used as a nonbitter control without the ultrafiltration.

(b) Ultrafiltration Unit. A Prep/Scale-TFF ultrafiltration unit and cartridge membranes with molecular weight cutoffs (MWCO) at 10000 (10K), 5000 (5K), 3000 (3K), and 1000 (1K) were purchased from Millipore, Inc. (Marlborough, MA). The ultrafiltration unit and TFF membranes were assembled, installed, and prepared according to manufacturer manuals. Prior to operation, the cartridge and holder were flushed and cleaned according to the procedure described in the Pep/Scale-TFF Cartridge Maintenance Procedures. Following flushing, the cartridge was measured for normalized water permeability (NWP) and subjected to an integrity test. The ultrafiltration (UF) pump rate, pressure, process time, and a general operating procedure were established for each UF cartridge using the experimental proteins before the test peptide fractions were prepared for the study.

(c) Fractionation of Peptides by Ultrafiltration. Five fractions were prepared using four membranes with MWCO at 1K, 3K, 5K, and 10K. A preliminary test using the actual Supro 710 and FP 900 samples was carried out to determine the optimum protein concentration of the starting material for separation and to check overall performance of the ultrafiltration unit toward protein solution under the recommended operating condition. This allowed optimization of separation efficiency of peptides and estimation of yields for preparing sufficient samples for evaluation.

The operating procedure of the ultrafiltration unit included the following steps: (1) drain the holder, cartridge, and tubing completely; (2) reinstall the cartridge in the holder; (3) place the feed tubing in the feed solution: (4) turn the retentate valve counterclockwise to the completely open position; (5) turn the circulation pump speed control to the lowest speed and turn on the pump; (6) set the circulation pump flow rate to between 1 and 3 L/min depending on the feed solids and membrane MWCO; (7) turn the retentate valve clockwise until the pressure reads 138 kPa (20 psi); (8) filter the sample until the targeted retentate volume is obtained; (9) open the retentate valve all the way and pull the feed tubing out of the feed container; (10) pump the retentate out of the tubing and cartridge and back into the feed container. The retentate was recovered as the greater than the designated MWCO (1−10K) fraction, and the permeate was further processed with the next smaller MWCO membrane cartridge by repeating steps 1−10.

The flow diagram for the UF fractionation process is shown in **Figure 1**. The soluble fractions of Supro 710 and FP 900 prepared by centrifugation were passed through the membrane starting with the largest MWCO membrane cartridge (10K). The retentate and permeate were collected separately. The retentate was recirculated to the feed at 1.5−3.0 L/min to repeat the separation process until a maximum permeate yield was obtained, indicated by decreased permeate flow rate. The permeate from the 10K membrane was then subjected to filtration with the 5K membrane, and the process was repeated until the maximal separation was obtained. In the same fashion, the 5K permeate was passed through the 3K membrane and the 3K permeate was treated with the 1K membrane. Inclusions of the smaller MW fractions in the larger MW fractions were minimized while enough retentates and permeates were collected each step. The same fractionation procedure was used for Supro 710 and FP 900 except that a 2× diafiltration process was employed for fractionation of FP 900 with the 10K and 5K membranes. The 2× diafiltration (a total of three UF cycles) was achieved by adding enough fresh water to the retentates to restore the original volume for the second and third filtration cycles. This was needed mainly because FP 900 is a highly hydrolyzed product with relatively low molecular weight peptides and cannot be fractionated well without diafiltration. The ultrafiltration process parameters obtained are shown in **Table 1**.

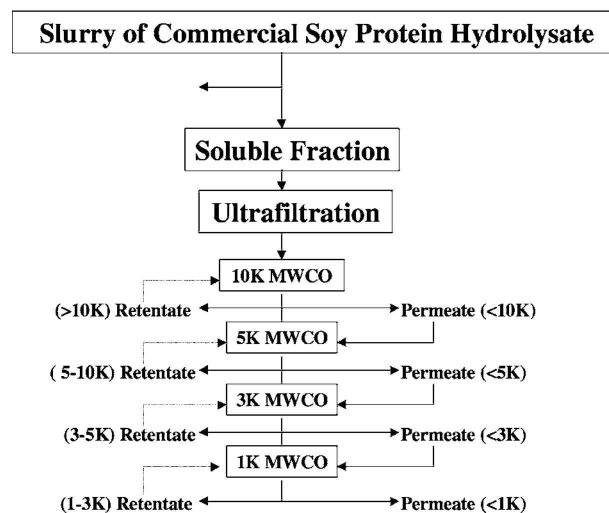


Figure 1. Process flow diagram of peptide sample preparation.

Table 1. Ultrafiltration Process Parameters

UF membrane MWCO	NWP[a] (mL/min)	outlet pressure[b] (kPa)	recycle flow rate (L/min)	total process time (min)	permeate rate (g/min)
Supro 710					
10K	3.940	103.4	3.0	225	14–180
5K	1.321	113.8	3.0	170	18–125
3K	0.992	113.8	1.5	150	32–108
1K	1.021	120.7	1.7	100	51–125
FP 900					
10K	3.940	134.5	2.0	465	20–80
5K	1.321	131.0	2.0	440	38–90
3K	0.992	134.5	2.0	350	50–101
1K	1.021	134.5	2.0	340	50–90

[a] Normalized water permeability. [b] Inlet pressure is 137.9 kPa (20 psi) for all.

The five peptide fractions were prepared and designated >10K (10K retentate), 5−10K (10K permeate−5K retentate), 3−5K (5K permeate−3K retentate, 1−3K (3K permeate−1K retentate), and <1K (1K permeate). All peptide samples including the pretest samples were freeze-dried using a Virtis Freezemobile, model 25XL (The Virtis Co., Gardiner, NY). The <1K peptide fractions of both Supro 710 and FP 900 were concentrated to 5−10% solids using a Rototap laboratory-scale evaporator at ambient temperature before the freeze-drying. The freeze-dried samples were used for all analyses.

Methods. *Evaluation of Relative Bitterness.* The relative bitterness intensity of the UF peptide fractions was determined quantitatively by a bitterness expert panel of 9−12 people and expressed as caffeine equivalent (CE) value. The samples were scored on a scale of 0−100 calibrated with 0−1000 ppm of CE. The scale was extended to 150 or 1500 ppm of CE for those who were more sensitive to protein bitterness. The peptide samples were dispersed at 1.0% protein for the Supro 710 fractions and at 0.15% protein for the FP 900 fractions. A set of two to three samples was presented to the panel as well as the caffeine standard solutions at 0, 200, 500, and 1000 ppm. The panel tasted the samples in comparison with the caffeine solutions and scored them on the scale accordingly. The samples were evaluated in duplicate, one set in the morning and the other in the afternoon.

Table 2. Molecular Mass and Free Energy (Δf) Values of Side Chains of Amino Acids

amino acid	mol mass (g/mol)	Δf^{a} (cal/mol)
alanine	89	500
arginine	174	750
aspartic acid	133	540*
cysteine	121	1000
glutamic acid	147	550*
glycine	75	0
histidine	155	500
isoleucine	131	2950
leucine	131	1800
lysine	146	1500
methionine	149	1300
phenylalanine	165	2500
proline	115	2600
serine	105	300
threonine	119	400
tryptophan	204	3400
tyrosine	181	2300
valine	117	1500

[a] From Bigelow and Channon (*8*) cited from Adler-Nissen (*9*), except for those with *, which are from Tanford (*2*).

HPLC Molecular Weight Profile. All peptide fractions from the ultrafiltration were analyzed for molecular weight distribution (MWD) using an HPLC system (Hewlett-Parkard, Palo Alto, CA). The HPLC system employed two columns tandemly connected to cover a wide molecular weight range, a TSKG3000SWXL column from ToSoHass Co. and a GPC Peptide column from Synchrom (Lafayette, IN). This HPLC system also equipped with a UV detector, an autosampler, an HPLC software program from Hewlett-Parkard (Palo Alto, CA), and M_w calculation software from Polymer Labs (Amherst, MA). The mobile phase was 6 M guanidine hydrochloride (GuHCl) with dithiothreitol (DTT) in 0.1 M phosphate buffer. This mobile phase was designed for dissociating protein completely to its subunit. The M_w standard proteins (all from Sigma Aldrich Chemicals, St. Louis, MO) used for calibrating the columns were phenylalanine (165 Da), cytidine (243 Da), hexapeptide (686 Da), vitamin B_{12} (1355 Da), insulin (5734 Da), aprotinin (6500 Da), cytochrome *c* (12400 Da), myoglobulin (17000 Da), α-chymotrypsin (25700 Da), ovalbumin (44000 Da), and bovine serum albumin (BSA, 66000 Da). Designated amounts (0.500−2.500 mg) of the protein standards were dispersed together in 5.0 mL of the mobile phase. The peptide samples were completely dispersed in mobile phase at 0.5% and centrifuged at 31300*g* for 20 min to remove the insoluble fraction. Both standard protein and peptide samples were filtered through a 0.45 μm cellulose acetate membrane. The filtered samples were transferred to the autosampler for the analysis.

Amino Acid Composition. A total of 18 amino acids was determined by Ralston Analytical Laboratory (St. Louis, MO) using three hydrolysis methods: (1) conventional acid hydrolysis for alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine; (2) oxidative acid hydrolysis for cysteine/cystine and methionine; (3) alkaline hydrolysis for tryptophan.

Hydrophobicity Based on Amino Acid Composition. The hydrophobicity of the fractionated bitter peptides in terms of *Q* value was calculated using two bases: (1) the mole fraction of amino acid residues based on the average chain length (ACL) from the actual average M_w data and (2) the total moles of amino acid residues per 100 kg of protein directly calculated by dividing each amino acid content with its molecular weight. The mole fraction based on the average peptide chain length and the mole concentration were calculated from the amino acid composition and molecular weight of each amino acid shown in **Table 2**. The total number of amino acid residues of a peptide is the sum of mole concentration of each amino acid. The *Q* value is the average free energy for the transfer of the amino acid side chains from ethanol to water ($Q = \Sigma\Delta f/n$), where *n* is the average chain length or the number of total amino acid residues of a peptide used in the calculation.

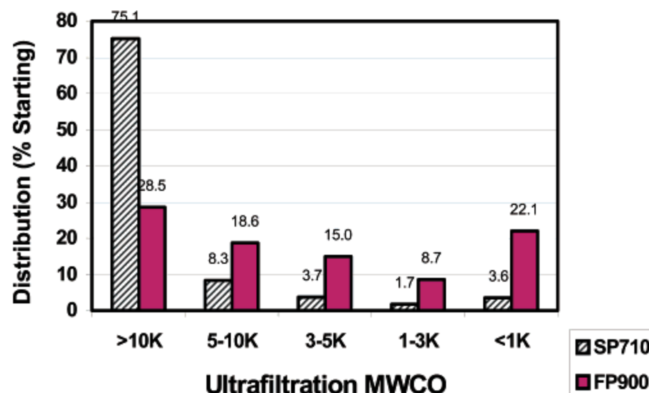


Figure 2. Yield distribution of peptide fractions.

Table 3. Average Molecular Weights of Fractionated Peptides from Supro 710 and FP 900

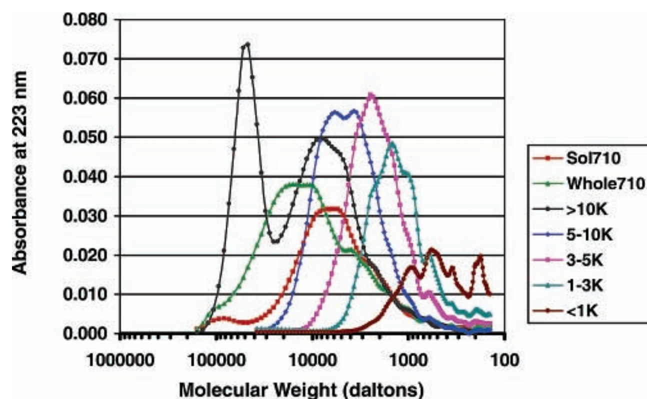
MWCO fraction	ToSoHass TSKG3000[a] GPC Peptide columns		
	280 nm M_w	280 nm M_n	280 nm PD
Supro 500E			
whole	32560	4120	7.86
soluble	30350	3820	7.94
Supro 710			
whole	16830	3630	4.64
soluble	10170	2750	3.70
>10K	11300	3980	2.39
5–10K	3310	1870	1.77
3–5K	1970	1250	1.58
1–3K	1350	900	1.49
<1K	610	460	1.32
FP 900			
whole	3140	980	3.21
soluble	3100	970	3.20
>10K	4600	1700	2.70
5–10K	1910	1190	1.60
3–5K	1180	820	1.44
1–3K	900	680	1.31
<1K	580	500	1.18

[a] This HPLC system used a combination of TSKG 3000 and GPC Peptide columns to cover a wide molecular weight range. The molecular weight data are from the Polymer Lab HPLC molecular weight calculation program. M_w, weight-average molecular weight; M_n, number-average molecular weight; PD, polydispersibility (M_w/M_n).

RESULTS AND DISCUSSION

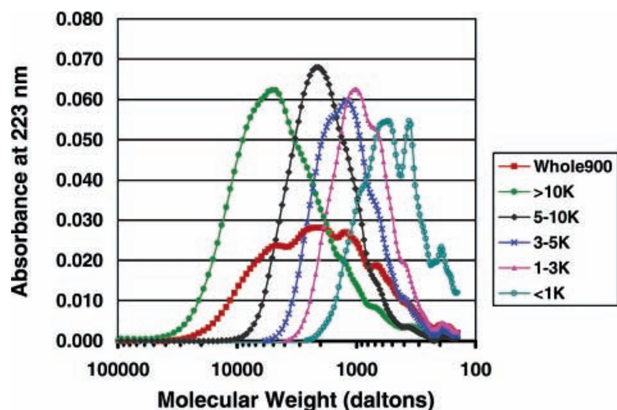
Fractionation of Peptides by Ultrafiltration. Figure 2 compares the yield of each peptide fraction from Supro 710 and FP 900. The yield for each MWCO peptide fraction was calculated on the basis of the total solids recovered and the amount of starting material for UF (i.e., the soluble fraction). The soluble fraction of Supro 710 showed very low yields for the peptides below the 10K MWCO, because Supro 710 is a slightly hydrolyzed protein as indicated by its relatively high M_w (16830 versus 32560 Da average for the intact protein, **Table 3**). About 76% of the total soluble fraction of Supro 710 was above 10K MWCO followed by 8.7% for the peptide between the 5K and 10K MWCO. The fraction between 1K and 3K MWCO showed the lowest yield (1.7%).

On the other hand, FP 900 showed a wider distribution of yield throughout various MWCO ranges. The >10K fraction showed the highest yield with 31.2% followed by the <1K fraction with 22.3%, and the 1−3K fraction had the lowest yield with 9.5%. However, it was expected to see less distinct separation for FP 900 compared to Supro 710 at the 1K-5K MWCO range, because FP 900 is a much more hydrolyzed



Sol710: Soluble fraction of Supro 710 (UF Starting material)
Whole 710: Parent Supro 710 before centrifugation
>10K: Retentate of 10K MWCO
5-10K: Fraction between 5K and 10K MWCO
3-5K: Fraction between 3K and 5K MWCO
1-3K: Fraction between 1K and 3K MWCO
<1K: Permeate of 1K MWCO

Figure 3. HPLC-MWD profiles of Supro 710 peptides (TSKG 3000/GPC Peptide columns).



Whole: Initial FP 900
>10K: Retentate of 10K MWCO
5-10K: Fraction between 5K and 10K MWCO
3-5K: Fraction between 3K and 5K MWCO
1-3K: Fraction between 1K and 3K MWCO
<1K: Permeate of 1K MWCO

Figure 4. HPLC-MWD profiles of FP 900 peptides (TSKG3000/GPC Peptide columns).

product (3140 Da average, **Table 3**) with a relatively narrow molecular mass range (580−3140 Da, **Table 3**).

Molecular Weights of the UF Fractionated Peptides. Table 3 summarizes the HPLC data from the TSKG3000/GPC Peptide columns. The average molecular weights (M_w) from Superdex Peptide 10/30 column were comparable to those of TSKG3000/GPC columns. The molecular weight distribution (MWD) profiles of Supro 710 and FP 900 peptides are shown in **Figures 3** and **4**, respectively. There are significant overlaps between the peptide fractions, and the actual average M_w values did not fall exactly within the expected M_w ranges from the MWCO membranes used. However, the MWD profiles indicate that the UF process with various MWCO membranes effectively fractionated Supro 710 and FP 900 proteins into the peptides with well-differentiated molecular weight ranges to study.

The retentate of the 10K MWCO (>10K fraction) of Supro 710, which is theoretically >10000 Da, indeed showed the average M_w of 11300 Da. The retentates of 5K MWCO (5−10K fraction), 3K MWCO (3−5K fraction), and 1K MWCO (1−3K fraction) from Supro 710 showed average M_w values of 3310, 1970, and 1350 Da, respectively, compared to 610 Da for the lowest M_w peptides (<1K fraction).

Table 4. Bitterness of Fractionated Peptides of Supro 710 and FP 900

UF MWCO fraction	av mol mass[a] (Da)	av bitterness[b,d] (ppm of CE)	rank sum[c]	av rank[d]
Supro 500E				
whole	32360	<100		
soluble	30350	<100		
Supro 710				
whole	16830	307		
soluble	10170	620		
>10K	11300	660b	33	1.8b
5–10K	3310	787a	22	1.2a
3–5K	1970	514c	55	3.1c
1–3K	1350	344d	73	4.1d
<1K	610	274d	87	4.8e
FP 900				
whole	3140	3720b	53	2.9b
>10K	4600	3940b	48	2.7b
5–10K	1910	5200a	23	1.3a
3–5K	1180	3870b	52/11	2.9b/1.0a
1–3K	900	1580c	94/54	5.2c/2.2b
<1K	580	1340c	103/89	5.7d/2.8c

[a] The rounded average M_w values are based on TSKG/GPC Peptide columns. [b] Bitterness (ppm, caffeine equivalent) on 1% protein slurry in water. FP 900 peptide samples were evaluated on 0.15% protein solution due to its strong bitterness, and the result was multiplied by the dilution factor for the bitterness of 1% protein solution. [c] Rank (from the most bitter to the least) sum of duplicate data by 9 panelists for Supro 710 and FP 900 peptides, and another set by 12 panelists for three FP 900 fractions (3–5K, 1–3K, and <1K). [d] Values with the same letter are not statistically different ($\alpha = 0.05$).

The FP 900 peptide fractions showed lower M_w ranges than the Supro 710 fractions from the same MWCO membrane process. This is simply due to the fact that FP 900 is a considerably modified protein primarily composed of peptides with M_w below 5000 Da. The UF starting material of FP 900 showed an average M_w of 3100 Da by the TSKG3000 column. This was fractionated into the average molecular mass ranges at 4600, 1910, 1180, 900, and 580 Da (**Table 3**). As mentioned, the MWD profiles of the FP 900 showed considerable overlaps between the fractions, but overall the peptide fractions exhibited fairly differentiated M_w ranges.

Table 3 also includes M_n (number-average M_w) and PDI (polydispersibility index = M_w/M_n), measuring the breadth of M_w distribution. The higher the PDI value, the more heterogeneous the molecular size. FP 900 with lower average M_w had lower PDI than Supro 710, and the peptides from lower MWCO fractionation had lower PDI values than the higher MWCO peptides.

Bitterness of the UF Fractionated Peptides. Table 4 summarizes the average intensities of bitterness in terms of caffeine equivalent (CE), and the bitterness rank sum and average values of the peptide fractions along with their starting materials and the intact protein, in comparison with their M_w values. As expected, the intact soy protein (Supro 500E) was evaluated to be not bitter for both whole and soluble fractions as indicated by <100 ppm of CE.

The bitterness of the Supro 710 peptide samples measured for a 1.0% protein solution in water ranged from 274 to 787 ppm of CE (**Table 3**). The 5−10K fraction, which is the 10K permeate but the 5K retentate with an average M_w of 3310 Da, presented the most bitterness (787 ppm of CE) followed by the 10K retentate (>10K) with 11300 Da (660 ppm) and the 3−5K

fraction with 1970 Da (514 ppm). The 1K permeate (<1K) with the lowest M_w at 610 Da showed the least bitterness (274 ppm) followed by the 1K retentate (3K permeate, 1−3K) with 1350 Da. The parent whole Supro 710 was less bitter (307 ppm of CE) than its soluble fraction (after removal of the insolubles by centrifugation) used as the starting material for the UF fractionation. This was probably related to the fact that the whole fraction had larger M_w. The Supro 710 UF starting material was similar to its >10K fraction in bitterness. This was expected because the >10K fraction retained ∼75% of the total solubles.

FP 900 is much more bitter than Supro 710 due to its higher degree of hydrolysis that resulted in lower M_w as mentioned. An optimal concentration for the FP 900 peptide samples to yield their bitterness within a measurable range between 0 and 1000 ppm of CE was 0.1−0.2%. Therefore, the bitterness of the FP 900 peptide fractions was measured for a 0.15% protein solution. The average bitterness values were 200−780 ppm of CE and were translated to be 1340−5200 ppm of CE for a 1.0% protein solution (**Table 4**). Among the FP 900 peptide fractions, the 5−10K fraction (10K permeate or 5K retentate) with an average M_w of 1910 Da showed the most bitterness (5200 ppm of CE). The second most bitter fraction was the >10K fraction (3940 ppm of CE) followed by the 3−5K fraction (3870 ppm of CE), the same trend as for the Supro 710 peptides (**Table 3**). However, the bitterness of the >10K and 3−5K fractions is not statistically different. These two peptide fractions showed bitterness similar to that of the parent FP 900 (3720 ppm of CE). The <1K fraction with the lowest M_w at 580 Da showed the least bitterness (1340 of ppm CE) followed by the 1−3K fraction peptide with 900 Da (1580 ppm of CE). The FP 900 peptide fractions showed 5−11 times higher bitterness than their corresponding M_w fractions of the Supro 710 peptides.

The bitterness gradually increased as M_w decreased (or the degree of hydrolysis increased), and then it sharply decreased as M_w further decreased. For both Supro 710 and FP 900 peptides, the 5−10K fraction showed the highest bitterness and the <1K had the lowest bitterness. A similar bell-shape relationship between M_w and bitterness was also observed by (*18*).

Amino Acid Profiles of UF Fractionated Peptides. Protein contents of the Supro 710 peptides varied from 37.6% on a dry basis for the <1K to 70.6% for the 1−3K, 87.6% for the 3−5K, 95.3% for the 5−10K, and 93.1% for the >10K compared to 91.6% for the soluble starting material. For FP 900 peptide fractions, the protein varied from 72.5% for the <1K to 95.9% for the 1−3K, 95.0% for the 3−5K, 97.0% for the 5−10K, 97.3% for the >10K compared to 95.9% for the starting FP 900.

The amino acid (AA) compositions of the Supro 710 peptide fractions based on protein and their moles in 100 kg of protein are shown in **Table 5**. The <1K fraction showed the lowest AA recovery among the peptide fractions, and this might be simply due to its high content of nonprotein nitrogen. Among 18 amino acids, glutamic acid, lysine, serine, and alanine were most significantly changed in level, decreased or increased, by the UF fractionation, whereas aspartic acid, valine, methionine, and tryptophan were least affected.

The seven hydrophilic amino acid profiles (glutamic acid, aspartic acid, arginine, lysine, serine, threonine, and histidine) of the Supro 710 peptide fractions had slightly different decreasing or increasing trends from each other as a function of molecular weight. As expected from the yield, the >10K fraction showed a similar amino acid profile to its starting material, whereas the lower MWCO fractions exhibited more different profiles. The <1K fraction had the most different AA profile from the starting material. Lysine, glutamic acid, and histidine showed a decreasing trend as the M_w decreased, whereas arginine, serine, and threonine had an increasing trend. It was unexpected to see that the 5−10K fraction with the most bitterness had the highest level of glutamic acid, but the lowest levels of the three most hydrophobic amino acids, leucine, phenylalanine, and tryptophan.

Among the hydrophobic amino acid (leucine, proline, phenylalanine, valine, isoleucine, alanine, tyrosine, and tryptophan) profiles of the Supro 710 peptide fractions, proline had a decreasing trend and alanine had an increasing trend as the M_w decreased. No significant difference was observed among the various peptide fractions above the 3K MWCO. The 1K fraction again exhibited the hydrophobic amino acid profile most different from those of the other peptide fractions. Overall, the amino acid profiles of the Supro 710 peptide fractions did not vary much from each other, compared to the FP 900 peptides.

The AA compositions on a protein basis and their mole values of the FP 900 peptide fractions are listed in **Table 6**. The FP 900 peptide fractions were much more altered by the UF fractionation than the Supro 710 peptides, resulting in quite different AA compositions from their starting material and from

Table 5. Amino Acid Profiles of Fractionated Peptides Samples from Supro 710

amino acid	soluble Supro 710	UF fractionated peptide fractions: >10K MWCO	5–10K MWCO	3–5K MWCO	1–3K MWCO	<1K MWCO
		Grams per 100 g of Protein				
alanine	3.39	3.39	3.44	3.78	4.72	5.40
arginine	7.72	7.78	8.03	8.37	9.69	9.80
aspartic acid	10.87	11.03	12.09	11.75	11.17	7.71
cysteine	1.15	0.90	0.88	0.52	0.43	0.46
glutamic acid	24.19	24.64	27.89	24.67	23.54	18.80
glycine	3.78	3.86	4.04	3.85	3.94	3.71
histidine	2.28	2.43	2.08	1.94	1.91	1.40
isoleucine	3.73	3.89	3.64	3.62	3.68	2.66
leucine	6.15	6.29	6.06	6.44	6.65	6.31
lysine	6.64	6.98	7.03	5.99	5.25	2.71
methionine	1.10	1.11	1.21	1.25	1.18	0.83
phenylalanine	4.09	4.18	3.94	4.22	4.57	4.26
proline	5.22	5.45	4.85	4.71	5.14	3.71
serine	4.70	4.62	5.54	5.92	6.45	5.97
threonine	3.06	2.95	3.37	3.82	4.42	3.91
tryptophan	0.96	0.99	0.76	0.96	0.95	0.71
tyrosine	2.84	2.84	2.90	3.38	3.74	3.37
valine	3.76	3.80	4.04	4.30	4.26	3.29
total	95.6	97.1	101.8	99.5	101.7	85.0
		Moles per 100 kg of Protein				
alanine	38.1	38.1	38.7	42.5	53.1	60.7
arginine	44.4	44.7	46.1	48.1	55.7	56.3
aspartic acid	81.7	82.9	90.9	88.3	84.0	58.0
cysteine	9.5	7.5	7.3	4.3	3.6	3.8
glutamic acid	164.6	167.6	189.7	167.8	160.1	127.9
glycine	50.5	51.4	53.9	51.4	52.5	49.5
histidine	14.7	15.7	13.4	12.5	12.3	9.0
isoleucine	28.4	29.7	27.8	27.6	28.1	20.3
leucine	46.9	48.0	46.2	49.2	50.7	48.2
lysine	45.5	47.8	48.1	41.0	35.9	18.6
methionine	7.4	7.5	8.1	8.4	8.0	5.6
phenylalanine	24.8	25.3	23.9	25.6	27.7	25.8
proline	45.4	47.4	42.2	41.0	44.7	32.3
serine	44.8	44.0	52.8	56.4	61.4	56.9
threonine	25.7	24.8	28.3	32.1	37.1	32.9
tryptophan	4.7	4.9	3.7	4.7	4.7	3.5
tyrosine	15.7	15.7	16.0	18.7	20.7	18.6
valine	32.2	32.5	34.5	36.8	36.4	28.1
total	725	735	772	756	777	656

Table 6. Amino Acid Profiles of Fractionated Peptide Samples from FP 900

amino acid	starting FP 900	UF fractionated peptide fractions >10K MWCO	5–10K MWCO	3–5K MWCO	1–3K MWCO	<1K MWCO
		Grams per 100 g of Protein				
alanine	3.67	2.03	2.96	4.13	4.73	6.26
arginine	7.96	10.30	8.25	7.13	6.47	4.75
aspartic acid	11.49	10.40	11.27	13.13	13.31	10.51
cysteine	1.09	1.72	1.30	0.75	0.50	0.28
glutamic acid	24.77	28.22	25.78	23.37	22.44	19.66
glycine	3.81	3.33	3.81	3.86	3.98	4.44
histidine	2.28	3.00	2.38	1.84	1.72	1.49
isoleucine	3.80	2.84	3.56	4.41	4.60	4.33
leucine	6.27	3.96	5.46	6.79	7.51	9.75
lysine	6.81	7.37	7.78	6.78	5.95	5.00
methionine	1.04	0.78	0.93	1.12	1.18	1.43
phenylalanine	4.35	3.12	3.47	4.42	4.98	6.69
proline	5.22	6.73	5.10	4.94	4.77	2.83
serine	4.90	4.05	4.56	4.81	5.41	6.54
threonine	3.28	2.38	3.04	3.70	3.99	4.26
tryptophan	0.87	1.22	0.66	0.65	0.65	0.77
tyrosine	3.11	2.27	2.66	3.27	3.61	4.45
valine	3.91	2.55	3.55	4.76	5.05	4.96
total	98.62	96.26	96.53	99.86	100.86	98.41
		Moles per 100 kg of Protein				
alanine	41.2	22.8	33.3	46.4	53.2	70.4
arginine	45.8	59.2	47.4	40.9	37.2	27.3
aspartic acid	86.4	78.2	84.8	98.7	100.1	79.0
cysteine	9.0	14.2	10.8	6.2	4.1	2.3
glutamic acid	168.5	192.0	175.4	159.0	152.7	133.8
glycine	50.8	44.4	50.8	51.4	53.1	59.2
histidine	14.7	19.4	15.4	11.9	11.1	9.6
isoleucine	29.0	21.7	27.2	33.6	35.1	33.0
leucine	47.9	30.2	41.7	51.9	57.4	74.4
lysine	46.6	50.5	53.3	46.5	40.8	34.2
methionine	7.0	5.2	6.3	7.5	7.9	9.6
phenylalanine	26.4	18.9	21.0	26.8	30.2	40.6
proline	45.4	58.5	44.3	43.0	41.5	24.6
serine	46.6	38.6	43.4	45.8	51.5	62.3
threonine	27.5	20.0	25.5	31.1	33.6	35.8
tryptophan	4.3	6.0	3.2	3.2	3.2	3.8
tyrosine	17.2	12.5	14.7	18.1	19.9	24.6
valine	33.4	21.8	30.3	40.7	43.2	42.4
total	748	714	729	763	776	767

each other. The FP 900 peptides showed a stronger decreasing or increasing trend in the AA values as a function of molecular weight than the Supro 710 peptides. Among 18 amino acids, glutamic acid, arginine, cysteine, histidine, lysine, and proline decreased as the M_w of the peptide fraction decreased, and except for a few all of the other amino acids increased. Glutamic acid, arginine, leucine, phenylalanine, and alanine were the most significantly affected by the UF fractionation, whereas aspartic acid, glycine, and tryptophan were the least affected.

As observed in the Supro 710 peptide fractions, the <1K fraction of FP 900 with the least bitterness contained the highest levels of several hydrophobic amino acids. However, the 5−10K fraction with the most bitterness did not show the same trend observed in the Supro 710 fraction with the highest level of glutamic acid and the lowest levels of leucine, phenylalanine, and tryptophan. Instead, the >10K fraction contained the highest glutamic acid and lowest levels of several hydrophobic amino acids. Among hydrophobic amino acids, only proline decreased as the M_w decreased.

Hydrophobicity Based on Amino Acids. The hydrophobicity of the fractionated peptides of Supro 710 and FP 900 based on the Q value is shown in **Table 7**. The Q values based

Table 7. Hydrophobicity (Q Values) of Fractionated Peptides of Supro 710 and FP 900

MWCO fraction	av peptide chain length	sum of Δf value[a]	Q value[b]	amino acid residues[c]	sum of Δf value[d]	Q value[e]
Supro 500E						
whole	286	331185	1157	772	843748	1093
soluble	269	303176	1127	723	773119	1069
Supro 710						
whole	149	171220	1150	759	825875	1087
soluble	90	97005	1078	725	745662	1029
>10K	100	108522	1086	735	762998	1037
5–10K	29	30096	1028	772	757624	982
3–5K	17	18403	1053	756	756266	1000
1–3K	12	12670	1060	777	778495	1002
<1K	5	5561	1029	656	633419	966
FP 900						
whole	29	29825	1074	748	767020	1026
soluble	28	29775	1063	749	766021	1025
>10K	41	42301	1038	714	719691	1008
5–10K	17	17604	1042	729	728684	1000
3–5K	10	11307	1086	763	789105	1035
1–3K	8	8722	1098	776	806424	1040
<1K	5	5826	1127	767	798873	1042

[a] Sum of the transfer free energy of single amino acid residues from ethanol to water based on the free energy values of amino acids by Tanford (*2*) and Bigelow and Channon (*8*) and the mole fraction of individual amino acids in the average peptide chain length of each peptide. [b] Q value is the average free energy for transfer of the amino acid side chain from ethanol to water. $Q = \Sigma\Delta f/n$, where n is the average chain length of the peptide. [c] Estimated total moles of amino acid residues in 100 kg of protein. [d] Sum of the transfer free energy of single amino acid residues from ethanol to water based on the free energy values of amino acids by Tanford (*2*) and Bigelow and Channon (*8*) and the mole concentration of individual amino acids of each peptide fraction. [e] $Q = \Sigma\Delta f/n$, where n is the total amino acid residues in 100 kg of protein.

on the average peptide chain length (fourth column in **Table 7**) were slightly higher than those based on the total amino acid residues (seventh column in **Table 7**), but overall they were comparable to each other (1028−1157 versus 966−1093 cal/mol). The Q values based on ACL will be used in the following discussion.

Supro 500E (an intact soy protein) and Supro 710 presented very similar Q values of 1157 and 1150 cal/mol, respectively. These values are comparable to literature values for soy isolate (1154−1163 cal/mol) by Adler-Nissen (*9*, *10*). The soy protein has a lower Q value than milk proteins, casein (1399 cal/mol), and whey protein concentrate (1320 cal/mol), making the soy less hydrophobic than casein. The Q values for other proteins are 1181 cal/mol for beef, 1124 cal/mol for fish protein, and 1026 cal/mol for gelatin (*9*, *10*). The Q values have been extensively used not only to compare the protein hydrophobicity but also to explain different bitterness intensities among various protein hydrolysates.

As expected, the soluble fractions of Supro 710 and Supro 500E showed lower Q values than their whole fractions, mainly because they contain less hydrophobic amino acids. The difference between the soluble and whole fraction was more clearly observed in the Supro 710 fractions (1078 versus 1150 cal/mol). However, this difference in Q value did not seem to have a correlation with the bitterness as the soluble Supro 710 was evaluated to be more bitter than its whole fraction. This may be related to the fact that the higher Q value of the whole Supro 710 was contributed by hydrophobic amino acids

contained in its insoluble fraction, which was less exposed to taste buds when relative bitterness tests in slurry were conducted.

The UF fractionated peptides of Supro 710 showed Q values ranging from 1028 to 1086 cal/mol compared to 1078 cal/mol of their starting material. All peptide fractions but the >10K fraction had lower Q values than their starting materials. Among the UF fractionated peptides, the >10K fraction showed the highest Q value (1086 cal/mol), and then the Q values slightly decreased as the M_w decreased. The 5−10K fraction with the most bitterness had the lowest Q value (1028 cal/mol), and this value was very close to that of the <1K fraction with the least bitterness among the peptides. These Q values are much lower than those of small bitter peptides isolated from a soy protein hydrolysate by Lovsin-Kukman (*16*, *17*) ranging from 1440 to 2340 cal/mol. The Q values did not show any correlation with the peptide bitterness in Supro 710 ($R^2 = 0.072$; $p = 0.56$).

The UF fractionated peptides of FP 900 showed a slight increasing trend in Q value from 1038 cal/mol for the 10K fraction to 1127 cal/mol for the <1K fraction as their M_w decreased. Except for the lowest M_w fraction, all showed lower Q values than their starting materials (1063 cal/mol). Overall, the FP 900 peptide fractions showed Q value ranges similar to the Supro 710 fractions except for the <1K fraction being higher, although they were much more bitter. The higher Q value of the <1K fraction was related to its higher concentration of the hydrophobic amino acids. For the FP 900 peptides, the Q values showed a negative correlation with their bitterness, where Q values increased as bitterness decreases ($R^2 = 0.76$; $p = 0.024$). All soy bitter peptides here had Q values lower than 1300 cal/mol. This trend is somewhat contrary to those reported by Ney (*1*) and Guigoz and Solm (*19*), who reported that the bitter peptides had Q values >1300−1400 cal/mol and nonbitter peptides had Q values <1300 cal/mol. However, the soy bitter peptides could have lower Q values compared to casein bitter peptides because soy protein contains fewer hydrophobic amino acids than it does casein. The hydrophobicity data from this study do not support Ney's Q rule as a predictor of bitterness of soy peptides.

Conclusions. Soluble peptides fractionated from commercial soy protein hydrolysates into various molecular mass ranges between 400 and 10000 Da have different bitterness and amino acid compositions from their parent proteins and from each other. The bitterness intensity of the fractionated peptides depends on the degree of hydrolysis of their parent proteins. The bitterness of the soy peptides is predominantly associated with the medium molecular mass range peptides at 1000−4000 Da. The small peptide fractions below 1000 Da are much less bitter than the higher molecular weight fractions. The average hydrophobicity of the soy peptides based on Q values calculated from the amino acid composition and Δf values does not correlate with their bitterness. The data from this study do not support Ney's Q rule as a predictor of bitterness of soy peptides.

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