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Effect of Dietary Fiber on the *In Vitro* Digestibility of Fish Protein

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Abstract

***In vitro* digestibility of filefish, protein was substantially decreased by fiber constituents in the following order : pectin (9.97%), gum karaya (7.03%), sodium alginate (6.12%),and cellulose (1.52%). The order of reduction by fibrous residues from vegetables ranked as follows : sea tangle (12.36%), Romaine lettuce (11.12%), perillar leaf (8.96%), and green pepper (5.15%). The inhibitory effect of the dietary fibers towards filefish protein digestion, expressed as soybean trypsin inhibitor equivalents, increased with added levels, but the inhibition differed with the sources of dietary fibers. Sea tangle and sodium alginate were most active in decreasing the concentration of essential amino acid from filefish protein hydrolysis. Sodium alginate exerted an inhibitory effect on the activity of trypsin, but the other fiber constituents did not have an inhibitory potency on trypsin and bacterial protease (*Streptomyces griceus*). Results supported that dietary fiber components may reduce protein digestibility through the interaction of dietary fiber components with filefish protein.**

Key words : *In vitro* digestibility, filefish protein, dietary fiber, sodium alginate, sea tangle, inhibitory potency, enzyme activity

INTRODUCTION

Fish and other seafoods have long been recognized by Koreans as major dietary sources of high quality protein. Most of those seafoods are consumed with vegetables for diminishing fish odor and/or endowing its peculiar flavor. Therefore, vegetables provide Korean seafood dishes with a great variety of flavors, balancing not only their culinary appeal but also their nutritional value. On the other hand, the inclusion of vegetables as dietary fiber sources within a diet containing these seafood proteins may provide beneficial aspects to the consumer's overall health. Although dietary fiber improves some aspects of gastrointestinal functioning[1,2], it can alter digestibility of food proteins, fats, carbohydrates and other micronutrients[3-9].

Interaction between dietary fiber and protein sources have been studied in humans and animals by many authors[4-6]. In general, nitrogen digestibility is decreased, and fecal nitrogen excretion and pancreatic secretions of digestive enzymes are increased when various fiber constituents or fiber-containing materials are added to diet. Effects of dietary fiber on a protein diet have also been established as prolonging chewing time of food, extending satiety associated with the extra volume of bowel contents, and changing the glucose level in blood[10-13]. Most studies of protein digestibility and quality have involved evaluations of the effects of cellulose and other fiber materials on casein as the sole dietary protein source except San Miguel *et al*[9].

The objective of the present study was to investigate the effect of favorite vegetables, such as Ro-

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maine lettuce (*Lactuca sativa*), perillar leaf (*Sesamum indicum*), green pepper (*Capsicum annum*), and sea tangle (*Laminaria japonica*) used in enjoying Saengsun Hwae (sliced raw fish ; "sashimi"), and isolated fiber constituents from those vegetables on the *in vitro* protein digestibility of fish. In addition, several marketed fiber materials (cellulose, pectin, sodium alginate and gum karaya) were also tested with enzymatic *in vitro* digestion of fish protein to ascertain the effect of the presence of the fiber materials on the concentration of the free essenual amino acids from casein and fish protein hydrolysis.

MATERIALS AND METHODS

Source and preparation of sample

Romaine lettuce (*Lactuca sativa*), perilla leaf (*Sesamum indicum*), green pepper (*Capsicum annum*), and sea tangle (*Laminaria japonica*) for dietary fiber sources were purchased at a local grocery. Trimmed and seeds removed (green pepper), vegetables were freeze dried and then reduced to a powder (60~80 mesh) in a micromill (IKA WERK A-10). Powdered samples were stored in sealed glass containers until used for compositional analysis and preparation of fibrous food residues. Cellulose, pectin, sodium alginate, and gum karaya were purchased from Sigma Chemicals. Fibrous food residues (extracted dietary fiber) were prepared from the freeze dried vegetables (60 mesh) by the enzymatic digestion procedure of Hellendoorn *et al.*[14]

Fish myofibril was prepared according to the procedure of Katoh *et al.*[15] To obtain the contaminants eliminated myofibril, a minced ordinary filefish muscle (*Novodan modestrus*) was homogenized in 8 volume of 0.04M Tris-HCl buffer (pH 7.0) containing 0.10M KCl and 1% Triton X-100. The homegenate was centrifuged at 1,200 x g for 20 min. and the precipitate collected. After repeated homogenization followed by centrifugation, the precipitate was freeze dried. Freeze-dried myofibril and the original ordinary filefish muscle were ground to pass through an 80 mesh screen and stored at −20 °C in sealed glass containers until experiments.

Compositional analyses

Moisture, fat, protein ($N \times 6.25$) and ash of fiber sources and fish protein sources were determined following AOAC methods[16]. Neutral detergent fiber (NDF) content of vegetables was determined by the method of Van Soest[17], and acid detergent fiber (ADF) contents were measured by AOAC method[16]. Total soluble dietary fiber was determined using the method of Mongeau *et al*[18].

Protein digestibility procedure

The *in vitro* digestibility was conducted following the 4-enzyme assay of AOAC[19]. The enzymatic assay consists of 37° C hydrolysis by a mixture of trypsin, chymotrypsin, and peptidase, followed by a 55° C hydrolysis by bacterial protease from *Streptomyces griceus*. ANRC sodium caseinate was used for reference protein.

Filefish protein-fibrous food residue mixture were evaluated at residue weight-to-protein weight ratios of 0.5 and 1.0. The filefish protein and fiber materials were mixed in the dry form, and then both substances were hydrated for at least 2 hours before digestion at 37° C[7,8]. The significance of digestibility data was analyzed by the least significant difference (LSD) test.

Assay of inhibitory activities

The inhibitory activity of dietary fiber sources against trypsin was determined according to the modified procedure of Ryu[20] from the method of Rhinehart[21]. Calculation of the inhibitory activity was conducted using a standard curve made from the relationship between the level of soybean trypsin inhibitor and pH drop in sodium caseinate solution(pH 8.0). Results of inhibitory effect were expressed in trypsin inhibitor equivalents, which equals the mg of purified trypsin inhibitor (Sigma) per gram sample.

Free amino acid release upon digestion

The 4-enzymes (trypsin, chymotrypsin, peptidase and bacterial protease from *Streptomyces griceus*) digestion asssy of AOAC[19] were used to determine the effect of the presence of fiber sources on the concentration of free amino acids released upon filefish protein hydrolysis. Free amino acid concentrations in the hydrolyzates were determined by the procedure of Resnick *et al.*[22] on a LKB (4150-α Type) amino acid analyzer.

Measurement of proteolytic enzyme activity

The isolated dietary fiber of 0.5% was added to the Hammarstein casein substrate solution made by the procedure of Anson[23], then compared changing of bacterial protease (*Streptomyces griceus*) and trypsin(from porcine pancreas) activities with casein substrate solution in the absence of isolated dietary fiber. Results were expressed as the percent of relative activity toward to activity for casein solution solely, in the absence of isolated dietary fiber.

RESULTS AND DISCUSSION

Proximate analysis

The proximate analysis of each vegetable used for a dietary fiber source is presented in Table 1. Green pepper (seed removed) contained higher TDF(total dietary fiber) and lower ash than the other dietary fiber sources. Sea tangle showed the lowest level in TDF while it had the highest ash content. The low TDF in sea tangle was probably due to the loss of soluble dietary fiber, such as alginates, during neutral detergent extraction. The crude fat in all samples was less than 10% on a dry basis, and crude protein content ranged from 13.76% (green pepper) to 24.14% (Romaine lettuce).

Dietary fiber content and composition

The crude fiber method is generally based on the extraction with detergents and enzymatic digestion. The neutral detergent fiber (NDF) primarily estimates the structural components of the cell wall and lignin ; the soluble dietary fiber (SDF) must be estimated by other methods. By itself, NDF underestimates the total fiber content of food has a higher content of SDF. Acid detergent fiber (ADF) covers the insoluble dietary fiber (lignin and pectin), and the difference between NDF and ADF provides a rough estimate of the hemicellulose content. The difference between NDF, ADF and SDF of vegetables are shown in Fig. 1. The dietary fiber composition of lettuce was similar to that reported by others[24,25] and there was a slight discrepancy between NDF and ADF with SDF. Raw perillar leaf contained the higher NDF, whereas it showed a lower ADF and SDF. The NDF content in seed removed green pepper was higher than the 5 to 7g/100g dried sample reported for chinese peppers[26], and the difference in content between NDF and ADF with SDF could reflect some loss of SDF during neutral detergent extraction. Sea tangle contained the lowest NDF among the samples used, and could be accounted for by the most soluble dietary fiber as algi-

Table 1. Proximate composition and total dietary fiber content of the vegetables used

% wet basis (% dry basis)

Sample	Moisture	Crude protein (N×6.25)	Crude fat	Crude ash	Total dietary fiber
Romaine lettuce (*Lactuca sativa*)	93.66	1.55 (24.41)	0.56 (8.80)	0.97 (15.26)	1.09 (17.25)
Perilla leaf (*Sesamum indicum*)	88.92	2.09 (18.87)	0.67 (6.06)	1.6 (14.42)	2.56 (23.08)
Green pepper (*Capsicum annum*)	89.65	1.42 (13.76)	0.41 (3.97)	0.57 (5.48)	2.51 (24.21)
Sea tangle (*Laminaria japonica*)	91.91	1.28 (15.84)	0.41 (5.07)	3.69 (45.65)	0.79 (9.75)

nates lost during neutral detergent extraction.

Protein digestibility

The *in vitro* protein digestibility of filefish protein in the absence of marketed fiber materials was 94.6 ±0.4%. This is higher than the 6% reported for raw filefish flesh utilizing the multienzyme method[27]. The higher degree of this digestibility is probably due to the purification of protein during sample preparation. In the presence of the various fiber , materials, a wide range of protein digestibilities for filefish protein were noted (Fig. 2). Cellulose had a slight (1.4~1.5%) decrease on the enzymatic hydrolysis of filefish protein. Pectin, sodium alginate and karaya significantly ($p < 0.05$) decreased the *in vitro* protein digestibility of filefish protein when present at the 0.5 ratio, and caused even some greater reduction at a fiber-to-protein weight ratio of 1.0. The reduction in digestibility by fiber materials ranked as follows : pectin>karaya>sodium alginate>cellulose. Cellulose is generally considered nonreactive with protein due to its linear structure, insolubility and lack of electrostatic side groups. The fiber matrix slightly retarded the substarte-enzyme reaction when the cellulose was tested at the higher levels (Fig. 2). Pectin, a slightly branched galacturonic acid-containing polysaccharide, did substantially reduce the filefish protein digestibility at all levels tested. It is possible that pectin's negatively charged acidic residues (uronic acid residues) interfered with the enzymes and/or substarates, thus reducing the extent of protein hydrolysis[7,8,28,29], although karaya's inhibition on digestibility may be attributed to a high content of glucuronic acid (37%) and its possible interaction with protein. As hydrocolloid, sodium alginate may form gel matrix and/or impart viscosity to the aqueous system through water absorption and colloidal interaction. Thus, sodium alginate caused a comparable reduction in filefish protein digestibility with pectin or karaya[30].

Of the vegetable fibers studied, green pepper fiber constituents showed a slight reduction in filefish protein digestibility to a maximum 5.15% at the 1.0 ratio, intermediate in effect to perillar leaf and Ro-

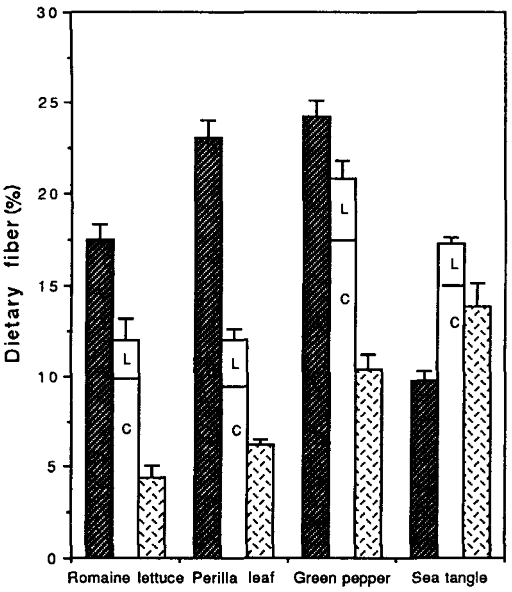

Fig. 1. Dietary fiber content in freeze dried vegetables.
NDF : neutral detergent fiber
ADF : acid detergent fiber
SDF : water-soluble dietary fiber
▨ NDF ▩ SDF
□ ADF L : Lignin C : Cellulose

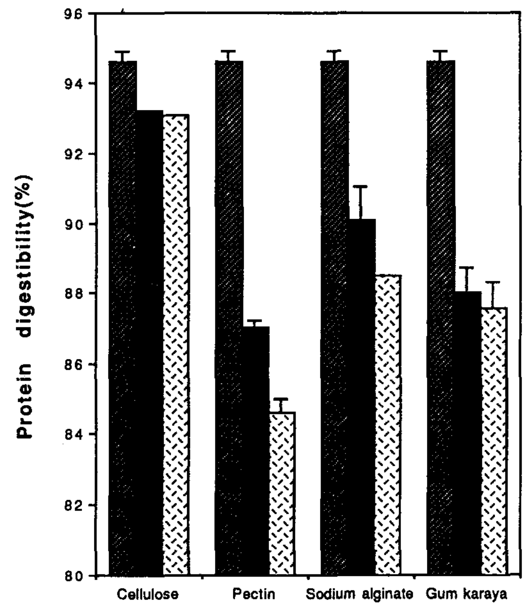

Fig. 2. Comparison of filefish protein digestibility in the presence of various marketed dietary fibers.
▨ Filefish protein
■ Fiber : Protein ratio (wt/wt) = 0.5 : 1
▩ Fiber : Protein ratio (wt/wt) = 1 : 1

maine lettuce. Sea tangle significantly ($p<0.05$) reduced digestibility from 7.89% at the 0.5 ratio, to 12.36% at the 1.0 ratio (Fig. 3). From the *in vitro* filefish protein digestibilities obtained in the presence of various dietary fiber sources (Fig. 2 and 3), it is possible to suggest that the lower cellulose and higher lignin content found in vegetables, the greater will be the reduction in filefish protein digestibility. Based on he findings with sea tangle, filefish protein digestibility significantly decreases as the fiber level or ratio to protein weight increases,

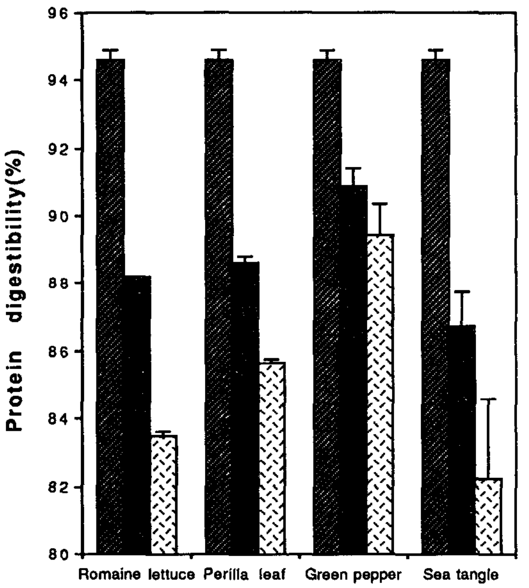

Fig. 3. ***In vitro* digestibility of filefish protein digestibility in the presence of various fiber constituents.**
▨ Filefish protein
■ Fibrous constituent : Protein (wt/wt) = 0.5 : 1
▩ Fibrous constituent : Protein (wt/wt) = 1 : 1

Table 2. Percent decrease in digestibility of filefish (*Novoden modestrus*) protein after interaction[a] with freeze-dried vegetables

Vegetables	Filefish	
	Raw flesh	Myofibrils
Romaine lettuce	5.17	8.45
Perilla leaf	5.82	8.89
Green pepper	6.46	9.12
Sea tangle	3.66	6.89

[a] Interaction for 2 hours at 37°C, filefish : vegetables (wt/wt) = 1 : 1

and the magnitude of the digestibility reduction is dependent on the source and/or constituents of the fiber material such, as ratio of SDF weight to TDF weight. On the other hand, Romaine lettuce, which had the lowest SDF among the samples tested, also decreased protein digestibility as did sea tangle. The result with lettuce indicates that complex lipid materials (cutin and suberin) in leafy vegetables were involved in the digestibility interference[31].

The digestibility of filefish protein (freeze dried raw flesh and myofibrils) in the presence of the ground freeze dried vegetables is given in Table 2. With the exception of sea tangle with raw flesh, significant ($p<0.05$) reductions of digestibility were found in the presence of all vegetables with proteins tested. While each vegetable varies in its insoluble fiber constituents, the largest digestibility decrease was found for green pepper with both filefish proteins.

Inhibition of trypsin by dietary fiber sources

The inhibitory capacities of marketed fiber materials and vegetable fiber constituents against the hydrolytic activity of trypsin towards ANRC casein are shown in Fig. 4. Results of inhibitory potency are expressed in trypsin inhibitor equivalents, which equals the mg of soybean trypsin inhibitor per gram samples. The marketed fiber materials had relatively low inhibitory capacity against trypsin, and Romaine lettuce fiber exhibited the highest capacity among the substances examined ($p<0.05$). In view of the observed inhibitory potency of these dietary sources against protein digestion, the higher inhibitory potency possesed fiber sources resulted in the greater reduction in protein digestibility. Although sea tangle did not have higher inhibitory potency than Romaine lettuce, its viscous materials, mainly alginates, may hinder enzymatic hydrolysis of protein through colloidal interaction[32]. For purpose of discussion of the other factors on digestibility reduction, fiber sources had been added to the enzyme solutions prior to the mixing with ANRC casein (Table 3). A slight, but not significant ($p>0.05$) decrease in the protease and trypsin-inhibitory activity of all dietary fiber sources was noted. The

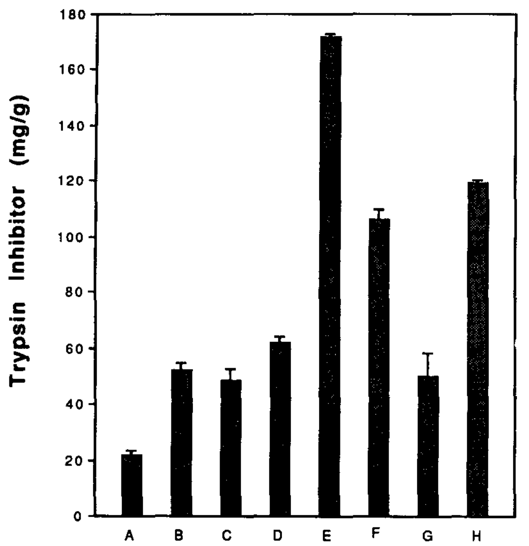

Fig. 4. Inhibitory potency[a] of dietary fiber sources towards the digestion of ANRC casein.
a : Soybean trypsin inhibitor equivalents
A : Cellulose B : Pectin C : Sodium alginate
D : Grum karaya E : Romaine lettuce F : Perilla leaf
G : Green pepper H : Sea tangle

Table 3. Effects of dietary fiber materials on protease activity (Relative activity %)

Fibers	Protease (*Streptomyces griceus*)	Trypsin
Control[a]	100.0	100.0
Cellulose	97.8	96.3
Pectin	100.8	98.5
Sodium alginate	87.8	118.5
Gum karaya	98.7	101.2

[a] Activity on ANRC casein in the absence of fiber materials

results in this study suggest that the reduction in protein digestibility by the dietary fiber sources may come mainly from their interaction with the substrate, not through their inhibition against enzyme activity[30,33,34].

Free essential amino acid release

The quantity of essential amino acids from filefish protein hydrolysis using the multienzyme digestion method in the presence of several fiber materials is given in Table 4. Sodium alginate was most effective in decreasing the concentration of essential amino acids, and pectin ranked second among the fiber materials used. Cellulose had minor effects, decreasing only valine by more than 40%. In the vegetable fiber constituents, sea tangle was most effective in decreasing essential amino acids from filefish protein-fiber complex through enzyme hydrolysis. Total concentration was reduced by more than 75% by sea tangle fiber constituents. There were few similarities among the dietary materials with respect to the specific amino acid reduced; isoleucine and valine were severely ($p<0.05$) affected. The severe decrease (>50%) of essential amino acids, compared to the previous report[8], could reflect that some free amino acids released at the first step of multienzyme hydrolysis had been bound by the fibrous matrix which was also removed with the larger peptides during precipitation of free amino acid assay.

Table 4. Essential amino acids from filefish protein-dietary fiber complex through enzymatic hydrolysis (mg/100g sample)

Amino acids	Dietary fibers								
	Control	A	B	C	D	E	F	G	H
Thr	89.15	67.03	ND	ND	52.01	82.01	22.29	44.58	29.72
Met	3.71	ND	ND	ND	ND	ND	ND	ND	1.49
Val	185.74	103.54	42.19	7.43	66.86	126.30	44.58	ND	14.86
Ile	74.29	74.71	28.13	ND	74.01	96.58	52.01	59.44	14.86
Leu	326.89	323.49	274.26	193.16	237.74	230.62	193.16	222.88	96.58
Phe	260.03	189.87	133.61	66.86	141.16	245.34	141.16	148.59	59.44
Lys	170.88	181.29	70.32	66.86	81.72	163.45	133.73	126.30	52.01

A : Cellulose B : Pectin C : Sodium alginate D : Gum karaya
E : Romaine lettuce F : Perilla leaf G : Green pepper H : Sea tangle
ND : not detected Control : Filefish protein solely used in enzymatic hydrolysis

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식이 섬유소가 어류단백 소화율에 미치는 영향

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요 약

단백소화율에 미치는 식이 섬유소의 영향에 대하여 알아보기 위해, 채소류(상치, 깻잎, 고추, 다시마)로 부터 추출한 식이 섬유소와 시판용 정제 식이 섬유소 (cellulose, pectin, sodium alginate, gum karaya)를 어류 단백질인 말쥐치 단백질(냉동건조육 및 myofibrils)에 첨가 반응시켜, 단백질의 소화율에 어느 정도 영향을 미치는가에 대해 알아보았다. 각 시료의 중성세제 추출섬유소 (neutral detergent fiber) 함량은 24.21%(고추) 9.75%(다시마)의 범위였고, 산성세제 추출섬유소 (acid detergent fiber) 함량은 20.85%(고추) 11.97%(깻잎)의 범위였으며, 수용성 섬유소 함량은 13.79%(다시마) 4.41%(상치)의 범위였다. 말쥐치 단백질에 대한 식이 섬유소의 반응 비율을 1 : 1 (wt/wt) 로 하고, 37°C에서 2시간 동안 반응시켰을 때, 말쥐치 단백소화율은 정제 식이 섬유소 첨가의 경우, 1.52%(cellulose) 9.97%(pectin)가 감소되었고, 추출한 식이 섬유소 첨가의 경우, 5.15%(고추) 12.36%(다시마)가 감소되었다. 섬유소의 trypsin 활성저해능은 단백소화율이 감소함에 따라 증가하여, ANRC casein에 대한 soybean trypsin inhibitor 22mg/g (cellulose) 61.82mg/g (gum karaya), 49.75mg/g(고추) 171.52mg/g(상치)에 상응하는 것으로 나타났다. 정제 식이 섬유소에 의한 단백분해효소의 활성 변화는 sodium alginate를 제외하고는 거의 없어, 어류 단백소화율의 저하는 식이 섬유소가 단백질에 직접 결합하여 비소화성 물질을 형성한 결과가 주도하리라 생각되었다. 말쥐치 단백질과 섬유소를 반응시킨 것을 효소 가수분해시킨 후에 측정한 유리 필수 아미노산의 함량은 sodium alginate와 다시마 섬유소의 경우 현저하게 저하하였으며(75% 이상), isoleucine과 valine이 크게 영향을 받았다.