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Physiological differences of soluble and insoluble dietary fibre fractions of brown algae and mushrooms in pepsin activity in vitro and protein digestibility

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Soluble and insoluble dietary fibre fractions were separated from Konbu, Wakame and Hijiki seaweeds and Shiitake, Hiratake and Yanagimatsutake mushrooms, respectively, and the effects of the fractions on pepsin activity in vitro and of those from Wakame on apparent protein digestibility in vivo were studied. Addition of each dietary fibre fraction inhibited pepsin activity in vitro in all the dietary fibre fractions tested, particularly the inhibition by soluble dietary fibre fractions being significantly greater, by 62-99%, than that by insoluble dietary fibre fractions, by 22-36% ($P < 0.01$ in each food). This suggests that soluble dietary fibres in algae and mushrooms are likely to play a different physiological role from insoluble dietary fibres. Measurement of viscosity of each soluble dietary fibre fraction resulted in the correlation of viscosity with the inhibition of pepsin activity by the soluble fraction. Young adult rats given a normal protein diet containing 5% of the soluble dietary fibre fraction derived from the Wakame seaweed showed a greater decrease in apparent protein digestibility by 9.4% than those given the diet containing 5% of the insoluble one ($P < 0.01$). This may have resulted in the significantly lower body weight gain of the former rats than that of the latter rats.

Introduction

We recently reported[1] that the soluble, insoluble and total dietary fibre contents of some traditional Japanese seaweeds and mushrooms were determined using the method of Prosky et al.[2] without the enzymes involved. The seaweeds showed high values of soluble dietary fibre, 40-60% on dry matter basis, while the mushrooms showed high values of insoluble dietary fibre, more than 90%. Some purified soluble dietary fibres are likely to exert physiological effects which are different from those of insoluble dietary fibres[3-6], and are summarised to have inhibitory effects on digestive proteolytic enzymes and protein digestibility in vitro[7,8]. Further, it has been suggested that the purified soluble dietary fibres with viscous property might have the inhibitory potency of protein digestion[6,9]. However, the physiological effects of mixed soluble or insoluble dietary fibres which could be separated from seaweeds and mushrooms are not well documented.

In the present study, soluble and insoluble dietary fibre fractions were separated from three seaweeds-- Konbu, Wakame and Hijiki, and from three mushrooms-- Shiitake, Hiratake and Yanagimatsutake, based on the method of Prosky et al.[2] modified by the authors[1]. The effect of each dietary fibre fraction on pepsin activity in vitro was examined. Then, viscosity of each soluble fraction was measured in relation to the inhibition of the pepsin activity in vitro. Further, to test the in vivo effect of the dietary fibre fractions, apparent protein digestibility was measured in rats fed normal protein diets containing the soluble or insoluble dietary fibre fraction separated from the Wakame seaweed.

Methods

Separation and purity of soluble and insoluble dietary fibre fractions

Separation of both fractions was carried out using the method of Prosky et al.[2] modified for algae and mushrooms by the authors[1] with no use of enzymes needed, which is useful to control contamination of the enzymes used in the fractions. Three traditional Japanese foods of brown algae, Konbu, Wakame and Hijiki, packed in the dried state, and three of raw mushrooms, Shiitake, Hiratake and Yanagimatsutake, were purchased at shops in Nagoya and Okazaki in Japan. The brown algae, as they were, and the fungi, after freeze-drying, were milled to pass through a 0.35mm mesh sieve and kept in a dessicator. The powdered sample with 0.05M phosphate buffer, pH 6.0,(brown algae, 0.3%; mushrooms, 2%) were heated at 97°C for 30 minutes. After being adjusted to pH 4.5 with phosphoric acid, the contents were centrifuged at 3,000 rpm, the supernatants, precipitated with 95% ethanol (60°C), were centrifuged, washed with ethanol and acetone and dried for soluble dietary fibre fraction (SDF). Residues were washed with ethanol and acetone and dried for insoluble dietary fibre fraction (IDF).

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Each dried fraction contained considerable amounts of Kjeldahl nitrogen, 0.5-1.0% for SDFs and 2.4-6.1% for IDFs, from the brown algae, and 2.4-4.0% for SDFs and 2.7-6.7% for IDFs, from the mushrooms. Treatment of each fraction with protease (No.A-3910, Sigma) for four hours showed no reduction of its Kjeldahl nitrogen content, which probably indicates that the nitrogen in the SDFs and IDFs is not derived from protein but presumably from nitrogen containing dietary fibre such as chitin. Considerable amounts of chitin, which is a nitrogen containing dietary fibre, in the mushrooms, 8.88% for Shiitake, 4.99% for Hiratake, are reported[1]. The nitrogen content of ethanol precipitates from Shiitake fungus[1], 0.72% is nearly consistent with the chitin nitrogen. 0.61%[10], on dry weight basis of the food.

Pepsin activity in vitro determination

Pepsin activity was measured basically by the method of Anson[11] with minor modifications[12]. The enzyme system of 2ml of pepsin aqueous solution with 1ml of 2% haemoglobin in 0.06N HCl (pH 1.5) was incubated at 37°C for 3 minutes. After stopping the reaction with the addition of 5% trichloroacetic acid, the contents were centrifuged and the phenol reagent positive materials of the supernatant were determined colorimetrically to serve as controls. To test the effect of each dietary fibre fraction, powdered dietary fibre fraction, 5 to 15mg from brown algae, 5 to 30mg from mushrooms were added to the enzyme solution. The SDF from the seaweeds made the solution nearly solid at more than 10mg added, and the SDF from the mushrooms did so at more than 15mg. The pepsin activity was assayed in the same manner as described above. Each assay was carried out in quadruplicate. We expressed here a decrease in pepsin activity as an 'inhibition' since Houck[7] had evidenced enzymologically that carrageenan were able to inhibit the pepsin activity in vitro. An inhibition rate (%) was computed by subtracting the latter activity from the controls.

Measurement of viscosity of the soluble dietary fibre fractions

Viscosity of samples was measured by the E Type Viscometer (Toki Sangyo, Tokyo, Japan) in the conditions close to the pepsin solution. Each sample was made a solution or a suspension in 1ml of water at 37°C and its viscosity was measured. The rotatory speed of the corn, 50rpm was adequate. Samples of insoluble dietary fibre fraction were impossible to measure. The value for viscosity was expressed as m Pa.s (mega Pascal second).

Measurement of apparent protein digestibility in rats

Fourteen young adult rats of Wistar strain were purchased commercially. They were housed in individual, suspended, wire-mesh stainless cages in a room maintained at approximately 25°C with alternate 12-hour periods of light and dark. The rats were provided diets and water ad libitum. They were fed a basal diet[13] containing 20% of casein until they attained a body weight of 270 to 300g, then divided randomly into two groups of 7 animals each. Rats of each group were given the normal protein diet containing 5% of SDF or IDF fraction separated from Wakame seaweed for seven days. The test diets contained the following ingredients, as percentage weights: fe-starch from potato, 60; casein, 20; corn oil containing 100mg of vitamin E, 10; mineral mixturel3, 4; vitamin mixturel3, 0.85; choline chloride 0.15; SDF or IDF fraction, 5. Food intakes for the last three days were recorded, and faeces excreted for the same period were collected in metabolic cages. Their nitrogen contents were analysed by the semi-micro Kjeldahl method. To determine apparent digestibility of dietary protein, absorbed nitrogen and intake of protein nitrogen were computed using the measured nitrogen of faeces excreted and that of food intake. The true nitrogen intake for dietary protein was estimated by subtracting the nitrogen from SDF or IDF contained in the diet from the total nitrogen intake.

Results and discussion

Effect of dietary fibre fractions on pepsin activity in vitro

When 5 to 15mg of the SDFs or IDFs from the three seaweeds were added to the enzyme system, pepsin activity in vitro was inhibited as the added amount increased (Figure 1). The inhibition was significantly greater in the SDFs than in the IDFs of the seaweeds ($P < 0.01$), and particularly, the inhibition by the Konbu's SDF was greatest amongst the seaweeds tested. A similar relation was seen in the three mushrooms when 5 to 30mg of each dietary fibre fraction was added to the enzyme system (Figure 2). The inhibition by the Hiratake's SDF was greatest amongst the mushrooms tested. The strong inhibition of pepsin by the soluble fractions is similar to another finding[7] regarding a purified soluble dietary fibre of carrageenin inhibiting pepsin activity in vitro.

The authors observed that the addition of dried powders of the seaweeds and mushrooms (final concentration of each powder in the enzyme system, 5% w/ v) inhibited the enzyme activity[12]. The inhibitions by the three seaweed powders were significantly greater than those by the three mushroom powders. This can be elucidated by the effects of the SDFs and IDFs involved in these foods. Thus, the soluble dietary fibres of brown algae and mushrooms are considered to have different inhibitory roles from insoluble dietary fibres in protein availability in the gastrointestinal tracts. However, whether the inhibition of pepsin activity in vitro is related or not to a decrease in protein digestibility in vivo remains unclear.

Correlation of the pepsin activity inhibitions by soluble dietary fibre fractions to their viscosities

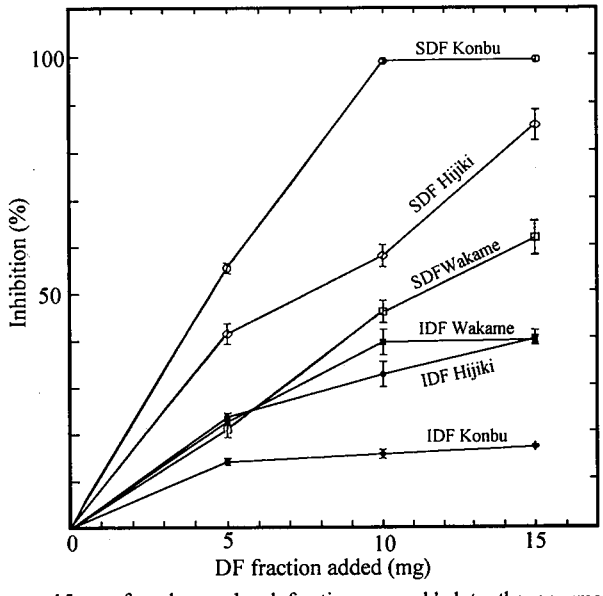
As shown in Table 1, the higher the concentration of SDF in the enzyme system, the greater its viscosity and pepsin activity inhibition. Consequently, a close correlation has been observed between viscosity and pepsin activity inhibition in the six foods. The inhibition of pepsin activity in vitro by soluble dietary fibre fractions might be attributed to their viscous property. We demonstrated here the association between SDF and IDF preparations or the viscosity of SDF preparations and the in vitro inhibition of pepsin. However, there is a possibility that both preparations might be contaminated with cell wall components which might be responsible for the observed inhibition of the enzyme or viscosity. However, we have no suitable method to detect and separate them from dietary fibres at present. This deserves further investigation.

Table 1. Viscosities and inhibitions of pepsin activity in vitro by the soluble dietary fibre fractions from seaweeds and mushrooms as a function of concentration of sample added and their relations.

Food Name		Concentration of sample added					Correlation!
Seaweeds:		0.15%	0.25%	0.35%	0.50%	0.75%	
Konbu	Viscosity (x)*	1.632	2.076	2.928	4.308	7.332	y = 52.2 + 5.25x
	Inhibition (y)#		55.3		98.9	99.1	γ = 1.862 !
Wakame	Viscosity (x)		3.072	6.072	11.39		y = 16.6 + 2.19
	Inhibition (y)		21.0		45.8	61.6	γ = 0.983
Hijiki	Viscosity (x)		0.888	0.996	1.152	1.296	y = -52.4 + 102.4x
	Inhibition (y)		41.3		57.8	85.4	γ = 0.952
Mushrooms:		0.25%	0.50%	0.75%	1.00%	1.50%	
Shiitake	Viscosity (x)		3.012	3.672	5.280	8.220	y = 28.5 + 22.9x
	Inhibition (y)	0.00	63.0	67.1	88.0	97.8	γ = 0.954
Hiratake	Viscosity (x)		2.376	2.532	2.844	2.988	y = 44.4 + 68.5x
	Inhibition (y)	0.00	82.6	87.1	94.1	96.7	γ = 0.997
Yanagimatsutake	Viscosity (x)		1.944	2.112	2.088	2.424	y = -85.3 + 70.5x
	Inhibition (y)	0.00	51.5	54.2	70.8	86.1	γ = 0.885

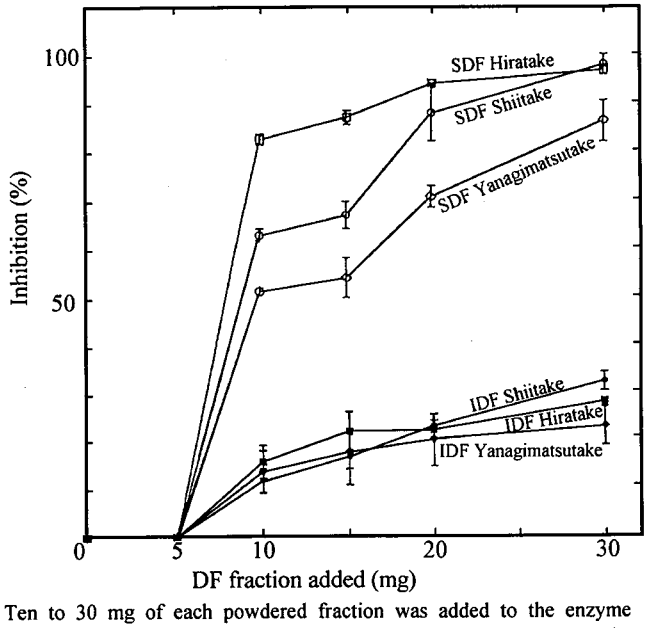
*: m Pa.S #: % of inhibition ! γ: Coefficient of correlation

Figure 1. Inhibitory effects of adding soluble (SDF and insoluble (IDF) dietary fibre (DF) fractions separated from Konbu, Wakame and Hijiki on pepsin activity.



Five to 15mg of each powdered fraction was added to the enzyme system, which was incubated for 3 minutes at 37° C at pH 1.5. The inhibition by SDF was significantly higher ($p < 0.01$, by t-test) than by IDF in each food. Vertical lines indicate standard deviation.

Figure 2. Inhibitory effects of adding soluble (SDF) and insoluble(IDF) dietary fibre (DF) fractions separated from Shiitake, Hiratake and Yanagimatsutake on pepsin activity.



Ten to 30 mg of each powdered fraction was added to the enzyme system which was incubated for 3 minutes at 37° C at pH 1.5. The inhibition by SDF was significantly higher ($p< 0.01$, by t-test) than by IDF in each food. Vertical lines indicate standard deviation.

Table 2. Body weight gain and apparent digestibility of protein in young adult rats given normal diets containing a 5% level of the soluble (SDF) or insoluble (IDF) dietary fibre fraction separated from Wakame seaweed.

Diet	Wakame SDF	Wakame IDF
Number of rats	7	7
Body weight gain (g)[a]	11.8±4.8[c]	17.2±4.0
Total N intake (mg)[b]	655±62	813±125
Faecal N excretion (mg)[b]	91±29c	153±23
Protein N intake-A (mg)[b]	632±65	669±111
Absorbed protein N-B (mg)[b]	564±54	660±113
Apparent protein digestibility-B/A x 100 (%)[b]	89.2±4.6[c]	98.6±1.5

a) for 7 days. b) for the last 3 days. c) Significantly lower than the IDF group by the student t-test ($P < 0.01$)

Effect of the dietary fibre fractions from Wakame seaweed on apparent protein digestibility in vivo

To test the relationship between the inhibition of pepsin activity in vitro and the digestibility of dietary protein in vivo, young adult rats were given a normal protein diet containing the SDF or IDF from Wakame seaweed, as a representative of the seaweeds and mushrooms used, for seven days. Apparent protein digestibility for the last three days was determined. As shown in Table 2, both nitrogen intake and faecal nitrogen excretion for the last three days were larger in the IDF group than in the SDF group. There is a possibility that the difference in the nitrogen intake derived from the IDF and SDF might contribute to this, because the nitrogen content of the IDF and SDF was different as described in the methods. Therefore, the true nitrogen intake of dietary protein was estimated by

subtracting the nitrogen intake of SDF or IDF from the total nitrogen intake. On the other hand, absorbed protein nitrogen was computed by subtracting faecal nitrogen from total nitrogen intake. Subsequently, apparent protein digestibility (%) was estimated by dividing absorbed protein nitrogen by protein nitrogen intake.

As shown in Table 2, total nitrogen intake of the SDF diet group tended to be smaller than that of the IDF diet group. This is not due to the difference in preference for the diets but to the difference in the nitrogen from the fibre fraction included in the diet because protein nitrogen intakes estimated as above in both groups were similar. On the other hand, absorbed nitrogen of the SDF diet group tended to be smaller than that of the IDF diet group. Consequently, rats given the SDF diet showed a significantly lower protein digestibility, 89.2% ($P<0.01$) compared to rats given the IDF diet, 98.6%. The value for the apparent casein digestibility particularly in the IDF group seemed somewhat higher compared to the data summarised by Gallaher et al.[8]. but Hove and King[15] also reported that the fairly high apparent digestibility of casein, 95-96% when diets of 22% casein level with 0-5% levels of cellulose were administered to rats. Though we did not determine here the digestibility of casein in a diet with no fibre fraction since the present objective was the comparison between the SDF and IDF from the Wakame seaweed, the present animal experiment has been appropriately conducted to see the difference between the SDF and IDF diets. The lowered digestibility might be associated with the findings that a highly viscous polysaccharide, sodium alginate, which constitutes a greater part of soluble dietary fibre in seaweeds, decreased the digestibility of protein in growing rats[6] and that soluble dietary fibres decreased trypsin activity in vitro[14]. The lowered protein digestibility by the SDF from the Wakame seaweed may have resulted in a significantly lower body weight gain of the SDF group compared with that of the IDF group (Table 2).

Conclusion

From the present results of the in vitro and in vivo experiments, it can be concluded that soluble dietary fibres with the viscous properties of seaweeds and mushrooms, which are different from insoluble fibres, can lower the availability of dietary protein. This finding may support the notion that soluble fibres in foods are likely to decrease in protein utilisation since some other purified soluble dietary fibres such as pectin and guar gum decreased not only protein digestibility but also nitrogen retention in less amounts than insoluble fibres[8]. The importance of this finding in human nutrition is that we should take into consideration the low availability of protein, particularly when dietary protein intake is not adequate. Japanese people are known to eat several kinds of seaweeds including the brown algae and their intakes for the past twenty years are estimated to be about 5g per capita per day[16]. The daily intake of soluble and insoluble fibres from seaweeds might be presumed to be 1.25g per capita per day when the amounts are computed by our previous data[1] on the assumption that the contents of both total dietary fibre and soluble dietary fibre as a percentage of total dietary fibre would be approximately 50%. However, since mushroom consumption by the Japanese is estimated to be about 10g per capita per day in 1991[16] the dietary fibre content would be about 0.18g for soluble fibre and 2.82g for insoluble fibre. Thus, the total intake from seaweeds and mushrooms account for 1.43g of soluble fibre and 4.07g of insoluble fibre. Of the total dietary fibre intake of 17.33g per capita per day[17], the soluble fibre represents 8.25% while the insoluble represents 23.48%. To what extent such fibre intakes can affect dietary protein availability is a problem that needs further study.

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褐藻和蘑菇的水溶性和非水溶性食物纖維對試管内胃蛋白酶的抑制及大鼠體内蛋白質消化率的差異。

摘要

作者將褐藻中的海帶、裙帶菜、狸藻、及蘑菇中的香菇、柳松蘑菇、平菇的水溶性食物纖維和非水溶性食物纖維分離，研究其對試管内胃蛋白酶的抑制和大鼠體内蛋白質表觀消化率的差異。以上兩種食物纖維，對胃蛋白酶活性均有抑制作用，特別是水溶性食物纖維（抑制 62-99%）的作用強於非水溶性食物纖維（抑制 22-36%）（統計差異顯著 $p < 0.01$）。顯示了褐藻和蘑菇的水溶性食物纖維和非水溶性食物纖維具有不同的生理功能。通過各水溶性食物纖維的粘度同胃蛋白酶抑制率關係的比較，發現褐藻、蘑菇的食物纖維粘度和其對胃蛋白酶活物的抑制率相關。分別將含 5% 裙帶菜的水溶性或非水溶性食物纖維的正常蛋白飼料飼養近成熟的大白鼠，結果表明，含水溶性食物纖維的蛋白飼料的表觀消化率（9.4%）低於含非水溶性食物纖維的蛋白飼料的（$p < 0.01$）；攝取含水溶性食物纖維蛋白飼料的大白鼠的體重增加量亦低於攝取含非水溶性食物纖維蛋白飼料的大白鼠。

褐藻とキノコの水溶性および不溶性食物繊維画分の試験管内ペプシン活性および消化吸収率への影響の差異

褐藻のコンブ、ワカメ、ヒジキ、およびキノコのシイタケ、ヒラタケ、ヤナギマツタケより、それぞれ、水溶性および不溶性食物繊維を分離し、これらの試験管内ペプシン活性におよぼす影響を調べるとともに、これらの食品の代表として、ワカメの水溶性および不溶性食物繊維分画をそれぞれ含む正常飼料をラットに投与した場合の、食餌性タンパク質のみかけの消化吸収率への影響を調べた。全ての食品で、両食物繊維画分とも、ペプシン活性を阻害したが、特に水溶性食物繊維による阻害（６２－９９%）は不溶性食物繊維（２２－３６%）よりも強力であった（$P < 0.01$）。これは、褐藻とキノコの水溶性食物繊維は不溶性とは異なった生理的役割があることを示すものである。各水溶性食物繊維画分の粘度を測定し、ペプシンの阻害率との関係をみると、褐藻、キノコともに、両者に相関が認められた。ワカメの水溶性または不溶性食物繊維画分を５%含む正常タンパク飼料を成熟間近のラットに投与した結果、食餌性タンパク質のみかけの消化吸収率は水溶性食物繊維で不溶性よりも有意に低下（９．４%）した。このために、水溶性食物繊維摂取ラットの体重増加量が不溶性に比べて少なかったものと考えられた。