Effects of Dietary Ethanol Extracts from Sake Rice and Sake Lees on Intestinal Impairment in Mice

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Abstract: Glucosylceramide (GlcCer), a major sphingolipid in plants and fungi, is known to have food functions, such as preventing intestinal impairment and enhancing the moisture content of skin. This study investigated the influence of fermentation on the composition and function of lipophilic components containing GlcCer in plant-based foods; we compared the effects of ethanol extracts from sake rice (SR) and sake lees (SL) on colon impairment in mice. GlcCer and ceramide (Cer) levels in SL were much higher than those in SR, and GlcCer in SL contained 9-methyl-trans,trans-8-sphingadienine as a fungi-specific sphingoid base. 1,2-dimethylhydrazine (DMH) treatment markedly increased the formation of aberrant crypt foci (ACF) and the levels of TNF-α and lipid oxidation in mice colons. However, dietary SR or SL significantly suppressed these DMH-induced changes, and SR demonstrated stronger effects than SL. In addition, dietary SR or SL suppressed the expression of apoptotic and anti-apoptotic proteins induced by DMH treatment. This study suggests that SR or SL intake could reduce colon ACF formation via the suppression of inflammation and oxidation-induced cell cycle disturbances. When compared to SR, the weakened effects of SL rich in GlcCer may be the result of the changes in sphingolipid composition (sphingoid base and Cer) and differences in the concentration of other bioactive compounds produced or digested during fermentation.

Key words: ceramide, colon cancer, glucosylceramide, rice, sake

1 Introduction

Complex sphingolipids are composed of a sphingoid base with an amide-linked fatty acid (i.e., ceramide, Cer) and a polar head group, such as a phosphocholine or a hexose (1). Sphingolipids are primarily found in the cell membrane of most eukaryotes and some prokaryotes. The compositions of the classes and sphingoid bases are highly diverse in nature. Mammalian sphingolipid classes consist of sphingomyelin, galactoceramide, Cer, and gangliosides. In contrast, plant and fungal sphingolipid classes are mainly composed of glucosylceramide (GlcCer), Cer, inositol phosphoceramide (IPC), and glycosyl IPC (GIPC). In the mammalian sphingolipids, trans-4-sphinganine (sphingosine, d18:1) is the most prevalent sphingoid base; whereas sphinganine (d18:0) and 4-hydroxysphinganine (phytosphingosine, t18:0) also occur frequently in smaller amounts. Plant sphingolipids have diverse sphingoid base structures such as trans-8-sphingenine (d18:1), cis-8-sphingenine (d18:1), trans-4,trans-8-sphingadienine (d18:1), trans-4,cis-8-sphingadienine (d18:1), 4-hydroxy-trans-8-sphingenine (t18:1), and 4-hydroxy-cis-8-sphingenine (t18:1). Specifically, 9-methyl-trans,4,trans-8-sphingadienine (9-Me d18:2) is a unique base found only in fungi.

Sphingolipids affect membrane fluidity by the different composition in sphingoid bases (6). Sphingolipids also play important roles in various biological functions (e.g., apoptosis, autophagy, cellular differentiation, and cell proliferation) (3). In addition to their biological functions, the administration of GlcCer has been reported to prevent atopic dermatitis (9), improve skin-barrier function (8), and enhance lipid metabolism (6). Our previous studies showed that dietary GlcCer from plant sources alleviates colon inflammatory injuries in the inflammatory bowel disease mice.
model treated with dextran sulfate sodium (DSS), and suppresses the formation of colon aberrant crypt foci (ACF) as well as the production of inflammation-related cytokine induced by 1,2-dimethylhydrazine (DMH). In vitro experiments indicated the possibility that GlcCer protects the colon surface from the harmful effects of various drugs. In addition, sphingoid bases themselves in digested GlcCer have been shown to have an apoptosis-inducing effect on colon cancer cells in vitro.

Many reports based on purified sphingolipids demonstrated that sphingolipids have very interesting dietary functions, however, their functions of complex mixtures as extracts are still not known. Usually, the commercial resources of plant sphingolipid GlcCer are byproducts rich in lipids like rice bran, wheat bran, or strained lees of maize or soybeans without its edible oil. These resources are abundant not only in GlcCer but in other functional lipophilic compounds as well. In contrast, polished rice has a lower amount of GlcCer but a higher purity in the lipophilic fraction when compared to rice bran. Previously, our investigations into the effects of plant-based lipophilic fraction have focused on GlcCer in polished rice. We reported that when compared to polished rice containing the same level of GlcCer, dietary ethanol extract from polished rice had higher suppressive effects on ACF formation and inflammation in the colon of mice treated with DMH. We also described the low utilization of GlcCer from plant-based foods and the possibility of synergism with other lipophilic components.

Sake (i.e., rice wine) is a traditional Japanese alcoholic beverage, produced using highly polished sake rice and parallel multiple fermentation with koji (Aspergillus oryzae) and sake yeast (Saccharomyces cerevisiae). Sake lees (sake-kasu, sake cake) is a byproduct of sake and is used as a bed for pickling (kasu-uke), a material of soup and drink (kasu-jiru and amazake), and a source for Japanese spirits and vinegar (kasutori-shochu and kasu-zu). Sake lees has been reported to improve hepatic lipid accumulation and has been shown to contain GlcCer with a fungi-specific sphingolipid base.

In this study, we investigated the influence of fermentation on the composition and function of lipophilic components in plant-based foods, by comparing the effects of the ethanol extracts from sake rice (SR) and sake lees (SL) on colon impairment in mice treated with DMH. In addition, we analyzed the lipophilic components of SR and SL, especially the sphingolipid composition.

2 Experimental Procedures
2.1 Preparation of extracts of sake rice (SR) and sake lees (SL)

We purchased sake rice (Oryza sativa cv. Yamadanishiki and Gohyakumangoku) from a rice wholesaler, Japan; koji (Aspergillus oryzae cv. Byakuya) from a koji maker (Hishiroku, Kyoto, Japan); and sake yeast (Saccharomyces cerevisiae cv. M310) from MEIRISHURUI Co., Ltd. (Ibaraki, Japan). Yamadanishiki and Gohyakumangoku rice were polished to 45 %, and used as koji rice (koji-mai) and adding rice (kake-mai), respectively. Sake and sake lees were produced during a 31-day fermentation, according to the shubo-shoryaku procedure, in which much more cultured sake yeast than normal is added during shubo preparation (starter culture; mixture of rice, koji rice, sake yeast, and lactic acid). The resultant sake lees was freeze-dried and crushed. To make the sake rice extract (SR), Gohyakumangoku rice was cooked, freeze-dried, and crushed. These powders were stirred in 80% ethanol at 20°C for 1 h. After filtration, the residue was treated in the same way by using 90% ethanol and 80% ethanol successively. The amount of diluted ethanol used each time was three times the amount of the powder. The filtrates were combined and dried using a rotary evaporator. The products were used as SR or sake lees extract (SL), and stored at −30°C until further use.

2.2 Lipid analysis in SR and SL

Lipid classes in SR and SL were separated using thin-layer-chromatography (TLC) with a mobile phase suitable for neutral lipids or polar lipids. Moreover, GlcCer and Cer in SR and SL were separated and their sphingoid bases were analyzed as described previously. Briefly, crude lipids were extracted using a chloroform/methanol/water solvent system and saponified with 0.4 M KOH in methanol at 38°C for 2 h to obtain the alkali-resistant lipids (e.g., sphingolipids and sterols). GlcCer and Cer were separated using TLC. The isolated sphingolipids were hydrolyzed with or without d18:0 (Cayman Chemical, Michigan, USA) as the internal standard using aq. methanolic 1 N HCl at 80°C. The reaction mixture was washed with hexane and adjusted to a pH of more than 9 with 6 N KOH. The liberated sphingoid base component was extracted with diethyl ether and converted to fatty aldehydes using NaIO4 oxidation. The resultant fatty aldehydes were analyzed with a gas chromatography (GC) - mass spectrometry (MS). The GC-MS system was equipped with a GC-2030 and QP2020NX instruments (Shimadzu, Kyoto, Japan).

2.3 Analysis of the other lipophilic components in SR and SL

We quantified the levels of the other lipophilic components in SR and SL (Table 1B). Ferulic acid levels were analyzed by high-performance-liquid-chromatography with a fluorescence detector (excitation at 390 nm, emission at 450 nm). Total fatty acid composition was determined by GC after direct methylation.
2.4 Experimental diets

The control diet was based on AIN-76, which does not contain sphingolipids. The SR group diet was supplemented with SR (0.94 g extracted from 150 g of sake rice), and the SL group was given SL (42.1 g extracted from 150 g of sake lees) instead of corn starch and sucrose (Table 1A).

2.5 Animals

Four-week-old female BALB/c mice were obtained from Japan SLC, Inc. (Shizuoka, Japan), and housed in isolator cages at 22°C under a 12 h light/dark cycle. The mice were randomly divided into four groups (n = 10 in each group). The details of the diets of the various groups were as follows: blank group (control diet with intraperitoneal (i.p.) vehicle); control group (control diet with i.p. DMH); SR group (SR diet with i.p. DMH); and SL group (SL diet with i.p. DMH). In addition, each group was subdivided into two subgroups (n = 5) for ACF formation or other analyses of the colon. After acclimation to the experimental diet for 2 weeks, each mouse was i.p. administered 15 mg/kg body weight of 1,2-DMH-HCl (Tokyo Chemical Industry Co., Ltd., Japan) once a week for 7 weeks. DMH treatment induces development of ACF which precursors of colon cancer, as well as chronic colon inflammation which is a risk factor of cancer; therefore, DMH-induced model is suitable for investigating potential preventive effects of food compounds in colon cancer. All protocols involving animals were approved by the Animal Care and Use Committee and conducted in accordance with the Obihiro University Guidelines (Permit Number: 18-161).

2.6 ACF identification

ACF from the large intestinal crypts were identified and quantified as previously described. Colonies of five mice in each group were prepared as specimens for counting ACF. The large intestine was excised from the mice under pentobarbitone anesthesia, and a portion of the intestine from the cecum to the vent was separated and rinsed with cold saline. This was followed by cell fixation overnight in PBS containing 4% paraformaldehyde and stained with 0.3% methylene blue solution in saline for 30 min at 20°C. ACF were counted throughout the large intestine under a microscope. During counting, ACF and AC in the colon were divided by AC degree and counted as: AC1, AC2, and AC3; to indicate ACF formed by 1 crypt, 2 crypts, and 3 or more crypts, respectively.

2.7 Tumor necrosis factor (TNF)-α levels and protein profiles for apoptosis

The colons of four mice in each group were analyze for the presence of TNF-α and apoptosis-related proteins. TNF-α levels were measured using a Mouse TNF-α ELISA kit (FUJIFILM Wako Shibayagi Corp., Gunma, Japan). Levels of apoptosis-related proteins were examined using a Mouse Apoptosis Array Kit (R&D Systems, Minneapolis, MN), respectively. Briefly, the colon mucosa was scraped, homogenized in PBS containing proteinase inhibitors (Protease inhibitor cocktail set III, Fujifilm Wako Pure Chemical Corp., Osaka, Japan), and mixed with Triton X-100. According to the manufacturer’s protocol, after freezing and thawing, the proteins were detected. The detected apoptosis-related proteins were denoted as follows: B-cell lymphoma 2 (Bcl-2); Bcl/leukemia x (Bcl-x); catalase; claspin;
(MCL-1); p27 cyclin dependent kinase 4 inhibitor 1B (p27/Kip1); X-linked inhibitor of apoptosis (XIAP); Bcl-xL/Bcl-2 associated death promotor (Bad); cytochrome c; second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI (SMAC/Diablo); fibroblast-associated (Fas); TNF receptor 1 (TNF R1); TNF-related apoptosis-inducing ligand receptor 2 (TRAIL R2); cleaved caspase-3; p53; hypoxia-inducible transcription factor (HIF)-1α; heme oxygenase (HO)-1; HO-2; heat shock protein (HSP)27; HSP60; and HSP70.

2.8 Lipid peroxidation and protein quantification assays
Thiobarbituric acid-reactive substances (TBARS), an indicator for lipid peroxidation was determined as equivalent to malondialdehyde (MDA) according to the method by Ohkawa et al.17 Protein amount of colon mucosa was measured by DC Protein Assay kit (Bio-rad, CA, USA).

2.9 Statistical analysis
The data between blank and control groups were evaluated by Student’s t-test. The data among DMH-treated groups were analyzed using variance analysis and Scheffé’s test. In all analyses, differences were considered statistically significant when $p < 0.01$ or $p < 0.05$.

3 Results
3.1 Composition of the ethanol extracts from SR and SL
Figure 1 shows SR and SL content evaluated by TLC. When SR and SL are compared, SL contained more lipophilic components including GlcCer, Cer, free fatty acids (FFA), and triacylglycerols (TG). Fatty acid ethyl esters (FAEE) were only observed in SL. Table 1B shows the amount of each of the lipophilic components in SR and SL that were added to the experimental diets. It is known that ferulic acids has an anti-ACF formation and anti-inflammatory effects18, 19, and diets low in n-6/n-3 ratios suppress inflammation20. Instrumental analysis revealed that the amount of total ferulic acids, GlcCer, Cer, and total fatty acids were much higher in SL than in SR. In addition, the ratio of Cer/GlcCer, saturated

![TLC profiles of lipid classes in SR and SL on silica thin-layer chromatography.](image)

**Fig. 1** TLC profiles of lipid classes in SR and SL on silica thin-layer chromatography. A, Neutral lipids of SR and SL; B, Polar lipids of SR and SL; C, Alkali-resistant lipids of SR and SL. To remove lipid components except for alkali-resistant lipids (sphingolipids and sterols), SR and SL were treated with an alkali, KOH. SL and SR prepared from the same amounts of respective freeze-dried samples were applied, and SR × 5 and × 10 indicate 5 and 10 times of application, respectively. FAEE, fatty acid ethyl ester; TG, triacylglycerol; FFA, free fatty acid; MGDG, monoglycosyldiacylglycerol; SG, sterylglucoside; GlcCer, glucosylceramide; DGDG, diglycosyldiacylglycerol; ASG, acylsterylglucoside; Cer, ceramide.
fatty acids, and n-6/n-3 was higher in SL than in SR. In contrast, the GlcCer and Cer purities in terms of the total fatty acids were higher in SR than in SL.

When the total fatty acid composition was converted to TG, SR and SL contained 0.1 g and 4.6 g, respectively. SR and SL also comprised proteins and carbohydrates, with the caloric value for SR being 4.4 kcal and SL being 191.4 kcal. Thus, the total dietary calories for the control, SR, and SL diets were 3748 kcal/kg, 3748 kcal/kg, and 3780 kcal/kg, respectively.

3.2 Sphingoid base composition for GlcCer and Cer in SR and SL

GlcCer in SR and SL was predominantly made up of sphingoid base d18:2\textsuperscript{4t,8c}, while Cer was dominated by t18:0 (Table 2). The secondary base for GlcCer in SR was t18:1\textsuperscript{8c}, while it was 9-Me d18:2\textsuperscript{4t,8t} in SL, which was not detected in SR. Cer in SL contained a higher ratio of d18:0 when compared to SR.

3.3 Body, liver, and spleen weights, and colon length in the mice

No differences in final body or liver weight and colon length were noted between the control and blank groups, while spleen weight in the control group was higher than that of the blank group (Table 3). Among the DMH-treated groups, there were no significant differences between the control and the other groups, except for the liver weight in the SR.

3.4 Effects of dietary SR and SL on the incidence of DMH-induced ACF in mice colons

Figure 2 shows the degree and number of ACF in each experimental group. ACF and AC in the colon specimens were divided by the AC degree and counted as: AC1, AC2, and AC3; to indicate ACF formed by 1, 2, and 3 or more crypts, respectively. The observed numbers of AC1, AC2, AC3, and total ACF in the control group were significantly higher than those in the blank group. In contrast, the SR and SL groups showed significantly less amount of AC1, AC2, and total ACF compared to the control group. Furthermore, when we compared the SR and SL groups, the number of AC1 and total ACF in the SR group was significantly decreased.

3.5 Effects of dietary SR and SL on the induction of inflammation and lipid peroxidation in the colons of DMH-treated mice

The levels of TNF-α and TBARS in the colon mucosa of DMH treated mice fed various diets are shown in Fig. 3. DMH treatment significantly increased the levels of TNF-α and TBARS in the colon mucosa. Dietary SR or SL suppressed DMH-induced levels of colon TNF-α and TBARS. The SR group showed a lower tendency of TNF-α and TBARS levels when compared to the SL group.

3.6 Effects of dietary SR and SL on the expression of apoptosis-related proteins in the colons of DMH-treated mice

DMH treatment induced the expression of anti-apoptoti-
ic, apoptotic, and other proteins in colon mucosa (Fig. 4). Specifically, DMH injection markedly increased the expression of cleaved caspase-3 as an effector caspase on the downstream step of apoptosis pathway. Dietary SR or SL suppressed the DMH-induced expression of almost colon apoptosis-related proteins at the same levels in the blank group.

4 Discussion
Dietary habits have strongly been associated with colon cancer incidences. The onset and progression of colon cancer are thought to be related to chronic colon inflammation and oxidative stress which results in genetic mutation and the dedifferentiation of normal cells. In our previous studies, we reported that dietary GlcCer or ethanol extract containing GlcCer from polish rice suppressed inflammation and ACF formation in colon impairments in mice. However, it is not well-established whether the diversity of the sphingoid base in GlcCer or other food
Fig. 3  Effect of dietary SR or SL on levels of TNF-α and lipid oxidation in mice colon after 8 weeks of DMH injection. A and B indicate levels of TNF-α and MDA in colon mucosa of mice, respectively. Mean ± SEM (n = 4). Asterisks indicate significant differences among DMH-treated groups (only asterisks, vs control group; asterisks attached bar, between SR and SL groups) by one-way ANOVA with Scheffe’s test (**p < 0.01, *p < 0.05). Sharps indicate significant differences between blank and control groups by Student’s t-test (##p < 0.01, #p < 0.05).

Fig. 4  Effect of dietary SR or SL on the expression of apoptosis-related proteins in mice colon after 8 weeks of DMH injection. A, Anti-apoptotic proteins; B, Apoptotic proteins; C, Other proteins. Mean ± SEM (n = 4). Asterisks indicate significant differences among DMH-treated groups (only asterisks, vs control group; asterisks attached bar, between SR and SL groups) by one-way ANOVA with Scheffe’s test (**p < 0.01, *p < 0.05). Sharps indicate significant differences between blank and control groups by Student’s t-test (##p < 0.01, #p < 0.05).
components may alter the function of GlcCer. This study investigated the influence of fermentation on the composition and function of lipophilic components containing GlcCer in plant-based foods. We compared the effects of the ethanol extracts of SR and SL on colon impairment in mice. GlcCer and Cer levels in SL were much higher than those in SR (Fig. 1 and Table 1), and GlcCer in SL contained 9-Me d18:2[4,8] as a fungi-specific sphingoid base (Table 2). DMH treatment markedly increased the formation of ACF and the levels of TNF-α and lipid oxidation in the colon of mice (Figs. 2 and 3). Dietary SR or SL significantly suppressed these changes, with SR exerting a stronger effect than SL. In addition, dietary SR or SL suppressed the expression of apoptotic and anti-apoptotic proteins induced by DMH treatment (Fig. 4).

Sake lees contains higher levels of lipophilic components because koji amylases and sake yeast consume carbohydrates in sake rice during fermentation. SL also has higher levels of lipophilic components (e.g., total fatty acids, total ferulic acids, GlcCer, and Cer) when compared to SR (Table 1). In addition, the Cer/GlcCer ratio and sphingoid base composition of GlcCer and Cer in SL were different than those in SR. General yeasts possess Cer but not GlcCer, and fungi including koji have GlcCer with 9-Me d18:2[4,8] but absent in plants[2,23,24]. Therefore, it is noteworthy that SL increased its Cer/GlcCer ratio and contained GlcCer with 9-Me d18:2[4,8] (Tables 1 and 2). Also, Cer in SL displayed an increased d18:0 ratio than those in SR, and it has not been reported that d18:0 content is high in GlcCer and Cer of sake yeast or koji. The sphingoid base d18:0 may be produced through the degradation of IPC and GIPC, or oxidized during fermentation reactions as is the case with the increase in saturated fatty acid content.

Dietary SR or SL ameliorated increases in TNF-α and TBA5S induced by DMH, as well as ACF formation in the colons of treated mice (Figs. 2 and 3). Additionally, dietary SR or SL adjusted the colon expression of apoptotic and anti-apoptotic proteins when disturbed by DMH (Fig. 4). It is thought that chronic colon inflammation and oxidative stress cause cell dedifferentiation and genetic mutation to induce cell proliferation, and ACF formation is due to high expression levels of anti-apoptotic proteins; whereas cellular homeostasis expresses apoptotic proteins for the suppression of cell proliferation[25]. Therefore, suppression of ACF formation by SR or SL supplementation could be related to the suppression of inflammation and oxidative stresses.

SR and SL diets contain GlcCer and ferulic acid that possess the effects of anti-inflammation and anti-ACF formation in the colon[8,12,18,19], and GlcCer and ferulic acid content in the SL diet is much higher than that of the SR diet (Table 1). Nevertheless, SR intake showed stronger effects on the suppression of colon impairment than SL intake (Figs. 2 and 3). This is not thought to be as a result of an overdose of GlcCer or ferulic acid; it is reported that dietary GlcCer or ferulic acid suppresses ACF formation at concentrations of 5 g/kg diet (GlcCer 7.0 mmol, ferulic acid 25.8 mmol), and show a dose dependent response when compared to GlcCer 1 g/kg diet or ferulic acid 2.5 g/kg diet (GlcCer 1.4 mmol, ferulic acid 12.9 mmol)[14,25]. The difference in sphingoid base composition for the GlcCer in SR and SL may affect their impact on colon impairment (Table 2); however, to the best of our knowledge, there is still no description of the impact of sphingoid base composition for colon impairment; although many studies have revealed the sphingoid base itself, which is a digestive product of GlcCer, and the structure of the sphingoid base have different intestinal absorption ratios in vivo and in vitro[26,27], and exhibit different effects on the skin and the adipocytes in vitro[28,29].

The decrease in the effects on colon impairment following fermentation may be related to the production or elimination of other bioactive components. SL possesses higher FFA level and Cer/GlcCer ratio than SR (Fig. 1 and Table 1). High levels of FFA, especially saturated FFA can be cytotoxic[30,31] and ceramide is an apoptotic signal in cells[32,33]. Ceramide is an agonist of Toll-like receptor (TLR) 4, and TLR4 signaling is thought to enhance the incidence of colon inflammation and cancer[34,35]. Dietary sphingomyelin, a complex sphingolipid from animals, is reported to both suppress and stimulate colon inflammation[36,37], and those reports which support the inflammatory roles of this sphingolipid suggest that ceramide, which is the digestive product, is the core component of this response; ceramide accelerates apoptosis via the expression of cathepsin D[37]; the administration of a sphingomyelins inhibitor ameliorates DSS-treated colon inflammation[38]. In contrast, when compared to sphingomyelin, dietary GlcCer is reported to only suppress colon inflammation, although this is a newer field of research with fewer publications[8,11]. There is a possibility that dietary GlcCer is digested to ceramide, but this is unlikely as the activity of glycosylceramidase is weaker than that of sphingomyelins. In addition, sphingoid base composition from plants could exert a different effect than those from animals. Moreover, saturated FFA activates sphingosine kinase which exchanges sphingosine from ceramide for sphingosine 1-phosphate, and sphingo-gosine 1-phosphate is thought to induce chronic inflammation and colon cancer[31,30]. Overall, we are of the opinion that high FFA and/or ceramide concentrations in SL adversely affect the intestinal conditions.

In our previous study, we reported that the rice cell wall reduces the beneficial effects of rice’s lipophilic components on colon impairment[12]. In this study, SL contains highly viscous substances which cannot be totally liquefied by amylases. Some fungi containing koji secrete indigestible extracellular soluble polysaccharides[40], and sake lees have resistant-proteins and resistant-starches[31]; therefore,
these indigestible substances may reduce the overall benefit of the lipophilic components as a result of reduced contact with the intestinal epithelium. In contrast, there is also a possibility that these functional substances are degraded during fermentation. Tocotrienols, which prevent cancer, are known to have synergistic effects when used with ferulic acid[43]. In this study, owing to the use of highly polished rice, tocotrienols were not detected in either SR or SL, but other substances having synergistic actions may be degraded during fermentation.

The livers in the SR group showed a higher weight than those in the other groups(Table 3). The caloric content of the experimental diet and its intake in the SR group were almost the same as those of the other groups, and the SR livers appeared normal (color and shape). In this animal model, DMH is metabolized to a carcinogen in the liver, and the bile acids carry this carcinogen from the liver to the colon[45]. Therefore, dietary SR may change the liver metabolism and protect the colon. In contrast, dietary SR may cause liver impairment through metabolism dysfunction. To clarify the benefits of SR and SL, it is necessary to perform more detailed experiments on these animals including fecal bile acid level and liver levels of oxidative stress, TG, and cholesterol.

In conclusion, ethanol extract from SR or SL prevents colon impairment in DMH-treated mice. The suppressive effects were higher for SR intake than SL intake. The change in the lipophilic composition and concentrations during fermentation may affect the overall beneficial impact of these extracts.

Conflict of Interest
The authors have no conflicts of interest to declare.

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