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Effects of Gluten and Soybean Polypeptides on Textural, Rheological, and Rehydration Properties of Instant Fried Noodles

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Abstract We investigated how the addition of polypeptides to instant fried noodle dough affects the dough properties, starch gelatinization, and textural properties of cup-type instant fried noodles. After comparing farinograph results of 100% wheat flour with 1% wheat flour substituted with gluten, there was a small difference in the mechanical dough properties. However, in the case of 1% wheat flour substituted with gluten peptides, the dough development time increased, dough stability decreased, and weakness increased. On the other hand, when gluten or gluten peptides were added, starch gelatinization did not change significantly. At the steaming stage, substitution with gluten peptides or soybean peptides markedly changed the molecular weight distributions of extractable polypeptides. Especially in the case of wheat flour substituted with 1% gluten peptides, the relative portion of low Mw extractable polypeptides (2.5-50 kDa) decreased more compared to a control. Also, the hardness and chewiness decreased in cooked cup-type instant fried noodles containing gluten peptides. This suggests that the addition of gluten peptides can reduce the rehydration time of cup-type instant fried noodles.

Keywords: instant fried noodle, starch, gluten peptide, soybean peptide, texture, rheology

Introduction

The effects of various additives on dough properties have been extensively studied as indicators of bread quality using farinographic, extensigraphic, and amylographic analysis of dough (1-4). These studies have shown that the addition of wheat starch increases dough hardness (5). The maximum resistance of dough is strongly increased by adding high molecular weight subunits of glutenin, and this resistance is reduced by adding low molecular weight subunits of gliadin (6). Acids strongly influence the mixing behavior of dough, and dough with a lower pH requires a slightly shorter mixing time, and has less stability than normal dough (7). When salt is added to a flour-water mixture, it lowers the water activity and increases the starch gelatinization temperature. Netto *et al.* (8) studied the effect of molecular weight on the glass transition (T_g) of polypeptides, such as fish, whey, and casein peptides, and showed that T_g depends on the source of protein as well as on the degree of hydrolysis (8).

Compared with bread, there are relatively few studies of mechanical characteristics or other indicators related to noodle quality. Although the manufacturing process is similar for bread and noodles, instant fried noodles are made using a different process. Therefore, indicators for the quality of bread are somewhat different from those for instant fried noodles.

Our previous study (9) showed that the steaming process determines the molecular weight (M_W) distribution of extractable polypeptides during the manufacturing processes of cup-type instant fried noodles. Also, low Mw polypeptides during the steaming process were related to the decrease in hardness of the instant fried noodles, which was also related to the reduction in rehydration time. These results suggest that polypeptides affect texture, which is an important quality of instant fried noodles.

In this study, we determined the effects of added low Mw polypeptides on dough properties, final noodle quality, and noodle rehydration. Also, we analyzed the effects of polypeptide addition on the changes in extractable polypeptides and textural properties, especially properties related to noodle rehydration.

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Materials and Methods

Materials Commercial food-grade wheat flour, modified starch, and sodium chloride were used in this study. Commercial wheat flour graded No. 1 contained 10.2% moisture, 0.4% ash, 0.01% crude fat, and 9.28% crude protein, was obtained from Dong-A Milling Co. (Incheon, Korea). Commercial vital wheat gluten containing 7% moisture content, 76% crude protein, and 0.8% crude fat was obtained from Manildra Milling Co. (Sydney, Australia). Commercial isolated soy protein containing 6.5% moisture content, 90% crude protein, and 1% crude fat was obtained from IC Food Co. (Daejeon, Korea). Commercial gliadin and glutenin were obtained from Asama Co. (Yokohama, Japan). Commercial endo-peptidase (the commercial name of this enzyme cannot be listed here due to legal considerations) was obtained from Biocatalysts Co. (Mid Glamorgan, UK); optimum pH 7.5, optimum temperature 65°C. The other analytical reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Production of gluten and soybean peptides Dried gluten was dispersed in water to reach a 10%(w/w) suspension. After pre-heating at 95±2°C for 10 min,

cooling, and adjusting to a pH of 8.0±0.1 using NaOH, endopeptidase was added at 60°C. The enzyme to substrate ratio was 0.004 and reaction time was 2 hr. After digestion, heating the mixture to 95±2°C for 10 min stopped the enzyme reaction. The hydrolyzates were passed through a filter press (40 mesh) and evaporated using a rotary vacuum evaporator (EYELA Co., Tokyo, Japan) at 50±1 °C, 760 mmHg. The concentrate (20.0 °Bx) was spray-dried using a pilot spray dryer (L-8; Okawara Co., Haibara-Gun, Japan) under the following conditions: inlet temperature, 170±2°C; outlet temperature, 98±2°C; and atomizer speed, 12,000±100 rpm. Soybean peptides were made using the same method, except that the initial concentration of the solution was 7%.

Determination of hydrolysis and recovery yield Recovery yield refers to the ratio of the total nitrogen (TN) content of polypeptides to that of raw protein (TN/TN). Also, the degree of hydrolysis (DH) was expressed as the ratio of amino nitrogen (AN) to the total nitrogen of the hydrolyzate (AN/TN). Total nitrogen content was determined using the Kjeldahl procedure (10), and amino nitrogen content was determined using pH stat (Radiometer, Titalab Co., Denmark).

Analysis of size exclusion HPLC Size-exclusion high performance liquid chromatography (SE-HPLC) of extractable polypeptides was performed by the method of Borneo and Khan (11). Briefly, solutions of extractable polypeptides were filtered through a 0.45-μm Millipore membrane, and a 10 mL aliquot was injected on to an SE-HPLC column (Bio-Sil SEC-125 column, 300×7.8 mm; Bio-Rad Co., Hercules, CA, USA). The samples were analyzed using an HPLC system at 214 nm. The elution solvent was 0.05 M sodium phosphate buffer (pH=6.9) containing 0.15 M sodium chloride with flow rate of 0.7 mL/min. A molecular weight standard mixture consisting of tyroglobulin (670 kDa), γ-globulin (158 kDa), ovalbumin (44 kDa), myoglobulin (17 kDa), and vitamin B_{12} (1,350 Da) was used for calculation of the relationship between retention time and molecular weight.

Analysis of amino acids The total amino acid content of samples was determined using the method of Chang *et al.* (12). A 100 mg sample was hydrolyzed using 1 mL of 5.0 N HCl in a Pico-Tag Workstation (Waters Co., Milford, MA, USA). After dilution with water, the sample was passed through a Sep-Pak C_{18} cartridge. A 50 μL volume of sample was dried in a Pico-Tag Workstation. After dissolving the sample in 20 μL of Solution I (water: methanol:triethylamine = 2:2:1), the solution was re-dried. The re-dried sample was combined with Solution II (methanol:water:triethylamine = 7:1:1) and 10 μL of phenyl-thiocarbamyl. After incubation at room temperature for 20 min, the sample was re-dried in a Pico-Tag workstation. The re-dried sample was diluted with 250 μL of sample diluent (Waters Co.). A 10-μL aliquot of solution was used for HPLC analysis using a Waters 510 HPLC system, 486 turnable absorbance detector (detected at 254 nm), 746 data modules, and a Pico-Tag column (150×3.9 mm, Waters Co.) at 45°C. Eluent A and 60% acetonitrile were used as the eluent at a 1 mL/min flow rate.

Analysis of farinography and amylography Farinographs were conducted using a Brabender farinograph (Brabender Co., Detmold, Germany) in accordance with approved methods (13). A 300 g sample of wheat flour (based on 14% moisture content) was mixed with suitable water in a bowl. For treated dough, wheat flour was substituted at 1, 3, and 6.0%(w/w) with peptides or proteins. Amylographs were conducted using a Viscograph-2 (Brabender Co.) in accordance with the method of Medcalf and Gilles (14) with some modifications. The 12% suspension of wheat flour (based on dried weight) was heated and cooled as follows: the temperature was increased at 1.5°C/min from 30 to 95°C, then remained at 95°C for 15 min, and then decreased at 1.5°C/min until it reached 50°C. Treatments were prepared by substituting 1.0% of wheat flour with polypeptides or proteins.

Manufacture of model cup-type instant fried noodles The formula for preparing model cup-type instant fried noodles was as follows: 50.15% wheat flour, 21.50% modified starch, 1.15% sodium chloride, 0.29% alkaline salt, and 26.88% water. The sodium chloride and alkaline salt were dissolved in water. Wheat flour (5.6 kg on a 13.5 % moisture basis) and modified starch were premixed for 5 min prior to the addition of water-dissolved sodium chloride and alkaline salt. The mixing program was 5 min on rapid speed and 15 min on slow. The dough passed through a sheeting roll with a 3.0-mm gap. The sheet was folded in half and the two layers combined by passing again through a 3.0-mm gap. Successive passes through seven roller steps achieved a reduction in the thickness of the dough sheet. The final pass was through a 0.8-mm gap, which was precisely determined using a test piece of dough cut from the main sheet after the previous pass. The sheet was then passed through a cutting roller to produce noodle strands with cross sectional dimensions of 1.2×0.8 mm. The strands were passed through a steaming box with 1.0 kg/cm steam pressure for 2.5 min and cut into 30 cm lengths. The strands were put into a round cup-type mold and fried in palm oil at 147±2°C for 2 min. The resulting packed instant fried noodles weighed 55 g after cooling at room temperature for 60 min, and were then stored in the dark.

Texture profile analysis (TPA) Cooked instant fried noodles were tested using a texture analyzer, TA-XT25i (Stable Micro System Co., Surrey, UK) within 5 min of cooking. Compression was repeated twice with a groove-faced cylinder probe (20-mm diameter) at a probe speed of 2.0 mm/sec, and 90% compression ratio. The force threshold was 10.0 g and the distance between bites was 1.0 mm. Textural properties such as hardness, cohesiveness, springiness, and chewiness were measured from the force-time curve. Each sample property was expressed as the mean of 15 measurements.

Sensory evaluation Trained panelists conducted a sensory evaluation of the rehydration time of cup-type instant fried noodles. Each noodle sample (50 g) was placed in a cup made from propylene resin; these cups are used in commercial packages of noodles called Shin Cup Ramyon (Nong Shim Co., Gyeonggi, Korea). After pouring

boiling water (210 mL) into four cups containing instant fried noodle samples, the noodles were allowed to cook for 2, 3, 4, and 5 min, respectively. The samples were then drained and transferred to other cups. The panelists tasted and evaluated these test samples. Commercial cup-type instant fried noodles (Shin Cup Ramyon) prepared in 4 min according to the standard method served as reference controls. The degree of rehydration of the test samples and controls was evaluated as follows: 0-1, no rehydration; 2-3, weak rehydration; 4-5, moderate rehydration; 6-7, strong rehydration; 8-9, extreme rehydration. Three coded samples were tested in random order. Triplicate samples were statistically analyzed using analysis of variance (ANOVA) and least significant difference (LSD) multiple comparisons. Optimum rehydration time was determined as the shortest time that resulted in an evaluation that was not significantly different from that of the control. Six panelists from the Nong Shim Research Center (Nong Shim Co.) were selected and trained for 2 weeks prior to participating in the study.

Results and Discussion

Enzymatic hydrolysis of gluten and isolated soy protein The total nitrogen (TN) content of the gluten suspension and polypeptides was 1.25 and 1.01%, respectively. Thus, we expressed the recovery yield for gluten peptides as the ratio of the TN content of polypeptides to that of raw protein, which is 81%. The degree of hydrolysis expressed as the ratio of the amino nitrogen (AN) content to TN content was 11.2% because the AN content of gluten peptides was 0.14%. The recovery yield and degree of hydrolysis of soybean peptide were 70.1 and 25.4%, respectively. The free amino acid contents of soybean peptide were two times higher than that of gluten peptide. This suggests that both the enzyme susceptibilities and the compositions of the polypeptides differ between gluten and soy protein.

Characteristics of gluten and isolated soy protein (ISP) The SE-HPLC profile of each polypeptide of gluten and ISP was analyzed. Elution profiles were expressed as the ratio of each area, M1 (>250 kDa), M2 (250-50 kDa), M3 (50-10 kDa), M4 (10–2 kDa), and M5 (<2.5 kDa), to the total area of the SE-HPLC chromatogram (Table 1) based on the standard curve obtained using molecular weight standard markers. Although the portion of M1 and M2 of soybean peptides (17.9%) was higher than that of gluten peptides, both peptides had at least 60% of low molecular weight peptide, i.e., <10 kDa.

As expected, the amino acid compositions of polypeptides from gluten and soybean were extremely different (Table 2). Gluten peptides had high concentrations of glutamic acid (25.7%) and proline (16%). In contrast, soybean peptides had high concentrations of alanine (23 %), glutamic acid (12.9%), threonine (11.5%), and leucine (10.8%). This suggests that the portion of hydrophobic amino acids is higher in soybean peptides than in gluten peptides. In addition, the effects of peptides from these two different sources on the properties of dough or instant fried noodles may be different.

Table 1. Size exclusion high performance liquid chromatography (HPLC) elution profile of polypeptides from vital wheat gluten and isolated soy protein

Polypeptides	Molecular weight distribution (%)[1)]				
	M1	M2	M3	M4	M5
Gluten peptides	4.2	0.9	26.5	30.7	37.7
Soybean peptides	12.6	5.3	21.0	25.1	36.0

[1)]Degree of molecular weight distribution was expressed as the percentage of each area-integrating size exclusion HPLC chromatogram at retention time, which was calculated using the standard molecular weight markers for the total area of the supernatant of the sample. M1, ≥250 kDa; M2, 250-50 kDa; M3, 50-10 kDa; M4, 10-2.5 kDa; and M5, <2.5 kDa.

Table 2. Amino acid composition of peptides of vital wheat gluten and isolated soy protein

Amino acid	Composition (%, w/w)	
	Gluten peptides	Soybean peptides
Aspartic acid	4.1	3.6
Glutamic acid	25.7	12.9
Serine	7.4	2.9
Glycine	5.4	1.4
Histidine	2.7	2.2
Arginine	5.0	0.7
Threonine	4.0	11.5
Alanine	4.1	23.0
Proline	16.0	3.6
Tyrosine	1.3	5.8
Valine	4.0	4.3
Methionine	1.2	5.0
Cysteine	4.2	2.2
Isoleucine	2.4	4.3
Leucine	4.3	10.8
Phenylalanine	5.5	2.9
Lysine	2.7	2.9
Total	100.0	100.0

Characteristics of farinographs of polypeptide-enhanced dough The properties of dough with added proteins or polypeptides were analyzed using farinographs. When gluten, gluten peptides, ISP, and soybean peptides were substituted for 1% wheat flour, dough development time, stability, and weakness clearly changed, even though changes in water absorption may be explained by the increased protein content (Table 3). The addition of gluten peptides increased dough development time from 1.5 to 4.4 min, increased the weakness from 40 to 90 BU, and decreased the dough stability from 7.1 to 5.3 min. In contrast, the addition of gluten did not affect dough properties compared to control dough made with 100% wheat flour. Farinographs of gluten, ISP, and their polypeptides confirmed the difference in effects on dough properties of proteins and polypeptides. The addition of

Table 3. Farinographs of composite flour made with proteins and polypeptides

Protein or polypeptide content in flour (%, w/w)	Water absorption (%, w/w)	Dough development time (min)	Dough stability (min)	Weakness (BU)[1)]
0% (100% wheat)[2)]	59.8[a3)]	1.50[a]	7.10[a]	40[a]
1% gluten	60.6[a]	1.50[a]	7.30[a]	45[a]
1% gluten peptides	62.1[ab]	4.40[c]	5.30[c]	90[d]
1% ISP	63.2[b]	2.20[b]	5.70[bc]	60[b]
1% soybean peptides	62.1[ab]	5.90[cd]	6.00[b]	85[c]
1% glutenin	66.8[c]	2.00[b]	4.00[d]	60[b]
3% glutenin	66.3[c]	2.35[b]	5.00[c]	50[ab]
6% glutenin	66.6[c]	2.10[b]	5.00[c]	55[b]
1% gliadin	66.7[c]	4.10[c]	5.00[c]	60[b]
3% gliadin	68.7[cd]	4.40[c]	4.00[d]	70[bc]
6% gliadin	70.5[d]	4.15[c]	3.50[d]	100[d]

[1)]BU: Brabender unit.
[2)]Control.
[3)]Data followed by different lowercase letters and in the same column are significantly different (ANOVA, $p<0.05$).

ISP increased dough development time, but this result differs from the previously published findings of Bae and Rhee (3). Further studies of the effect of ISP concentration are needed to confirm any differences between control dough and dough with added ISP.

Farinographs were also used to compare the effects of gluten peptides with the additional effect of glutenin or gliadin, which are known as the major polypeptides of wheat gluten. As expected, water absorption increased with increasing amounts of gliadin (6). Dough development time was not affected by the addition of glutenin or gliadin in the range of 1-6%, even though the addition of gliadin yielded a greater dough development time compared with gluten. Dough stability and weakness changed with addition of gliadin, while there were no detectable effects of glutenin addition. For wheat gluten, previous studies have shown that glutenin increased dough strength and loaf volume, while gliadin decreased dough strength (6). However, the addition of isolated gliadin to flour resulted in weaker and less stable dough, as shown by a decrease in mixing time and maximum resistance (15). Isolated gliadin has relatively lower molecular weight than other proteins in wheat flour. Addition of gliadin caused decreasing molecular weight in composite flour and this made stability change in dough. As you can see from the Table 3, the addition of gluten peptides decreased water absorption and increased weakness compared to glutenin or gliadin.

Characteristics of amylographs of polypeptide-enhanced dough The properties of starch gelatinization for protein addition or polypeptide addition were compared with 100% wheat flour using amylographs. When gluten, gluten peptides, ISP, or soybean peptides were substituted for 1% wheat flour, there was little difference in the pasting temperature and peak time among the polypeptides (Table 4), while peak viscosity showed a slight decrease. Therefore, the polypeptides may not influence starch gelatinization, but rather the gluten network of the dough.

Table 4. Amylographs of composite flour types with vital wheat gluten, isolated soy protein, and their polypeptides

Protein or polypeptide content in flour (%, w/w)	Pasting temperature (°C)	Peak viscosity (BU)	Time at peak (min)	Set back (BU)[1)]
0% (100% wheat)[2)]	59.4[a3)]	883[a]	27.0[a]	588[a]
1% gluten	59.6[a]	847[b]	26.4[a]	567[b]
1% gluten peptides	59.4[a]	844[b]	26.5[a]	572[ab]
1% ISP	59.1[a]	860[ab]	26.6[a]	576[ab]
1% soybean peptides	59.3[a]	815[c]	26.5[a]	569[b]

[1)]BU: Brabender unit.
[2)]Control.
[3)]Data followed by different lowercase letters and in the same column are significantly different (ANOVA, $p<0.05$).

Molecular weight distribution of extractable polypeptides in noodles To elucidate the effects of polypeptides during the manufacture of cup-type instant fried noodles, the noodles were made with the addition of various polypeptides, such as gluten peptides, soybean peptides, and gliadin (one of the major polypeptides of gluten). The changes in extractable polypeptides were analyzed by size exclusion HPLC. During the mixing process, there were no significant changes in molecular weight distribution of extractable polypeptides with any polypeptide addition. However, during the steaming, there were changes in certain characteristics such as structure and bonding (Table 5). For controls manufactured without polypeptide addition, the relative amount of high M_W polypeptides (M1 and M2) was great, and that of low Mw polypeptides (M3 and M4) was low. When gluten peptide was added, the relative amount of M3 and M4 decreased compared to the control. Although a similar trend appeared when soybean peptide was added, the increase in M5 was larger than when gluten peptide was added. In contrast, the addition of gliadin resulted in no detectable change in extractable polypeptides during the steaming

Table 5. Molecular weight distribution of extractable polypeptides at the steaming stage in the manufacturing of cup-type instant fried noodles containing polypeptides

Composite[1)]	Storage	Molecular weight distribution (%)[2)]				
		M1	M2	M3	M4	M5
Control	Before steaming	5.9	23.6	34.4	19.4	16.7
	After steaming	24.8**	41.4**	14.1**	8.7**	11.0*
Gluten peptides	Before steaming	5.7	22.4	34.0	18.7	19.2**
	After steaming	33.6**	19.2*	4.4**	7.0**	35.8**
Soybean peptides	Before steaming	5.6	22.4	34.9	18.9	18.2
	After steaming	23.4**	13.9*	3.6**	8.6*	50.5**
Gliadin	Before steaming	6.0	23.6	35.2	18.7	16.5
	After steaming	5.5	24.5	33.9	13.3	22.8**

[1)]Control noodles were made following the model cup-type instant fried noodles formula; 1.0%(w/w) of each polypeptide was substituted for 1.0% wheat flour in the model formula.
[2)]Molecular weight distribution was expressed as the percentage of each area-integrating size exclusion HPLC chromatogram at retention time calculated using standard molecular weight markers to the total area of the supernatant of the sample. M1, ≥250 kDa; M2, 25-50 kDa; M3, 50-10 kDa; M4, 10-2.5 kDa; and M5, <2.5 kDa. * and ** indicate significant differences within each row at $p<0.05$ and $p<0.01$, respectively.

Table 6. Textural properties of cooked cup-type instant fried noodles containing peptides in the compression test

Composite[1)]	Hardness	Adhesiveness	Springiness	Cohesiveness	Chewiness
Control	1035±70[a]	-0.82±0.11[a]	1.09±0.17[a]	0.65±0.03[a]	726±52[a]
Gluten peptides	907±60[b]	-0.77±0.13[a]	1.34±0.17[ab]	0.66±0.02[a]	733±56[a]
Soybean peptides	1017±77[a]	-0.98±0.10[b]	1.61±0.19[b]	0.64±0.04[a]	1025±56[b]
Gliadin	1243±131[c]	-1.57±0.17[c]	1.04±0.08[a]	0.62±0.04[a]	798±71[a]

[1)]Control noodles were made following the model cup-type instant fried noodles formula; 1.0%(w/w) of each peptide was substituted for 1.0% wheat flour in the model formula.
[2)]Determined using a texture analyzer. Each value is the mean of 15 replicates. Different lowercase letters in the same column indicates significant differences (ANOVA, $p<0.05$).

Table 7. Sensory evaluation for rehydration time of cup-type instant fried noodles made with different types of flour

	Composite[1)]			
	Control	Gluten peptides	Soybean peptides	Gliadin
Rehydration time (min)[2)]	4.0	3.0	4.0	5.0

[1)]Control noodles were made following the model cup-type instant fried noodles formula; 1.0%(w/w) of each peptide was substituted for 1.0% wheat flour in the model formula.
[2)]Trained panelists evaluated the rehydration time of cooked cup-type instant fried noodles.

process. The amount and size distribution of extractable polypeptide play a role in determining flour physical dough properties and baking performance (16).

Textural profiles of instant fried noodles The texture profiles of cup-type instant fried noodles made with added polypeptides are shown in Table 6. The effects of polypeptide addition varied depending on the type of polypeptide that was added. The hardness and chewiness of the noodles decreased with the addition of gluten peptides. The addition of soybean peptides increased the adhesiveness, springiness, and chewiness. Addition of gliadin increased the hardness, adhesiveness, and chewiness. The results of gluten peptide addition may have occurred when the interaction of the relatively low Mw of gluten peptides with other polypeptides present in wheat flour, formed not only a higher M_W polymer, but also an extractable polymer, and the gluten network diminished in rigidity. However, in the case of gliadin addition, the unextractable and high Mw polymer formed during the mixing process sustained the rigid gluten network. Schofield *et al.* (17) reported that low-molecular weight proteins including gliadin involve polymerization by sulfide bonding at more than 70°C. This suggests that gluten peptides may reduce the rehydration time of cup-type instant fried noodles, but that gliadin has the opposite effect. In this study, we found that polypeptides can be used as texture changers for cup-type instant fried noodles. In particular, addition of soybean peptide improved springiness compared to control (Table 6). This implies that soybean peptides may improve the elasticity of noodle texture, which is an important property of noodles. However, further studies on sensory evaluation are needed.

Sensory evaluation for rehydration time The sensory evaluation for rehydration time reflected the expectations suggested by the TPA. The instant fried noodles made with added gluten peptides demonstrated the lowest

hardness and the fastest rehydration time (3 min). This suggests that the addition of 1% gluten peptides reduces the rehydration time by approximately 1 min compared to standard cup-type instant fried noodle control. Gliadin addition increased rehydration time by approximately 1 min (Table 7). These results demonstrated that the hard texture of the instant fried noodles was highly correlated with the rehydration of cup-type instant fried noodles.

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