Transit of Radical Scavenging Activity of Milk Products Prepared by Maillard Reaction and *Lactobacillus casei* Strain Shirota Fermentation through the Hamster Intestine

T. Nishino,[1] H. Shibahara-Sone, H. Kikuchi-Hayakawa, and F. Ishikawa
Yakult Central Institute for Microbiological Research, Yaho 1796, Kunitachi-shi, Tokyo 186-8650, Japan

ABSTRACT

Oxidative stress in the colon is associated with the incidence of colon cancer. In situ, the suppression of oxidative stress in the colon would be an effective form of prevention of the cancer. In this study we investigated the transit of the radical scavenging activity of milk products through the hamster intestinal tract. Two types of skim milk products were prepared by Maillard reaction and then lactic acid fermentation. Heat treatment enhanced the radical scavenging activity for 2,2- diphenyl-1-picrylhydrazyl radical of skim milk. The activity was further increased by fermentation with *Lactobacillus casei* strain Shirota. Normal hamsters were fed these milk products for 14 d. For potential radical scavenging activity per unit dry weight of feces and cecal content, the groups ranked in the order of fermented product-fed hamsters > heated product-fed hamsters > control hamsters, reflecting the order of the potential of the corresponding diets. Approximately 12% of the activity of the heated and the fermented product diets passed through the gastrointestinal tract. These results suggest that some of the radical scavenging activity generated by food processing reached the colon in nonabsorbable products.
(**Key words:** Maillard reaction, *Lactobacillus casei* strain Shirota, radical scavenging activity, intestine)

Abbreviation key: DPPH = 2,2-diphenyl-1-picrylhydrazyl, **FSM** = MSM fermented by *Lactobacillus casei* strain Shirota, **MSM** = skim milk treated by Maillard reaction; **MW** = molecular weight, **RS** = radical scavenging.

INTRODUCTION

Epidemiological studies indicate that iron status is involved in the pathogenesis of colon cancer (7, 16) and that intake of phytate-rich dietary fiber may prevent the incidence of cancer (9, 29). Erhardt et al. (12) reported that a dietary fiber-rich diet is associated with the suppression of hydroxyl radical formation in human feces, suggesting that the radicals contribute to an enhanced risk of colon cancer, which has a high mortality rate. Azoxymethane-induced colon cancer in rats was suppressed by phytic acid (22), which may indicate the inhibition of iron-mediated hydroxyl radical formation. Hydroxyl radicals may be generated in feces by the Fenton reaction driven by fecal iron and a superoxide radical that is produced by bacterial metabolism (3). High intake and excess accumulation in tissue of iron appear to influence colon carcinogenesis through hydroxyl radical production (19, 28).

Green vegetables, fruits, beans, and grains, which are thought to prevent oxidative stress, contain two types of antioxidative components; one is absorbable (e.g., some vitamins such as ascorbate, tocopherols, and carotenes and some polyphenols such as flavonoids and catechins), and the other is nonabsorbable (e.g., phytate). The former would mainly act in blood and tissues, and the latter in the gastrointestinal tract.

Functions of foods could be evaluated not only by inherent ingredients but also by secondary components generated from food processing. Heating and fermentation are basic processing methods to make food materials edible, to alter texture and flavor, and to sterilize food, among other purposes. The Maillard reaction frequently provides biofunctional characters (18) as well as sensory values in foods. The antioxidative function of Maillard reaction products is important for preventing lipid peroxidation in food preservation (5). Nondialyzable melanoidins have scavenging activity for reactive oxygen species such as hydroxyl radical and superoxide radical (13). Dairy products obtained by lactic acid fermentation improve lactose malabsorption and diarrhea and suppress colon carcinogenesis (14).

There are fewer reports about the physiological effects of the additional components of processed foods than for native ingredients. We have elucidated how the components of dairy products affect the health of the intestine. The aim of this study was to investi-

Received August 2, 1999.
Accepted December 2, 1999.
Corresponding author: F. Ishikawa; e-mail: fumiyasu-ishikawa@yalcult.cojp.
[1]Current address: Ocean Research Institute, University of Tokyo, Minamidai 1-15-1, Nakano-ku, Tokyo 164-8639, Japan.

gate the transit of the radical scavenging (**RS**) activity of milk products in the intestinal tract.

MATERIALS AND METHODS

Chemicals

All chemicals used in this study were purchased from Sigma-Aldrich Japan, Tokyo, Japan, unless otherwise stated. *Lactobacillus casei* strain Shirota, *Bacillus subtillis* M45 and *B. subtillis* H17 were obtained from the Culture Collection Research Laboratory of the Yakult Central Institute for Microbiological Research (Tokyo, Japan).

Treatment of Skim Milk by the Maillard Reaction

Spray-dried skim milk (160 g, Snow Brand Milk Products Co., Tokyo, Japan) and a reducing sugar mixture (67 g, glucose and fructose (31.5:35.5, wt/wt), Sanmatsu Kogyo Co., Tokyo, Japan) were dissolved with distilled water to 1 L. The solution was heated at 100°C. The skim milk product after heating at 100°C for 90 min was used as the medium (**MSM**: Maillard reaction skim milk) for lactic acid fermentation.

Fermentation by *Lactobacillus casei* Strain Shirota

Lactobacillus casei strain Shirota (1×10^9 cells), a popular starter for fermented milk products, was seeded in MSM (1 L), and cultured at 37°C until a final pH of 3.7. The fermented MSM (**FSM**) thus obtained was cooled down at 4°C overnight. The liquid MSM and FSM were mixed with pregelatinized corn starch (100 g/L), then lyophilized, powdered, and stored at –20°C, avoiding moisture.

Diets and Animals

The composition of the experimental diets (control, MSM and FSM diets) is shown in Table 1. The amount (30.6%) of pregelatinized corn starch contained in the powdered MSM and FSM was deducted from that in the MSM and FSM diets, respectively, to standardize the amount of starch among the diets. The protein, fat, and other components (except for starch in the powdered skim milk products) were replaced by casein, corn oil, and sucrose in the control diet, respectively.

Eighteen male golden Syrian hamsters (Japan S.L.C. Co., Shizuoka, Japan) were obtained at the age of 5 wk. The hamsters were fed a commercial nonpurified solid diet (MF diet, Oriental Yeast, Tokyo, Japan) for 2 d and then the powdered MF diet for 5 d. After this adaptation period, the hamsters were randomly divided into three groups of six each of similar mean body weights. They were fed one of the above three diets for 14 d and allowed free access to food and water. Food consumption and body weight were recorded every 2 or 3 d and every week, respectively. The hamsters were housed individually in stainless-steel wire-bottomed cages in a room with controlled lighting (lights on 0830 to 2030 h), temperature (24 ± 2°C), and humidity (60 ± 5%). The feces collected from noon of the ninth day to noon of the 12th day in the experimental period were lyophilized, milled, and stored at –20°C until the measurement of RS activity. On the 14th day, the hamsters were anesthetized with an intraperitoneal injection of sodium pentobarbital (Nembutal, Abbott Laboratories, Chicago, IL) at 25 mg/kg of body weight. Food was withheld for 4 h before euthanasia. The cecal content was collected, lyophilized, milled, and stored at –20°C until the measurement of RS activity. The animals were maintained in accordance with the guidelines of the Ethical Committee for Animal Experiments of Yakult Central Institute.

Table 1. Composition of the experimental diets fed to hamsters.

Component	Control diet	MSM[1] diet	FSM[1] diet
	(g/kg)		
Casein[2]	250.0	175.8	175.8
Corn oil[3]	100.0	98.5	98.5
Lard[3]	70.0	70.0	70.0
Cellulose[4]	50.0	50.0	50.0
Vitamin mixture[5]	10.0	10.0	10.0
Mineral mixture[5]	35.0	35.0	35.0
Choline bitartrate[6]	2.0	2.0	2.0
Pregelatinized corn starch[7]	233.0	90.0	90.0
Sucrose[8]	250.0	25.7	25.7
Lyophilized MSM[9]	0.0	443.0	0.0
Lyophilized FSM[10]	0.0	0.0	443.0

[1]FSM = MSM fermented by *L. casei* strain Shirota; MSM = skim milk treated by Maillard reaction.

[2]Acid casein (Murray Goulburn Co-Operative Co., Melbourne, Australia).

[3]Purchased from Hayashi Chemicals Co., Tokyo, Japan.

[4]Cellulose powder D (Toyo Roshi Kaisha, Tokyo, Japan).

[5]AIN-76 formulation (2), purchased from Oriental Yeast Co., Tokyo, Japan.

[6]Purchased from Wako Pure Chemical Industries, Osaka, Japan.

[7]α-Corn starch (Oriental Yeast Co., Tokyo, Japan).

[8]Powdered sugar (Tokukura Shokai Co. Ltd., Tokyo, Japan).

[9]Water, 1.6%; protein, 16.4%; fat, 0.3%; ash, 3.5%; fiber, 0%; carbohydrate, 78.2%. Contained 30.6% pregelatinized corn starch per dry weight.

[10]Water, 3.6%; protein, 16.0%; fat, 0.3%; ash, 3.6%; fiber, 0%; carbohydrate, 76.5%. Contained 30.6% pregelatinized corn starch per dry weight.

Measurement of Radical Scavenging Activity

Radical scavenging activity was measured by using a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (**DPPH**, Tokyo Kasei Kogyo Co., Tokyo, Japan), which

shows a strong absorption at 517 nm (deep violet color) because of its odd electron (8). When DPPH radical accepts an electron or a hydrogen radical, the absorption vanishes. The decolorization is stoichiometric for the number of electrons accepted (8). We modified the method of Takao et al. (26) as follows. Liquid samples (the skim milk-sugar mixture, the heated mixtures, the fermented mixture and the eluate from the Sephadex column), as they were or as they were diluted with distilled water, were used for the assay. Powdered samples (diet, feces, and cecal content) were suspended with distilled water, and the suspensions were used as samples for the assay. Sample suspension (1.0 ml) was mixed with 1.0 ml of DPPH solution (0.08 mg/ml of ethanol), then the mixture was left standing at room temperature for 30 min. Distilled water instead of sample suspension was used as the blank. Then 0.5 ml of chloroform was added to each mixture and then immediately mixed for collection of DPPH molecules. The chloroform fraction was obtained by centrifugation and diluted with three volumes of ethanol. Absorbance at 517 nm of the solution was measured. In some cases the degrees of RS activity were expressed as decolorizing degrees of the absorbance differences between blank and sample suspension. To evaluate the transit of RS activity, we estimated the RS activity of sample suspensions as follows. The absorbance difference of sample suspension was plotted against the $\log_{10}$ of dilution rate of the suspension. Thus the potential of the RS activity of the suspension was calculated by the extrapolation of the linear plot to the intercept at dilution rate = 1, where 1 U of the RS activity was defined as an absorbance difference of 1.0. The specific RS activity was defined as the RS activity per dry weight (mU/mg). The total RS activity (U) was calculated from the specific RS activity and the whole dry weight of the sample.

Digestibility of Dry Matter and Recovery of Radical Scavenging Activity

The digestibility of dietary dry matter was calculated as follows:

$$\text{dry matter digestibility (\%)} = (1 - A \div B) \times 100,$$

where A and B are dry weight (g) of the feces excreted and weight (g) of the food consumed for 3 d, respectively.

The recovery of dietary RS activity in feces was calculated as follows:

$$\text{recovery of RS activity (\%)} = (C \div D) \times 100,$$

where C and D are the total RS activity (U) of the fecal and the diet samples for 3 d, respectively.

Table 2. Change in radical scavenging activity of skim milk-sugar mixture during heating.

Treatment of samples	Color measurement values[1]			Radical scavenging activity[1,2]
	L	a	b	Δ517 nm
Untreated	92.4	–5.6	16.9	0.182
100°C, 30 min	77.8	4.1	18.3	0.235
100°C, 60 min	64.9	7.6	20.1	0.293
100°C, 90 min	58.2	8.9	20.6	0.331
100°C, 120 min	53.3	9.7	20.6	0.339

[1]Values are the means of triplicate measurements.

[2]Values are the absorbance differences at 517 nm between blank and 100-fold diluted sample.

Evaluating the Maillard Reaction

The Maillard reaction was evaluated by tristimulus color measurement using a color difference meter (NDJ-1001 DP, Nihon Densyoku Kogyo Co., Tokyo, Japan). Triplicate measurements of each sample were taken and the mean values of L, a, and b were determined, as described by Bates et al. (4).

Measurement of Mutagenicity

Because some mutagens are formed during cooking (24, 30), we examined the mutagenicity of MSM by using the *rec* assay on a plate (15) as follows. The plate consisted of two layers: the lower layer was a solid medium containing, 10 g (per liter) of Bacto Trypton (Difco Lab., Detroit, MI), 5 g of Bacto Yeast Extract (Difco), 5 g of sodium chloride and 12 g of agarose (Difco), and the upper layer was a mixture of bacterial spores [10^6 spores from *Bacillus subtillis* M45 (*rec*$^-$) or *B. subtillis* H17 (*rec*$^+$) per ml] and the above medium with a half content (6 g/L) of agarose, which was prepared at 55°C. Aliquots (5 μl) of the skim milk products shown in Table 2 were applied to a paper disc (6 mm in diameter, for antibiotic assay, Advantec, Tokyo, Japan) on the plate. The plate was incubated for 24 h at 37°C. Mutagenicity was judged by measuring the inhibitory area of the growth of the *rec*$^-$ strain, using *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine as a positive mutagen.

Molecular Weight Distribution Pattern of Radical Scavenging Activity

To know whether components with radical scavenging activity changed through the intestine, we measured to the molecular weight distribution pattern by gel filtration. Aliquots (1 g) of diet and fecal samples were suspended in 10 ml of 50 mmol of sodium-phosphate buffer/L (pH 7.0) and 15 ml of water, respectively, and stirred at room temperature for 1 h. The suspension

was centrifuged at 5000 × *g* for 5 min to remove precipitate prior to gel filtration. The supernatants (0.4 ml) of diet and fecal samples were applied to a Sephadex G-25 cartridge column (PD-10, Pharmacia Biotech, Tokyo, Japan) previously equilibrated with the above buffer and water, respectively. To determine the molecular weight (**MW**) distribution pattern of RS activity, we measured the absorbance difference at 517 nm of each fraction (1 ml) as described above. BSA (67,000 Da) and L-Cys (121 Da) were eluted at fraction 3 and 8 on the column, respectively.

Statistical Analysis

Values are expressed as the mean and SD, unless otherwise stated. Means were compared using VisualStat software (StatSoft Inc., Tulsa, OK), by variance analysis and, subsequently, the honestly significant difference test of Tukey. In the case that the variance was significantly different by the Bartlett test, after logarithmic transformation to stabilize the variance, the above comparison was made. For nonparametric data, the Kruskal-Wallis test and nonparametric Tukey's test were used (32). The difference was considered to be significant when P was less than 0.05.

RESULTS

Effect of the Maillard Reaction on the Radical Scavenging Activity and Mutagenicity of Skim Milk Products

The mixtures of spray-dried skim milk and reducing sugar were used for the Maillard reaction, because we wanted dairy products with RS activity. The color measurement values and the absorbance difference at 517 nm of the mixtures are shown in Table 2. Browning of the mixture and the potential of RS activity were increased as heating time was prolonged. Ninety minutes of heating time rendered the mixture brownish and slightly aromatic, but not bitter. The mixture prepared under this condition was used as the material (MSM) for the below experiment in this study. With longer heating, L values decreased (more darkish) and values of a and b increased (more brownish), but RS activity was virtually constant. None of the heated mixtures inhibited the growth of the *rec*⁻ strain of *B. subtillis*, indicating that they did not have mutagenicity. Because *Lactobacillus casei* strain Shirota is not mutagenic (25) and has the antimutagenicity for heterocyclic amines (17), FSM that was the MSM fermented by this bacterium was considered to be a safe MSM.

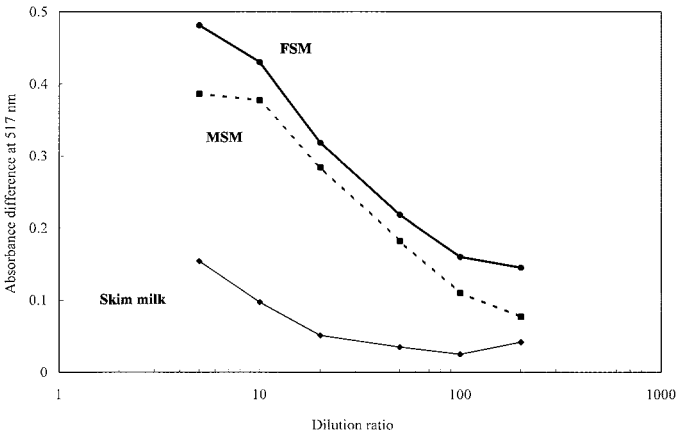

Figure 1. Radical scavenging pattern for intact mixture of skim milk and sugar, heat-treated skim milk product (MSM), and MSM fermented by *Lactobacillus casei* strain Shirota (FSM). The radical scavenging activity of each sample is shown as the absorbance difference at 517 nm of 2,2-diphenyl-1-picrylhydrazyl. Each value is expressed as the mean obtained from three separate samples.

Effect of *Lactobacillus casei* Strain Shirota Fermentation on Radical Scavenging Activity

To determine the effect of fermentation on RS activity, we cultured *L. casei* strain Shirota in MSM. As shown in Figure 1, the absorbance difference at 517 nm of FSM was higher than that of MSM, indicating that lactic acid fermentation was associated with an increase in RS activity. The bacterium secreted L-lactic acid (290 mmol/L) as it grew, thus we investigated whether the increase was due to this acid. Radical scavenging activity remained constant even when L- lactic acid was added to MSM at the same concentration as to FSM.

Transit of Radical Scavenging Activity Through the Gastrointestinal Tract

To find the path of the RS activity of MSM and FSM through the intestine, we examined the recovery of the RS activity in feces following the oral feeding of MSM or FSM. Prior to the feeding experiment, we checked the effect of protein sources on RS activity. Lactose, glucose, and fructose were mixed with casein to give values equivalent to spray-dried milk. The casein and skim milk mixtures had an equivalent RS activity, thus we used the casein-based control diet as a standard diet. MSM and FSM diets were prepared by replacing casein, sucrose and slight corn oil in the control diet with the powdered MSM and FSM, respectively. By Sephadex G-25 column chromatography, MSM and FSM diet samples were found to have a similar MW distribution pattern with two sharp peaks at void and

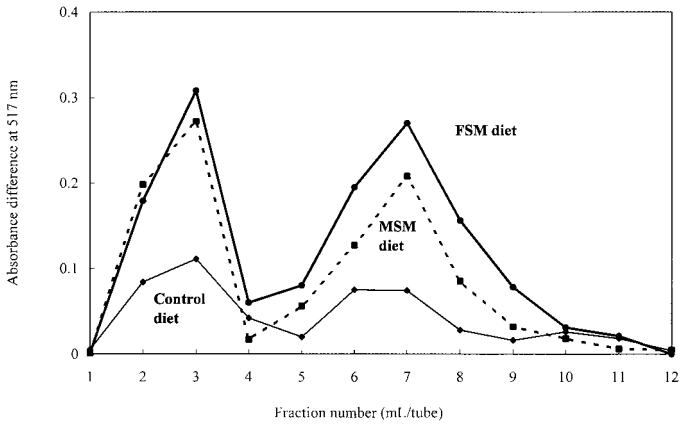

Figure 2. Molecular weight distribution on a Sephadex G-25 column of the radical scavenging activity in the control diet, the Maillard reaction skim milk (MSM) diet containing heat-treated skim milk product, and the FSM diet containing MSM fermented by *Lactobacillus casei* strain Shirota. The radical scavenging activity of each fraction (1 ml) is expressed as the absorbance difference at 517 nm of 2,2-diphenyl-1-picrylhydrazyl. The sample for gel filtration was prepared from an aliquot of the well-mixed experimental diet. BSA (67,000 Da) and L-Cys (121 Da) were eluted in fraction 3 and 8, respectively.

later volumes (Figure 2), which resembled the figures for the powdered MSM and FSM, respectively. Control diet sample had a similar pattern to MSM and FSM diet samples, but with less RS activity.

Body weight gain, food intake, food efficiency, and liver weight were not significantly different among the three diet groups (Table 3), but the body weight gain of FSM hamsters showed a downward tendency. Table 4 shows the digestibility of diet dry matter and the recovery of RS activity in feces. There was no significant difference in the digestibility among the three diet groups because of the similar amount of food intake and fecal weight. Compared with the control diet group, the MSM and FSM groups had higher total RS activities. In both MSM and FSM groups, approximately 12% of dietary RS activity was recovered from feces. In cecal content and feces, the specific RS activities of the FSM and MSM groups were significantly higher than that of the control group, and, furthermore, the specific RS activity of the FSM group was slightly but significantly high ($P < 0.05$ in cecal content and $P < 0.001$ in feces) compared with that of the MSM group.

Figure 3 shows the MW distribution pattern of the RS activity of fecal sample obtained from a representative hamster for each diet. In fecal samples for MSM and FSM, a broad peak of RS activity was drawn from void (at the position eluting BSA) to later volumes (at the position eluting L-Cys). The fecal sample of the control hamster had only a low level of the activity; the pattern was similar to the MSM and FSM groups.

DISCUSSION

Maillard reaction products, such as mellanoidins, glycated proteins, and glucose-glycine reaction products, have antioxidative and radical scavenging activities (13, 20, 31). Furthermore, these products can chelate metal ions (27). However, it is unclear whether the activity generated from food processing contributes to the health of the body, particularly the gastrointestinal tract. Thus we examined the transit of the RS activity in milk products through gastrointestinal traffic as a first step to investigating the physiological effect.

The assay for RS activity with DPPH was simple and reproducible. This compound is known to be stoichiometrically decolorized by potent reducing substances and antioxidants such as cysteine, glutathione, ascorbic acid, and tocopherols (8). As shown in Table 2, the mixture of skim milk and sugar acquired RS activity through heating. Prolonging the heating time conferred higher RS activity to the mixture up until 90 min, but overheating brought about some disadvantages such as a bitter taste and dark color. Because we investigated the actual foodstuffs, the heating condition used in the present study was selected on the basis of the criteria of sensory satisfaction (nonbitter taste and aromatic flavor) and safety (no mutagenicity). Lactic acid fermen-

Table 3. Body weight, food intake, food efficiency, and liver weight in hamsters fed skim milk products with radical scavenging activity.

	Control diet n = 6		MSM[1] diet n = 6		FSM[1] diet n = 6	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Initial body weight, g	90.0	4.2	89.5	3.4	90.0	4.8
Final body weight, g	115.1	7.4	114.6	9.7	111.6	5.5
Body weight gain, g/14 d	25.1	5.2	25.1	8.2	21.6	2.9
Food intake, g/d	7.1	0.7	7.2	1.1	6.8	0.5
Food efficiency[2]	0.25	0.03	0.24	0.05	0.23	0.02
Liver weight, g	5.58	0.74	6.26	0.75	5.94	0.6

[1]FSM = MSM fermented by *L. casei* strain Shirota; MSM = skim milk treated by Maillard reaction.

[2]Body weight gain for 14 d/food intake for 14 d.

Table 4. Radical scavenging activity in diet, cecal content, and feces and the recovery of the activity in hamsters.

	Control diet		MSM[1] diet		FSM diet	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Food consumed, g of dry diet/3 d, n = 6	20.5	2.7	19.7	3.2	19.3	2.9
Feces excreted, g of dry feces/3 d, n = 6	1.52	0.24	1.48	0.28	1.46	0.26
Dry matter recovery,[2] %, n = 6	7.39	0.66	7.49	0.56	7.65	1.69
Total RS activity of diet taken,[3] U, n = 6	102.5[b]	13.5	191.4[a]	30.9	204.3[a]	31.1
Total RS activity of feces,[4] U, n = 6	16.5[b]	2.2	22.6[a]	4.3	23.7[a]	4.5
Recovery of RS activity in feces,[5] %, n = 6	16.1[a]	1.3	11.8[b]	0.9	11.8[b]	2.7
Specific RS activity of diet, mU/mg, n = 3	5.01[c]	0.25	9.72[b]	0.11	10.58[a]	0.39
Specific RS activity of cecal content, mU/mg, n = 6	19.9[c]	0.4	25.4[b]	0.6	26.6[a]	1.0
Specific RS activity of feces, mU/mg, n = 6	10.9[c]	0.4	15.3[b]	0.3	16.2[a]	0.3

[a,b,c]Means within a row without common superscripts differ ($P < 0.05$, a > b > c).

[1]FSM = MSM fermented by *L. casei* strain Shirota; MSM = skim milk treated by Maillard reaction; RS = radical scavenging activity.

[2](Feces excreted for 3 d/food consumed for 3 d) × 100.

[3]Mean value of specific RS activity of each diet × food consumed for 3 d (mg).

[4]Specific RS activity of feces × feces excreted for 3 d (mg).

[5](Total RS activity of feces/total RS activity of diet) × 100.

tation by *L. casei* strain Shirota raised the RS activity of the MSM, but L-lactic acid as a major metabolite of the bacterium did not enhance the activity. It is reported that *L. casei* is a proteolytic (caseinolytic) species (10, 21) and that the casein-derived peptides possess antioxidative activity (23). Although casein hydrolysates in the FSM might be one of the factors enhancing RS activity, it is unclear what increases the RS activity during fermentation.

The total fecal RS activities of the MSM and FSM groups were higher than that of the control group. This would reflect the potential of the activity in the corresponding diet. Dietary RS activity partially disappeared through the gastrointestinal tract, and some of the activity was recovered in feces. Diet samples of the three groups had a similar MW distribution pattern of RS activity. This was also the case for fecal samples. Thus, the RS activities of these diets would be the result of digestion, decomposition, and absorption in a common fashion. The small intestine has the main function in digestion and absorption of the major components of food such as proteins, fats and carbohydrates, whereas the large intestine consisting of cecum, colon, and rectum absorbs water, electrolytes, and short chain fatty acids as bacterial metabolites (6). Assuming that the large intestine affects the digestibility of dry matter less than does the small intestine, the difference between the specific RS activities of cecal content and feces would reflect the change in the activity itself rather than the change in whole dry matter. Therefore, in all groups the fecal specific RS activity was 40 to 45% lower than that of cecal sample, suggesting a loss of the activity through the lower intestinal tract (from cecum to rectum). Taking the low recovery of dietary RS activity into consideration, a large part of the RS activity of these diets would be expected to be lost through the small intestine. These results reveal that although in normal hamsters much of the RS activity of the processed dairy products would be digested, absorbed and degraded in the small intestine, part of the activity would arrive at the large intestine in a nonabsorbable product. Because free radicals in feces may be associated with the incidence of colon cancer (11, 19, 22, 28), the RS activity of intestinal content or feces

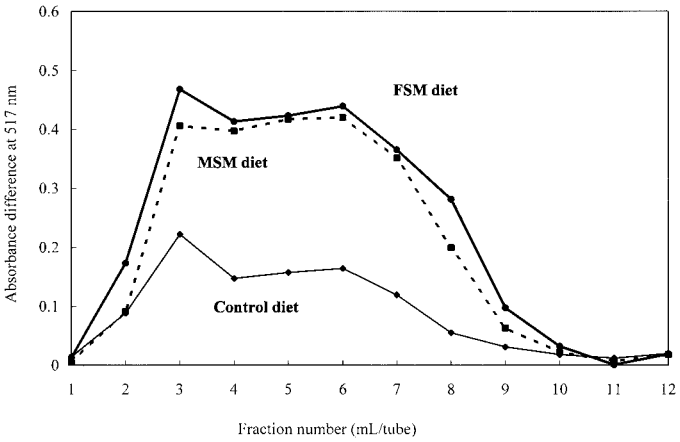

Figure 3. Molecular weight distribution on a Sephadex G-25 column of the radical scavenging activity in feces obtained from a representative hamster fed the control diet, the Maillard reaction skim milk (MSM) diet, and the FSM diet containing MSM fermented by *Lactobacillus casei* strain Shirota. The radical scavenging activity of each fraction (1 ml) is expressed as the absorbance difference at 517 nm of 2,2-diphyenyl-1-picrylhydrazyl. BSA (67,000 Da) and L-Cys (121 Da) were eluted in fraction 3 and 8, respectively.

would be expected to suppress oxidative stress in the colon as nonabsorbable antioxidants (3, 22).

Because the diet composition would be important to an evaluation of the transit of RS activity, we prepared diet samples taking care to avoid any changes in physical and chemical properties. For example, diets were prepared under the same conditions, except for the addition of pregelatinized corn starch (usually called α-corn starch in Japan). The starch was mixed with the liquid MSM and FSM at low temperature and then lyophilized to prepare the powdered MSM and FSM, which were used as components of the MSM and FSM diets, respectively. Because this starch was a processed preparation without raw starch, the risk of changing the physiology of the intestine would be negligible. In fact, food efficiency and dry matter digestibility did not differ among the three diet groups. This similarity indicates that these diets have a similar nutritional value in spite of the compositional difference in fat (corn oil and perhaps milk fat in the MSM and FSM). However, we have been worried about the body weight of the FSM group and the vitamin content of the diets. Body weight gain trended downward in the case of the FSM group. This result might be explained by the somewhat low intake of food for 14 d of feeding because of lactic acid in the diet, but no evidence supports this. Although the content of vitamins (with RS activity) in the diets was based on the AIN 76 formulation (2), it is possible that these components affected the absorption and the recovery of the RS activity. Because the present data do not clear up these concerns, further investigations need to be conducted.

Oxidative stress is presumably involved in the pathogenesis of inflammatory bowel disease such as ulcerative colitis (1) and Crohn's disease (11), as well as associated with the incidence of colon cancer. Our goal is to clarify whether functional foods suppress oxidative stress in the gastrointestinal tract. On the basis of the transit of RS activity in FSM and MSM in the colon of normal animals, we have begun to further examine the antioxidative effects (including radical scavenging activity) of food products under oxidative stress.

CONCLUSIONS

Maillard reaction and lactic acid fermentation conferred RS activity to skim milk. Normal hamsters fed MSM and FSM had the higher recovery of the RS activity from feces, compared with control ones. This indicates that some of the RS activity generated by the processing of skim milk can reach the colon in nonabsorbable form. Products with RS activity might contribute to protection of the gastrointestine from oxidative stress. Further study is needed to document the physiological relevance of this study.

ACKNOWLEDGMENTS

The authors thank T. Osawa for advice on the *rec* assay and the staff members of our laboratory animal facility for the careful maintenance of the hamsters.

REFERENCES

1 Ahnfelt-Ronne, I., O. H. Nielsen, A. Christensen, E. Langholz, V. Binder, and P. Riis. 1990. Clinical evidence supporting the radical scavenger mechanism of 5-aminosalicylic acid. Gastroenterology 98:1162–1169.
2 American Institute of Nutrition. 1977. Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. J. Nutr. 107:1340–1348.
3 Babbs, C. F. 1990. Free radicals and the etiology of colon cancer. Free Radical Biol. Med. 8:191–200.
4 Bates, L., J. M. Ames, D. B. MacDougall, and P. C. Taylor. 1998. Laboratory reaction cell to model Maillard color development in a starch-glucose-lysine system. J. Food Sci. 63:991–996.
5 Bedinghaus, A. J., and H. W. Ockerman. 1995. Antioxidative Maillard reaction products from reducing sugars and free amino acids in cooked ground pork patties. J. Food Sci. 60:992–995.
6 Binder, H. J., and G. I. Sandle. 1994. Electrolyte transport in the mammalian colon. Pages 2133–2171 *in* Physiology of the Gastrointestinal Tract. 3rd ed. L. R. Johnson, ed. Raven Press, New York, NY.
7 Bird, C. L., J. S. White, M. E. Swendseid, J. M. Shikany, I. F. Hunt, H. D. Frankl, E. R. Lee, M. P. Longnecker, and R. W. Haile. 1996. Plasma ferritin, iron intake, and the risk of colorectal polyps. Am. J. Epidemiol. 144:34–41.
8 Blois, M. S. 1958. Antioxidant determinations by the use of a stable free radical. Nature (Lond.) 181:1199–1200.
9 Block, G., B. Patterson, and A. Subar. 1987. Fruit, vegetables and cancer prevention: A review of the epidemiologic evidence. Nutr. Cancer 18:1–29.
10 De Palencia, P. F., C. Pelaez, C. Romeo, and M. C. Martin-Hernandez. 1997. Purification and characterization of the cell-wall proteinase of *Lactobacillus casei* subsup. *casei* IFPL 731 isolated from raw goat's-milk cheese. J. Agric. Food Chem. 45:3401–3405.
11 Emerit, J., S. Pelletier, J. Likforman, C. Pasquier, and A. Thuillier. 1991. Phase II trial of superoxide dismutase (Cu Zn SOD) in the treatment of Crohn's disease. Free Radical Res. Commun. 12–13 (part 2):563–569.
12 Erhardt, J. G., S. S. Lim, J. C. Bode, and C. Bode. 1997. A diet rich in fat and poor in dietary fiber increases the in vitro formation of reactive oxygen species in human feces. J. Nutr. 127:706–709.
13 Hayase, F., S. Hirashima, G. Okamoto, and H. Kato. 1989. Scavenging of active oxygens by melanoidins. Agric. Biol. Chem. 53:3383–3385.
14 Hove, H., H. Norgaard, and P. Brobech Mortensen. 1999. Lactic acid bacteria and the human gastrointestinal tract. Eur. J. Clin. Nutr. 53:339–350.
15 Kada, T., Y. Sadaie, and Y. Sakamoto. 1984. *Bacillus subtilis* repair test. Pages 13–32 *in* Handbook of Mutagenicity Test Procedures. 2nd ed. B. J. Kilbey, M. Legator, W. Nichols, and C. Ramel, eds. Elsevier, Amsterdam, Netherlands.
16 Knekt, P., A. Reunanen, H. Takkunen, A. Aromaa, M. Heliovaara, and T. Hakulinen. 1994. Body iron stores and risk of cancer. Int. J. Cancer 56:379–382.
17 Morotomi, M., and M. Mutai. 1986. In vitro binding of potent mutagenic pyrolyzates to intestinal bacteria. J. Natl. Cancer Inst. 77:195–201.
18 Namiki, M. 1988. Chemistry of Maillard reactions: recent studies on the browning reaction mechanism and the development of antioxidants and mutagens. Adv. Food Res. 32:115–184.

19 Nelson, R. L. 1992. Dietary iron and colorectal cancer risk. Free Radical Biol. Med. 12:161–168.
20 Okamoto, G., F. Hayase, and H. Kato. 1992. Scavenging of active oxygen species by glycated protein. Biosci. Biotech. Biochem. 56:928–931.
21 Sasaki, M., B. W. Bosman, and P.S.T. Tan. 1995. Comparison of proteolytic activities in various lactobacilli. J. Dairy Sci. 62:601–610.
22 Shamsuddin, A. M., A. M. Elsayed, and A. Ullah. 1988. Suppression of large intestinal cancer in F344 rats by inositol hexaphosphate. Carcinogenesis 9:577–580.
23 Suetsuna, K. 1997. *Shinkina hekusa pepuchido oyobi kasseika sannso sogaizai*. Japan Pat. No. 9-59297.
24 Sugimura, T. 1985. Carcinogenicity of mutagenic heterocyclic amines formed during the cooking process. Mutat. Res. 150:33–41.
25 Takahashi, M., and M. Onoue. 1999. Safety of *Lactobacillus casei* strain Shirota. Section 2. Safety. Pages 263–281 *in Lactobacillus casei* strain Shirota. T. Yokukura, chief ed. Yakult Honsha Co., Ltd., Tokyo, Japan.
26 Takao, T., F. Kitatani, N. Watanabe, A. Yagi, and K. Sakata. 1994. A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci. Biotech. Biochem. 58:1780–1783.
27 Terasawa, N., M. Murata, and S. Homma. 1991. Separation of model melanoidin into components with copper chelating Sepharose 6B column chromatography and comparison of chelating activity. Agric. Biol. Chem. 55:1507–1514.
28 Toyokuni, S. 1996. Iron-induced carcinogenesis: the role of redox regulation. Free Radical Biol. Med. 20:553–566.
29 Trock, B., E. Lanza, and P. Greenwald. 1990. Dietary fiber, vegetables, and colon cancer: critical review and meta-analyses of the epidemiologic evidence. J. Natl. Cancer Inst. 82:650–661.
30 Wakabayashi, K., M. Nagao, H. Esumi, and T. Sugimura. 1992. Food-derived mutagens and carcinogens. Cancer Res. 52:2092s–2098s.
31 Yoshimura, Y., T. Iijima, T. Watanabe, and H. Nakazawa. 1997. Antioxidative effect of Maillard reaction products using glucose-glycine model system. J. Agric. Food Chem. 45:4106–4109.
32 Zar, J. H. 1984. Multiple comparisons. Pages 185–235 *in* Biostatistical Analysis. 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, NJ.