近畿大農紀要 23：55～61 (1990)
Mem. Fac. Agr. Kinki Univ. 23：55～61 (1990)

Desmutagenic Activities of Vegetables on Broiled Fish

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Synopsis

The extracts of certain vegetables (eggplant, broccoli etc.) reduced the mutagenicity of pyrolysate mutagens by inhibiting metabolic activation with rat liver preparation S-9. Juices prepared from 90 kinds of vegetables and fruit were almost all capable of irreversibly suppressing the mutagenicity of broiled fish by direct enzymatic inactivation of the mutagenic principle. Some pyrolysated fish products contained components resistant to vegetable desmutagenic action.

Introduction

Roasted meats and broiled fish contain highly mutagenic substances produced when amino acids and proteins are heated[1,2]. Crude extracts of many vegetables counteract the in vitro mutagenicity of the pyrolysates derived from certain amino acids[3,4]. Inoue *et al.*[5] isolated a hemoprotein from cabbages that has peroxidase and NADPH-oxidase activity, and that acts desmutagenically on tryptophan pyrolysate (Trp-P-2) both by on direct enzymatic inactivation and by suppression of metabolic activation. A similar substance has been isolated from broccoli[6].

Here, we did an extensive survey of the desmutagenic activities of vegetables and other foodstuffs to estimate their direct capability of inactivating the mutagenicity of broiled fish. Most vegetable extracts strongly inhibited metabolic activation or irreversibly inactivated mutagenicity. However, pyrolysated fish products contained substances that resisted vegetable desmutagenic capabilities.

Materials and Methods

To prepare a crude mutagenic extract, 100 *g* of raw *aji* fish (*Trachurus japonicus*) was broiled on a gas burner. About 20 *g* of the burned surface was extracted with a 1：1 mixture of chloroform and methanol. The solvent was evaporated at 45°C under reduced pressure and 5 *g* of the residue was dissolved in 5 m*l* of dimethyl-sulfoxide (Me_2SO).

Vegetable samples were purchased at a supermarket in Higashi-Osaka city. Each vegetable was purred in juicer and filtered through gauze. The juice obtained was centrifuged at 35,000× *g* (18,000 rpm) for 15 min at 0°C. The supernatant was separated and stored at −20°C until use.

In the experiment with vegetable juice, 1 *g* of the extract in Me_2SO was mixed with 5 m*l* of juice and kept at 37°C for 60 min. Then the mixture was extracted with 16 m*l* of ethyl acetate at

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pH 12 with vigorous shaking for 15 min. Eight milliliters of the supernatant was dried under reduced pressure at 0°C and the dry material was dissolved in 0.5 m*l* of Me_2SO. As a control, 5 m*l* of distilled water was used instead of the vegetable juice.

The rat preparation S 9 was prepared from rats treated with polychlorobiphenyl by the method by Ames *et al.*[7]. The Ames assay was done with 0.1 m*l* of the solution in Me_2SO by the pouring of a 0.1 m*l* suspension of *salmonella typhimrium* TA98 containing 0.3 m*l* of the S-9 mixture onto a selective agar plate. The plates were incubated at 37°C for 48 hr and the number of His^+ colonies was counted.

Separately, mutagenicity assays were also done with the Me_2SO extract of broiled fish without vegetable juice or with a mixture of broiled fish extract and a vegetable juice without ethyl acetate extraction. All experiments were done at least twice with triplicates each time, and results show the mean of two experiments.

Results

The addition of the S-9 preparation to the extract prepared from broiled *aji* fish conferred mutagenicity on the extract (Fig. 1). About 200 His^+ revertants were found per plate when 1 m*g* of dry pyrolysate was added. The effects of three vegetable juices on the mutagenicity of the *aji* fish extract are shown in Fig. 2. The mutagenicity of the *aji* extract was reduced by the addition of burdock, eggplant, or broccoli juice.

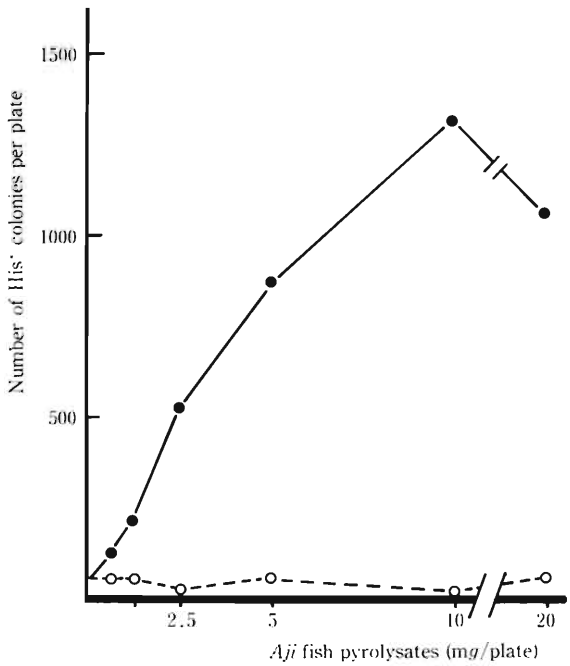

Fig. 1. Mutagenicity of the extract from broiled *aji* fish (*Trachurus japonicus*). See text for preparation of the sample. *Salmonella typhimurium* TA98 was used.
●, with S 9 preparation
○, without S-9 preparation

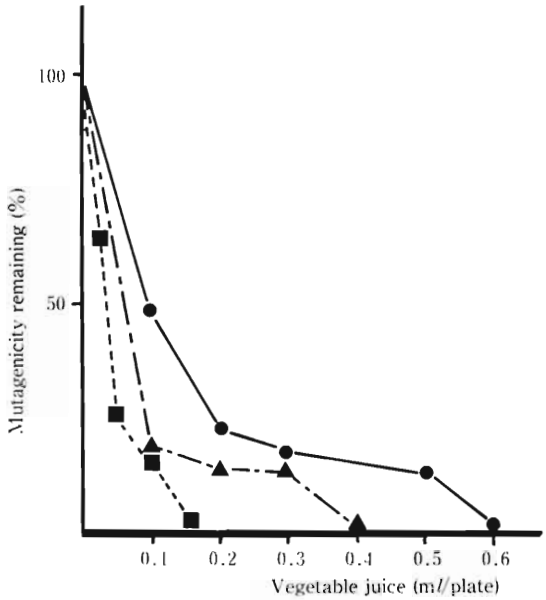

Fig. 2. Effects of vegetable juices on the mutagenicity of broiled *aji* fish. Juice was mixed with the fish extract, bacteria, and S-9 preparation in melted soft agar solution (3 ml, 43°C) and poured onto a solid agar plate.
●, Burdock
▲, Eggplant
■, Broccoli

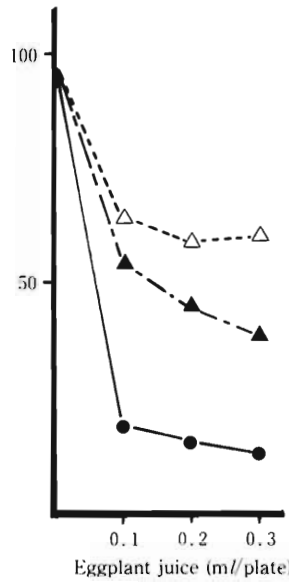

Fig. 3. Heat sensitivity of the desmutagenic activity of eggplant. See text for details of experiment.
●, Broiled fish extract was mixed with eggplant juice, and the mutagenicity of the mixture was assayed.
▲, Broiled fish extract mixed with eggplant juice was extracted with ethyl acetate, and the mutagenicity of the extract was assayed.
△, Broiled fish extract was mixed with eggplant juice that has been heated at 100°C for 30 min.

The suppression of mutagenicity might be caused by direct inactivation of mutagenic substances or by inhibition of metabolic activation by the S-9 preparation. Next, the mutagenic principle in the mixture of vegetable juice and fish extract was extracted with an organic solvent, ethyl acetate. The desmutagenic activity of crude unheated eggplant extract was higher than that of heat-treated juice or the fraction extracted with ethyl acetate, and about half of the mutagenicity that had seemed to be lost was recovered in the extracted fraction (Fig. 3). Some of the desmutagenic capacity of juices resisted heating; about 50% of the mutagenicity remained after heat treatment. A similar inactivation curve was obtained with heated juice (100°C for 30 min).

We wanted to identify vegetables that when made into juice would act directly on the mutagens of broiled fish before digestion. A survey was done, and results are shown in Table 1, which lists 90 vegetables, fruit, and other foodstuffs. Some foodstuffs (golden apple, pomegranate, chrysanthemum, etc.) had strong desmutagenic effects on *aji* fish pyrolysis products. More than half of the foods had moderate desmutagenic activity. However, the pyrolysate fish products included factors resistant to vegetable desmutagenic capabilities.

Inhibitors of metabolic activation and desmutagens are quite different. One example will be given. *Enokidake* mushrooms (*Flammulina velutipes*), which had a desmutagenic activity of 47%

Table 1. Desmutagenic effects of foodstuffs on the broiled *Aji* extract

Sample	Activity (%)
Golden apple (*Malus pumila athor* Mill)	84
Pomegranate (*Punica granatum* L.)	81
Garland chrysanthemum (*Chrysanthemum coronarium*)	78
Ponkan orange (*Citrus reticulate*)	76
Lily bulb (*Lilium auratum* Lindl.)	74
Grape (*Vitis spp.*)	73
Kidney bean (*Pisum sativum* L.)	71
Plantain (*Musa acuminata*)	71
Royal fern (*Osmunda japonica* Thunb.)	70
Corn (*Zea mays* L.)	69
Hime apple (*Malus pumila*)	69
Ginger (*Zingiber officinale* Rosc.)	67
Kaiware (*Raphanus sativus* L.)	65
Sweet pepper (*Piper nigrum* L.)	64
Red onion (*Allium ceoa* L.)	63
Garlic (*Allium satium*)	63
Shallot (*Alliumfistulasum*)	63
Small onion (*Allium cepa* L.)	61
Papaya (*Carica papaya* L.)	61
Ginkgo nut (*Ginkgo biloba* L.)	57
Radish (*Raphanus sativus* L.)	57
Mitsuba water dropwort (*Cryptotaenia japonica*)	56
Chinese quince (*Chaenomeles sinensis*)	54
Mugwort (*Artemisia vulgaris* L.)	53
Sunny lettuce (*Lactuca sativa* L.)	53
Sweet potato (*Ipomoea batatas*)	53
Onion (*Allium cepa* L.)	53
Japanese butterbur (*Petasites japonicus*)	51
Spinach (*Spinacia oleracea* L.)	51
Kiwifruit (*Actinidia chinensis*)	51
Fuji apple (*Malus pumila*)	51
Parslery (*Petroselinum* sativum)	51
Banana (*Musa sapientum* L.)	51
Kabosu citrus fruit (*Citrus sphaerocarpa*)	50
Japanese persimmon (*Diospyros Kaki* L.)	47
Enokidake mushroom (*Flammulina velutipes*)	47
Suikamodoki pumpkin (*Cucurbita* sp.)	45
Pine apple (*Ananas camasus*)	42
Touroku bean (*Faba* spp.)	42

Violet cabbage (*Brassica oleracea* L.)	42
Soybean sprout (*Glycine max.*)	41
Rape (*Brassica campestris* L.)	41
Eggplant (*Solanum melongena* L.)	40
Lemon (*Citrus limon*)	40
Soybean (*Glycine max.*)	40
Sudachi citrus fruit (*Citrus sudachi*)	38
Okra (*Abelmoschus esculentus* Moench)	38
Burdock (*Arctium lappa* L.)	37
Chinese cabbage (*Oleracea spp.*)	36
Navel orange (*Citrus sinensis*)	34
Shimeji mushroom (*Lyophyllum aggregatum* Kuhner)	30
Kumquat (*Fortunella spp.*)	30
Cauliflower (*Brassica oleracea* L.)	29
Uzura bean (*Phaseolus vulgaris*)	27
Tomato (*Lycopersicum esculentum*)	26
Radish (*Raphanus sativus* L.)	26
Carrot (*Daucas carota* L.)	25
Tsukuneimo (*Dioscorea batatas*)	25
Pumpkin (*Cucurbita moschata*)	25
Cherry (*Prunus avium* L.)	23
Pods, immature (*Pisum sativum* L.)	20
Kintoki bean (*Phaseolus vulgaris* L.)	20
Celery (*Apium graveolens* L.)	20
Cucumber (*Cucumis sativus* L.)	19
Green peas (*Pisum sativum* L.)	18
Mustard (*Brassica juncea*)	16
Broccoli (*Brassica oleracea* L.)	16
Budo bean (*Glycine max*)	14
Sweet pepper (*Piper nigrum* L.)	13
Azuki bean (*Vigna angularis*)	13
Head lettuce, butterhead type (*Lactuca sativa* L.)	9
Shaddock (*Citrus spp.*)	9
Buckwheat (*Fagopyron esculentun* Moench)	7
Mung bean (*Vigna radiata* Wilcz.)	7
Head lettuce (*Lactuca sativa* L.)	5
Mushroom (*Agraricus bisporus*)	2
Yamaneimo (*Dioscorea japonica*)	2
Indian lotus root (*Nelumbo nucifera*)	2
Kinusaya pea pods, immature (*Vigna sinensis*)	1
Fern bracken (*Wasabi japonica*)	1

Nameko mushroom (*Pholiata nameko*)	*
Grapefruit (*Citrus paradisi*)	*
Orange (*Citrus sinensis* Osbeck)	*
Strawberry (*Fragaria grandiflora*)	*
aji fish (*Trachurus japonicus*) pyrolysate (control)	0

*Less than 0 (activation of mutagenicity)
The results are shown as the activity relative to the control as 0%. Details of the procedure are in the text.

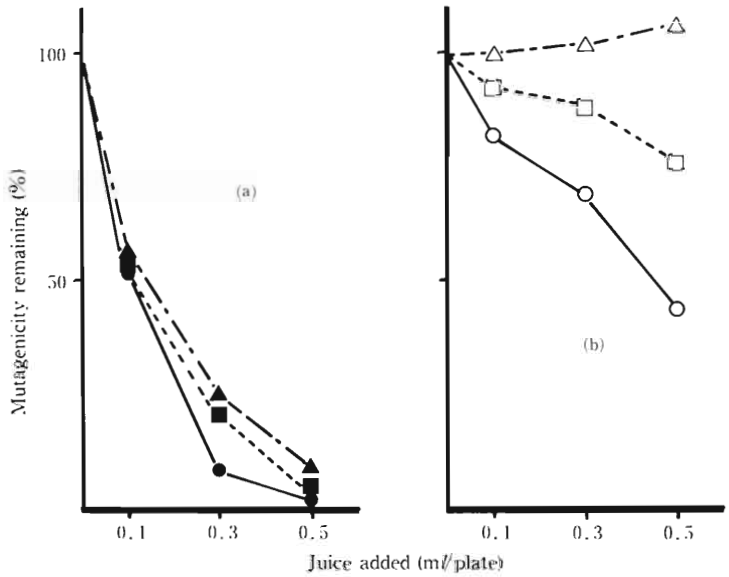

Fig. 4. Heat sensitivity of the desmutagenicity of *enokidake* mushrooms (*Flammulina velutipes*)
(a) Extract of broiled fish was mixed with fresh or heated juice, and the mutagenicity of the mixture was assayed.
●, mixed with fresh juice
■, mixed with heated juice (100°C for 10 min)
▲, mixed with heated juice (100°C for 30 min)
(b) Extract of broiled fish was mixed with fresh or heated juice, and ethyl acetate extraction was done. The mutagenicity of the extract was assayed.
○, mixed with fresh juice
□, mixed with heated juice (100°C for 10 min)
△, mixed with heated juice (100°C for 30 min)

was studied to identify the mechanism of the desmutagenic activity. The juice of these mushroom had a heat-resistant inhibitor of metabolic activation (Fig. 4a), but its desmutagen was heat-labile (Fig. 4b).

Discussion

Our results showed that most kinds of vegetables were capable of some degree of direct inactivation of the mutagenicity of broiled fish pyrolysates. With some vegetable, metabolic activation by the S 9 preparation was inhibited, and mutagenic substances were recovered by

extraction with an organic solvent.

Desmutagenic activities that affect tryptophan pyrolysate are involve the direct enzymatic inactivation of mutagens and also suppression of metabolic activation[8]. In our examination using broiled *aji* fish as the mutagen, desmutagenicity was found in some vegetable juices. The mutagenicity of this broiled fish arises from several highly mutagenic products of pyrolysis, such as Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, 2-Amino-3-methylimidazo[4,5-*f*]quinoline, 2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoline[9]. The results obtained here did not show what kind of action the vegetable juices had. It is not known whether the components of the pyrolysate from broiled fish unaffected by vegetable juice are carcinogenic or not.

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食品中の突然変異原性物質とその不活化

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摘　　要

約90種の野菜・果実について突然変異原性物質に対する不活化作用の検索を試みた。ナスやブロッコリー等の野菜ジュースは，ラットのS-9を用いる代謝活性を阻害するため，*Salmonella typhimurium* TA98における魚肉焼成物の変異原性を見かけ上減少した。

一方，約90種の野菜や果実のジュースのほとんどは，その酵素によって直接的に変異原性を抑制した。しかし，突然変異原性物質を完全に不活化できない事も認められた。