Effect of Proanthocyanidin-Rich Extract from Grape Seeds on Human Fecal Flora and Fecal Odor

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The aim of this study was to evaluate the effects of proanthocyanidin-rich extracts from grape seeds on human fecal flora and fecal odor. Proanthocyanidin-rich extract containing 38.5% proanthocyanidin was administered to nine healthy adults at a dose of 0.5 g/day (0.19 g/day as proanthocyanidin) for 2 weeks, and proanthocyanidin-rich extract containing 89.3% proanthocyanidin was administered to eight elderly inpatients at a dose of 0.43 g/day (0.38 g/day as proanthocyanidin) for 2 weeks. Green tea extract and/or champignon extract, both of which have been found to have a deodorant effect on fecal odor, were administered in a similar manner as controls. In healthy adults, marked decreases in fecal odor and concentrations of methyl mercaptan and hydrogen sulfide in feces were observed during proanthocyanidin-rich extract intake, but the effects of green tea extract and champignon extract were weak. After 2 weeks of proanthocyanidin-rich extract intake, the number of Bifidobacterium had increased significantly (p < 0.05), whereas the number of Enterobacteriaceae tended to decrease (p = 0.121). The level of putrefactive substances, including ammonia, phenol, p- cresol, 4-ethylphenol, indole, and skatol tended to decrease after proanthocyanidin-rich extract intake, and fecal pH also tended to decrease. Nurses and hospital aides performed organoleptic evaluations that showed less fecal odor in elderly inpatients with proanthocyanidin-rich extract intake than with champignon extract intake. In an in vitro study, the proanthocyanidin-rich extract reduced methyl mercaptan and hydrogen sulfide release from the feces of healthy adults, and also reduced methyl mercaptan release from methyl mercaptan solution. The absorptive ability of methyl mercaptan was stronger in procyanidin oligomers larger than decamer than procyanidin dimer to tetramer. These results suggest that proanthocyanidin-rich extract from grape seed intake induces a reduction in the level of putrefactive products in the intestine, which may be linked to the modest change in the numbers of Bifidobacterium and Enterobacteriaceae. They also suggest that the strong deodorant effect of proanthocyanidins on fecal odor is due to the decrease of putrefactive products and the absorption of malodorous compounds from feces by the larger molecular procyanidin oligomers in the proanthocyanidins. Key words: proanthocyanidin, grape seed extract, polyphenol, fecal flora, fecal odor.

INTRODUCTION

Epidemiologic studies have shown that wine consumption is associated with a reduced incidence of coronary heart disease, the so-called ‘French paradox’ (1, 2). Red wine is a rich source of polyphenols, and many studies have shown that these polyphenols have an important role in preventing cardiovascular disease (3–5).

Proanthocyanidins, which are oligomers or polymers of polyhydroxy flavan-3-ol units, such as (+)-catechin and (−)-epicatechin (6), are present in their greatest amounts in the polyphenols of red wine and the polyphenols of grape seeds. In grape seeds, only the procyanidin-type of proanthocyanidins have been detected (7).

Proanthocyanidins have been reported to have a variety of biological activities, including antibacterial (8, 9), antioxidant (10–12), antiatherosclerotic (13), anticarcinogenic (14, 15), and antiulcer (16) activity.

Consumption of tea catechin, monomeric flavanols (1.2 g/day for 4 weeks) changes the fecal flora of healthy adults (17), and intake (0.3 g/day for 3 weeks) changes the fecal flora and fecal odorous parameters of elderly residents in long-term care facilities (18). On the other hand, when ¹⁴C-labelled procyanidins were orally administered to rats, 6% of the dose was excreted as ¹⁴CO₂ in expired air, 19% was eliminated in urine, and 45% was excreted in feces (19); thus, a large portion of the procyanidins remained in the feces. Therefore, the dietary proanthocyanidins may be effective in improving the intestinal environment and may change odor parameters.

However, the influence of proanthocyanidin intake on fecal flora, fecal odor parameters, and fecal odor has never been assessed.

In this study, we investigated the effects of a proanthocyanidin-rich extract from grape seeds on the fecal flora
and fecal odor of healthy adults and on the fecal odor of elderly bedridden inpatients.

MATERIALS AND METHODS

Grape seed extracts, green tea extract, and champignon extract

The grape seed extracts used in our studies were Gravinol™ and Gravinol super™ (Kikkoman Co., Chiba, Japan), which contain 38.5% and 89.3% proanthocyanidins, respectively. The proanthocyanidin-rich extracts were prepared from grape seeds (Vitis vinifera L.). Gravinol super™ was composed of 89.3% proanthocyanidins, 6.6% monomeric flavanols, 1.7% fructose, 0.4% glucose, 1.7% ash, 3.6% moisture, 1.1% protein, and 0.2% fat. Gravinol™ was composed of 38.5% proanthocyanidins, 2.4% monomeric flavanols, 8.9% fructose, 7.8% glucose, 11.6% citric acid, 5.0% ash, 2.5% moisture, and 3.7% protein. The degree of polymerization of the procyanidins in the extracts ranged from 2 to 15; this was detected by mass spectrometry (Dr. M. Kameyama, National Food Research Institute, Ibaraki, Japan, unpublished data). A representative structure of procyanidins, the component of proanthocyanidins from grape seeds, is shown in Fig. 1. For the in vitro study using methyl mercaptan solution, procyanidin dimer to tetramer, procyanidin pentamer to heptamer, and procyanidin oligomers larger than decamer were produced by the grape seed extract containing 89.3% proanthocyanidins, followed by ethyl acetate extraction and separation using an LH20 sephadex column (Pharmacia Biotech, Uppsala, Sweden). Procyanidin oligomers more than decamer were also produced by the grape seed extract, followed by separation by regenerated cellulose (Millipore, Bedford, TX, USA). The green tea extract and champignon extract were used as controls. The green tea extract was Sunflavon HG (Taiyo Kagaku Co., Tokyo, Japan), which contains 50% catechins including (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin, and (-)-epigallocatechin gallate. The champignon extract was Bio-M BX100FPD (Ricom Co., Tokyo, Japan), which did not contain flavonoids. The main ingredients influencing fecal odor in the extract were not clear.

Human study in healthy adults

The subjects of this study were nine healthy male volunteers aged 37–42 years. The duration of the experiment was 10 weeks, divided into six periods: a first 14-day washout period, three 14-day administration periods, and two 14-day washout periods between each administration period. During each administration period, 0.5 g/day/subject of proanthocyanidin-rich extract from grape seeds (0.19 g/day/subject as proanthocyanidin), 0.5 g/day/subject of green tea extract (0.25 g/day/subject as catechins), and 0.5 g/day/subject of champignon extract were administered orally in two divided doses. Throughout the entire period of the experiment, the subjects did not consume any bifidogenic oligosaccharides, fermented dairy products prepared using lactic acid bacteria, red wine, or green tea, and they were free of antibiotics.

Human study in elderly inpatients

The subjects were 24 elderly hospital inpatients (five males and 19 females, aged 67–98 years), who wore diapers. They were divided into three groups of eight patients each. During the 14-day administration period, 0.43 g/day/patient of sugar, 0.43 g/day/patient of proanthocyanidin-rich extract from grape seeds (0.38 g/day/patient as proanthocyanidin), and 0.43 g/day/patient of champignon extract were administered orally once a day. The diagnoses of the patients included cerebral stroke (15 cases), bone fracture (five cases), senile dementia (three cases), and articular rheumatism (one case). None of the subjects had gastrointestinal or endocrine complications, and none were receiving antibiotics. Those who were on medication continued treatment. The subjects received almost the same daily diet (1467.7 ± 48.2 kcal/day of energy, 62.5 ± 4.7 g/day of protein, 38.0 ± 4.7 g/day of fat, 212.0 ± 15.0 g/day of carbohydrate, 9.4 ± 1.3 g/day of sodium, 803.9 ± 105.8 ml/day of moisture, and 568.1 ± 89.9 mg/day of calcium). Both studies were performed in accordance with the Helsinki Declaration as updated in Tokyo in 1975.

Collection of fecal specimens

Freshly voided fecal specimens were obtained from the healthy adults by direct defecation into 1.6-l airtight vessels on days 0, 2, 7, and 14 of administration for the organoleptic evaluation of fecal odor and determination of methyl mercaptan and hydrogen sulfide gas release by the feces. Freshly voided fecal specimens from six healthy adults were collected from healthy adults by direct defecation into 1.6-l airtight vessels on days 0, 2, 7, and 14 of administration for the organoleptic evaluation of fecal odor and determination of methyl mercaptan and hydrogen sulfide gas release by the feces.
adults were also collected into 1.6-l airtight vessels for in vitro examination. A portion of the specimens from the six healthy adults obtained on days 0 and 14 was immediately transported anaerobically at 4°C to the laboratory (Chemotherapy Division, Mitsubishi Kagaku Bio-Clinical Laboratories, Inc.) for microflora analysis. The analysis was performed within 12 h of excretion. The remainder of the samples was frozen at −30°C for later analysis of bacterial metabolites. For the elderly inpatients, freshly voided fecal specimens with soiled napkin were collected into 12-l airtight vessels every day during the administration period for the organoleptic evaluation of fecal odor.

Organoleptic evaluation of fecal odor

In the study on the healthy adults, three adult subjects (one female and two males, aged 40–59 years) evaluated degree of fecal odor according to the following 4-grade evaluation: grade 3, strong bad smell; grade 2, moderate smell; grade 1, smell almost undetectable; grade 0, no smell. In the study on the elderly inpatients, six nurses and hospital aides (six females, aged 32–58 years) evaluated fecal odor as described above.

Determination of methyl mercaptan and hydrogen sulfide gas release by the feces

In the study on healthy adults, the concentrations of methyl mercaptan and hydrogen sulfide gas in the headspace of the fecal specimen vessels were measured with gas detector tubes (Komyo Rikagaku Kogyo Co., Tokyo, Japan) (20, 21).

Fecal microflora analysis

The method of Mitsuoka et al. (22, 23) was used for fecal microbial analysis. The culture media and methods for isolation and identification of the microflora are described in detail elsewhere (24). Bacterial colonies on each medium were counted and identified according to their colonial and cellular morphology, gram-reaction, spore formation, and aerobic growth (24). The results are expressed as the log_{10} of the number of bacteria per gram wet weight of fecal material. The viable count was calculated from the sum of the counts of all bacterial species.

Determination of fecal putrefactive products and other characteristics

Fecal putrefactive products (phenol, p-cresol, 4-ethyl phenol, indole, and skatol) were measured by the methods of Yoshihara (25, 26). A 0.5-g fecal sample was homogenized in 5 ml of 1.0 M phosphate buffer (pH 6.0), and iso-propylphenol was added as an internal standard. The homogenate was steam-distilled and analyzed by Shimazu GC-9A gas chromatography. Fecal ammonia was analyzed using a high performance liquid chromatography (HPLC) system on a PCI-301S column, 4.6 × 125 mm (TOA, Tokyo, Japan). Fecal pH was measured directly by inserting a glass electrode into the feces, and fecal moisture content was measured in approximately 1-g samples by weighing before and after drying for 4 h in a vacuum oven at 105°C. The difference in weight was considered to be the moisture content.

In vitro studies using fecal specimens and methyl mercaptan solution

The ability of proanthocyanidin-rich extract from grape seeds to bind methyl mercaptan and hydrogen sulfide released from feces was studied using freshly voided fecal specimens (mean wet weight: 101.0 ± 63.1 g) from six healthy adults. The concentrations of these gases in the headspace of the 1.6-l airtight vessels containing the fecal specimens were first measured with gas detector tubes. The fecal specimens were immediately mixed with 0.5 g grape seed extract (0.19 g as proanthocyanidin), and the concentrations of the gases were measured.

The ability of proanthocyanidin-rich extract from grape seeds to bind methyl mercaptan was studied by incubating 5 ml of 5 × 10^{-14}% extract, containing 89.3% proanthocyanidins, in distilled water with 1.3 μl methyl mercaptan solution (1 μg/μl benzene solution; Wako Pure Chemical Co., Tokyo, Japan). The incubations were performed at 37°C in a 150-ml airtight sealable flask. Aliquots of gas were removed for analysis at 0, 1, 5, 30, and 60 min. Similar experiments were performed with distilled water and with distilled water containing procyanidin dimer to tetramer, procyanidin pentamer to heptamer, and procyanidin oligomers larger than the dimer. The concentrations of methyl mercaptan were determined using an Hitachi G-3000 gas chromatograph.

Statistical analysis

Results are shown as means ± standard deviation (SD). The statistical significance of differences was examined by the Student’s t-test, and p < 0.05 was regarded as significant.

RESULTS

Organoleptic evaluation of fecal odor and concentrations of methyl mercaptan and hydrogen sulfide gases released by feces

In healthy adults, the fecal odor and concentration of methyl mercaptan gas from the feces decreased significantly during the period of administration of proanthocyanidin-rich extract compared with before administration (Figs. 2 and 3). The concentration of hydrogen sulfide gas released from the feces tended to decrease slightly during the period of administration of proanthocyanidin-rich extract (Fig. 4). The fecal odor and concentrations of the gases from feces decreased more during proanthocyanidin-rich extract intake than during green tea extract intake or champignon extract intake (Figs. 2–4).
Fig. 2. Effect of the intake of proanthocyanidin-rich extract from grape seeds on the fecal odor of healthy adults. The degree of fecal odor was evaluated by organoleptic evaluation using a 4-grade evaluation scale (grade 0 = no smell; 1 = smell almost undetectable; 2 = moderate smell; 3 = strong bad smell). * ** Significant difference from the value of 0 days (before intake) at $p < 0.05$, $p < 0.01$, respectively. $n = 9$ /group.

Among the elderly inpatients, fecal odor also decreased more during proanthocyanidin-rich extract intake than during champignon extract intake (Fig. 5).

**Fecal microflora**

The number of *Bifidobacterium* had increased significantly ($p < 0.05$) on day 14 of administration of proanthocyanidin-rich extract compared with before administration (Table I). The number of *Enterobacteriaceae* tended to decrease ($p = 0.121$) on day 14 of administration of proanthocyanidin-rich extract. The changes in numbers of other bacteria were modest.

**Fecal putrefactive substances and other characteristics**

The putrefactive substances skatol, indole, 4-ethylphenol, $p$-cresol, phenol, and ammonia tended to decrease on day 14 of administration of proanthocyanidin-rich extract compared with before administration, but the differences were not significant (Table II). Fecal pH decreased slightly in proanthocyanidin-rich extract intake, but fecal weight and moisture did not change markedly at any time during the experimental period.

In the *in vitro* study using fecal specimens and methyl mercaptan solution

In the *in vitro* study using fecal specimens, the concentration of methyl mercaptan from the fecal specimens in the headspace of the vessels ($7.0 \pm 2.8$ ppm) was significantly lower ($1.8 \pm 3.4$ ppm) in the fecal specimens mixed with proanthocyanidin-rich extract. The concentration of hydrogen sulfide released from the fecal specimens ($8.8 \pm 5.1$ ppm) decreased to $3.0 \pm 1.4$ ppm, but the difference was not significant ($p = 0.054$).

In the *in vitro* study using methyl mercaptan solution, proanthocyanidin-rich extract reduced markedly methyl mercaptan release in the airtight flask. The reduction of methyl mercaptan release was greater with the procyanidin oligomers larger than decamer than procyanidin dimer to tetramer (Fig. 6).

Fig. 3. Effect of the intake of proanthocyanidin-rich extract from grape seeds on methyl mercaptan gas released by the feces of healthy adults. * ** Significant difference from the value of 0 days (before intake) at $p < 0.05$, $p < 0.01$, respectively. $n = 9$ /group.

Fig. 4. Effect of intake of proanthocyanidin-rich extract from grape seeds on hydrogen sulfide gas released by the feces of healthy adults. $n = 9$ /group.

Fig. 5. Effect of the intake of proanthocyanidin-rich extract from grape seeds on the fecal odor of elderly inpatients. The degree of fecal odor was evaluated by organoleptic evaluation using a 4-grade evaluation scale (grade 0 = no smell; 1 = smell almost undetectable; 2 = moderate smell; 3 = strong bad smell). * ** Significant difference from the value of 0 days (before intake) at $p < 0.05$, $p < 0.01$, respectively. $n = 8$ /group.
Table I

Effect of the intake of proanthocyanidin-rich extract from grape seeds on fecal microflora of healthy adults\(^a\)\(^,\)\(^b\)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Before intake (day 0)</th>
<th>Proanthocyanidin-rich extract intake (day 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bifidobacterium</strong></td>
<td>9.6 ± 0.4 (100)</td>
<td>10.1 ± 0.3* (100)</td>
</tr>
<tr>
<td><strong>Bacteroides</strong></td>
<td>10.1 ± 0.3 (100)</td>
<td>10.3 ± 0.4 (100)</td>
</tr>
<tr>
<td><strong>Escherichia</strong></td>
<td>9.7 ± 0.4 (100)</td>
<td>9.7 ± 0.5 (100)</td>
</tr>
<tr>
<td><strong>Peptostreptococcus</strong></td>
<td>8.9 ± 0.4 (83)</td>
<td>8.9 ± 0.4 (67)</td>
</tr>
<tr>
<td><strong>Clostridium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lecithinase (+)</td>
<td>5.7 ± 1.9 (50)</td>
<td>5.7 ± 1.8 (50)</td>
</tr>
<tr>
<td>Lecithinase (−)</td>
<td>7.1 ± 0.2 (83)</td>
<td>7.7 ± 0.4 (83)</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>6.6 ± 1.8 (67)</td>
<td>5.8 ± 2.0 (83)</td>
</tr>
<tr>
<td>Veillonella</td>
<td>6.1 (17)</td>
<td>6.9 ± 1.0 (67)</td>
</tr>
<tr>
<td>Fasobacterium</td>
<td>5.3 ± 0.2 (33)</td>
<td>5.0 (17)</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>7.3 ± 0.9 (100)</td>
<td>6.5 ± 1.0 (100)</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>4.4 (17)</td>
<td>4.5 ± 0.7 (67)</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>6.4 ± 1.8 (100)</td>
<td>6.5 ± 0.8 (100)</td>
</tr>
<tr>
<td>Streptococcus</td>
<td></td>
<td>8.3 (17)</td>
</tr>
<tr>
<td>Bacillus</td>
<td>6.1 ± 2.4 (83)</td>
<td>4.1 ± 1.0 (67)</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td></td>
<td>4.4 (17)</td>
</tr>
<tr>
<td>Yeasts</td>
<td>3.1 (17)</td>
<td></td>
</tr>
<tr>
<td>Total counts</td>
<td>10.5 ± 0.2</td>
<td>10.5 ± 0.4</td>
</tr>
<tr>
<td>Total anaerobes</td>
<td>10.5 ± 0.2</td>
<td>10.5 ± 0.4</td>
</tr>
<tr>
<td>Total aerobes</td>
<td>7.7 ± 0.8</td>
<td>7.3 ± 1.1</td>
</tr>
</tbody>
</table>

\(^a\) Bacterial counts expressed as mean ± SD of log\(_{10}\) colony-forming units/g wet feces (n = 6).

\(^b\) Frequency of occurrence (%). *Significantly different (p < 0.05) from the counts of day 0 (before intake).

**DISCUSSION**

The balance of intestinal microbiota is known to influence the host’s health (27, 28). In particular, the increase of bifidobacteria in the intestinal tract is thought to exert a beneficial effect on the host by the production of short chain fatty acids (SCFAs), lowering the pH in the bowel, decreasing putrefactive products, and suppressing the growth of putrefactive bacteria (29, 30).

In this study, we showed that the number of *Bifidobacterium* in healthy adults had increased significantly (p < 0.05), whereas the number of *Enterobacteriaceae* tended to decrease (p = 0.121) (Table I). The level of putrefactive substances and pH tended to decrease after the intake of proanthocyanidins, which are oligomers or polymers of (-)-catechin and/or (-)-epicatechin, rich extract in healthy adults (Table II). Goto et al. (18) reported that the intake of tea catechin, monomeric flavanols, led to the increase of the number of *Bifidobacterium*, and to the decrease of the number of putrefactive bacteria such as *Enterobacteriaceae* and clostridia in elderly residents receiving liquid alimentation for 14 days. It seemed that the changes in the fecal flora in elderly residents and healthy adults in our study showed an almost similar pattern, although the change of fecal flora was modest and clostridia did not change in our study. The reduction of fecal ammonia and other putrefactive products was thought to result from the suppression of putrefactive bacteria (31, 32), and the decrease of fecal pH was considered to have resulted from an increase in the levels of lactic acid-forming bacteria such as bifidobacteria and lactobacilli (33, 34). Tebib et al. (35) and Martín-Carrón & Góñi (36) observed a significant increment in the SCFA pool and a pH decrease in the cecal contents of rats fed proanthocyanidins from grape seeds compared with controls, respectively. These changes may be related to the increased levels of lactic acid-forming bacteria in the intestinal tract. We examined the ability of proanthocyanidin-rich extract from grape seeds to bind skatol, indole, and ammonia in feces. Fresh feces from healthy adults was mixed with proanthocyanidin-rich extract containing 89.3% proanthocyanidin at dose of 0.43 g/100 g fecal wet weight *in vitro*, and skatol, indole, and ammonia in the feces were analyzed and compared with feces without proanthocyanidin-rich extract. There were no differences

Table II

Effect of the intake of proanthocyanidin-rich extract from grape seeds on fecal putrefactive substances and other characteristics\(^a\)

<table>
<thead>
<tr>
<th>Metabolites in feces</th>
<th>Before intake (day 0)</th>
<th>Proanthocyanidin-rich extract intake (day 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skatol (μg/g)</td>
<td>4.3 ± 5.9</td>
<td>0.8 ± 1.3</td>
</tr>
<tr>
<td>Indole (μg/g)</td>
<td>18.8 ± 8.5</td>
<td>13.5 ± 5.4</td>
</tr>
<tr>
<td>4-Ethylphenol (μg/g)</td>
<td>0.9 ± 1.6</td>
<td>0.2 ± 0.5</td>
</tr>
<tr>
<td>p-Cresol (μg/g)</td>
<td>35.4 ± 30.3</td>
<td>17.2 ± 8.7</td>
</tr>
<tr>
<td>Phenol (μg/g)</td>
<td>8.4 ± 17.6</td>
<td>0.4 ± 0.6</td>
</tr>
<tr>
<td>Ammonia (μg/g)</td>
<td>804.3 ± 221.7</td>
<td>598.8 ± 125.3</td>
</tr>
<tr>
<td>Fecal characteristic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal weight (g)</td>
<td>116.0 ± 67.0</td>
<td>109.7 ± 63.4</td>
</tr>
<tr>
<td>pH</td>
<td>6.6 ± 0.5</td>
<td>6.4 ± 0.6</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>77.3 ± 7.8</td>
<td>77.9 ± 5.9</td>
</tr>
</tbody>
</table>

\(^a\) Data expressed as mean ± SD (n = 6).
in their levels in the feces with the extract and feces without the extract (data not shown); thus, the extract might not absorb skatol, indole, and ammonia in the feces. These results suggest that intake of proanthocyanidin-rich extract from grape seeds induces a decrease of putrefactive products in the intestine, which may be linked to the modest change in the numbers of *Bifidobacterium* and *Enterobacteriaceae*.

Changes in the intestinal microflora were not marked in this study, and the minor microbial changes may reflect stable microflora in our healthy subjects. However, the minor microbial changes might link the changes of putrefactive substances because similar findings were observed for tea catechin (17) and *Bifidobacterium longum* (37) feeding tests using healthy subjects.

In an *in vitro* study, Ahn et al. (38, 39) reported that tea catechins enhanced the growth of some *Bifidobacteria* and *Lactobacilli*, but inhibited the growth of putrefactive bacteria such as *Clostridium difficile* and *Clostridium perfringens*. We examined the growth-inhibitory activity of proanthocyanidin-rich extract from grape seeds against *C. perfringens* in a similar manner; however, the extract did not inhibit the growth of *C. perfringens* (data not shown). The extract might not inhibit directly the growth of *C. perfringens* in *vitro*. Vennat et al. (8) reported that procyanidins inhibited the growth of *Pasteurella*, *Proteus*, *Pseudomonas*, *Shigella*, *Staphylococcus*, and *Streptococcus* in *vitro*. The effect of proanthocyanidins on the growth of intestinal bacteria in *vitro*, however, is unclear. Further *in vitro* study on this topic is required.

We could not assess the effect of proanthocyanidin-rich extract on fecal flora in elderly inpatients because we could not obtain freshly voided fecal specimens for fecal microbial analysis from the inpatients who wore dispersers. Tea catechins might be more effective in improving the intestinal environment of elderly inpatients than of healthy adults because they changed markedly the fecal flora in elderly inpatients, but they only changed slightly the fecal flora in healthy adults (17, 18). The proanthocyanidin-rich extract may have a greater influence on the intestinal environment of elderly people compared with healthy adults.

Proanthocyanidin-rich extract from grape seeds had a strong deodorant effect on fecal odor in both healthy adults and elderly inpatients (Figs. 2 and 5). This deodorant effect of proanthocyanidin-rich extract was stronger than that of green tea extract and/or champignon extract, both of which have been found to have a deodorant effect on fecal odor (Figs. 2 and 5). Fecal odor is mainly due to the presence of mercaptans, hydrogen sulfide, skatol, indole, ammonia, volatile amines, and SCFAs. The level of putrefactive substances, such as skatol, indole, and ammonia, tended to decrease after proanthocyanidin-rich extract intake (Table II). The proanthocyanidin-rich extract reduced fecal methyl mercaptan and hydrogen sulfide release in *vitro*, and also reduced methyl mercaptan release from methyl mercaptan solution (Fig. 6). Proanthocyanidins always occur as a mixture of oligomers and polymers in plants; thus, we examined the absorptive ability of methyl mercaptan by each polymerization of procyanidins. The effect was stronger in procyanidin oligomers larger than decamer than in procyanidin dimer to tetramer (Fig. 6). These results suggested that the strong deodorant effect of proanthocyanidins on fecal odor was due to the decrease of putrefactive products and the absorption of malodorous compounds such as mercaptans. The deodorant effect of green tea extract, which contains monomeric flavonols, was weak in healthy adults. The strong deodorant effect of grape seed extract may be due to the larger molecular procyanidin oligomers.

In conclusion, proanthocyanidin-rich extract from grape seeds might change fecal flora and fecal odor parameters in healthy adults, and it markedly decreased fecal odor. The extract also decreased fecal odor in elderly inpatients. In particular, the fecal odor of bedridden old men who wear diapers is one of the biggest problems in homes and long-term care facilities. The fecal odor of these patients is offensive and makes the caregiver’s job difficult. Health foods containing grape seed extract will contribute to improving conditions in homes and long-term care facilities.

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