Effects of *Houttuyniae cordata* and Refinery Final Molasses on the Development of Offensive Odor in Porcine Small Intestine during Storage

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Summary Porcine small intestine evolved a specific offensive odor only 0.5 to 1 day after storage at 20°C. We investigated the effects of *Houttuyniae cordata* (dokudami), refinery final molasses (RFM), green tea, and brown sugar on the evolution of methylmercaptan and ethanol, which were the main components of the volatiles which evolved from porcine small intestine in storage. Furthermore, we determined their antibacterial effect and deodorant activity against methylmercaptan, as possible factors in reducing the offensive odor. Addition of those materials reduced the offensive odor during storage. In particular, dokudami, green tea, and RFM markedly suppressed the evolution of methylmercaptan. RFM was most effective in suppressing the growth of bacteria. Dokudami had the highest deodorant activity, comparable to that of perilla leaves. However, the retardation of methylmercaptan evolution in situ cannot be simply explained by either of deodorant or antibacterial effect. It seems likely that the combined action of both effects affects the evolution of methylmercaptan in situ.

Key Words porcine small intestine, storage, odor, methylmercaptan, *Houttuyniae cordata*, dokudami, refinery final molasses, antibacterial effect, deodorization

Elimination of offensive odor or control of its evolution is one of the essentials in increasing the food uses of edible meat by-products, especially entrails, of domestic animals. Although rich in nutrients, porcine small intestine has not been effectively utilized as human food. It is likely that the acceptability of porcine small intestine may depend, to a large extent, on the desirability of their fresh or storage flavor which develops rapidly on storage at room temperature.

We have shown in previous studies that development of offensive odor depends on storage conditions. The evolution of offensive odor during storage of porcine
small intestine was largely attributable to microbial growth and the concomitant production of sulfur compounds (especially methylmercaptan) and alcohols (especially ethanol) (1). Chemicals known as chelators and sterilizers, such as EDTA, HgCl₂, and phenol, retarded the evolution of offensive odor and the growth of bacteria (2). Under the storage condition, the amounts of volatiles evolved, especially those of sulfur compounds and alcohols, related closely to the total counts of bacteria and also paralleled with the sensory scores. Spices, herbs and seasonings, green tea, and brown sugar were some of the foodstuffs that showed suppressive effects on the offensive odor (2).

The present study was designed to further our previous findings. The materials tested in this study are obtainable easily and inexpensively, and may have a possibility of wide application. We tried to determine the efficiency of some natural materials in suppressing offensive odor development during storage of porcine small intestine.

MATERIALS AND METHODS

1. Materials. Porcine small intestines, which had been cut open and washed briefly in running water according to commercial practice, were obtained from a slaughter house and immediately frozen at −20°C. They were stored at −20°C and thawed at room temperature before use. Immediately after being thawed, they gave counts of total bacteria, yeast, and anaerobic bacteria of $2.2 \times 10^4 - 8.1 \times 10^5$, $9.6 \times 10^3 - 7.1 \times 10^4$, and $1.0 \times 10^4 - 8.0 \times 10^5$ colony-forming units/g, respectively, and their intestinal mucosa remained uncollapsed.

Green tea (Sen’cha), brown sugar, and perilla leaves (fresh) were purchased from local markets. Houttuyniae cordata, dried for medicinal use from fresh leaves, was purchased from a pharmacy. Houttuyniae cordata is called “juyaku” or “dokudami” in Japan. Therefore, we expressed Houttuyniae cordata as dokudami in this report. RFM was a kind gift from Nanso Togyo Ltd. (Kagoshima, Japan). Proximate composition of RFM was unknown except for water content of 23.0%.

2. Reagents. Culture media were purchased from the following companies: potato dextrose agar and plate count agar from Difco (Detroit, Michigan, U.S.A.), B.L. agar from Eiken Chemical Industries, Ltd. (Tokyo, Japan), and horse blood from Nikken Biochemical Laboratory (Kyoto, Japan). Standard solution of methylmercaptan (1 µg/µl in benzene) and other chemicals of guaranteed grade were purchased from Wako Pure Chemical Industries, Ltd. (Kyoto, Japan).

3. Preparation of samples. Thawed porcine small intestine (50 g) was cut into pieces of about 5 mm length and mixed with the additives (5 g in dry weight) in a capped 100 ml vial. Pretreatment of the additives is described in the legends of respective figures. After storage at 20°C for 1 day in the dark, the sample was transferred from the vial into a 500 ml beaker and homogenized with 150 ml of saline for 90s in a bio-mixer (Nihonseiki Kaisha Ltd., BM-1). The samples were treated under conventional laboratory conditions.

4. Analysis of volatile compounds. A portion (50 ml) of the homogenates was transferred into a 100 ml vial and heated in a water bath at 60°C for 30 min. Two milliliters of the head-space gas was withdrawn and injected directly into a gas chromatograph (Shimadzu Co. Ltd., GC-15APF). The analytical conditions for the gas chromatography were the same as employed in the previous study (1). Peak components were identified basing on their retention times.

5. Microbiological analysis. Remaining portion of the homogenates was diluted serially with saline. Plate count agar and potato dextrose agar were used as media for the counts of living cells of total bacteria and yeast, respectively. Counts of anaerobic bacteria were measured by steel-wool method (3) using B.L. agar medium added with horse blood. Colonies formed were counted after incubation at 37°C for 48 h.

The values of fresh and storage samples obtained in different experiments were of a wide range, probably because of the samples variation. Paired t-test and Wilcoxon matched pairs signed-rank test were applied to test the significance of differences between control and experimental values transformed into logarithm.

6. Determination of deodorant activity and odor score. Deodorant effects of the natural materials against odor of methylmercaptan, one of the main components of offensive odor evolved from porcine small intestine, were measured by the method of Tokita et al. (4) with the following modification: in a 100 ml vial, 50 ml of methylmercaptan solution (50 µl of the standard solution of methylmercaptan in distilled water) was mixed with each additive (1 g in dry weight). The mixture was then shaken for 1 min and heated at 60°C in a water bath for 30 min. A mixture without an additive was used as control. The head-space gas was analyzed as described previously.

From the counts of integrator for methylmercaptan in the chromatogram, deodorant activities of the additives were expressed as follows:

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\text{Deodorant activity (\%) = \frac{(C-S)}{C} \times 100,}
\]

C, counts for methylmercaptan in control; S, counts for methylmercaptan in samples of analysis.

In addition, deodorant effects of the additives in situ were measured by using homogenate of porcine small intestine, prepared as shown in the legend of Fig. 4, in place of the methylmercaptan solution, and expressed in terms of decrease in odor score defined as follows:

\[
\text{Odor score (\%) = \frac{S}{C} \times 100.}
\]

The experimental results were shown by the average of three measurements.

RESULTS

1. Effects of some natural materials on the evolution of methylmercaptan and ethanol during storage of porcine small intestine

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Figure 1 shows the effects of added natural materials on the evolution of methylmercaptan and ethanol from porcine small intestine on storage at 20°C for 1 day. In the control, ethanol and methylmercaptan evolved at sufficient levels after 1 day of the storage, and a strong offensive odor was organoleptically detected. On the other hand, in the samples treated with additives, the evolution of ethanol and methylmercaptan was suppressed and offensive odor was least detectable. Green tea and brown sugar showed similar effects as previously observed (2). Green tea, dokudami, and RFM were particularly effective in this series of experiments.

2. Effects of the additives on microbial growth

Figure 2 shows the effects of additives on the growth of total bacteria, yeast, and anaerobic bacteria. Experiments of respective groups were repeated twice and expressed as the result of one experiment in the figure. In group A, total bacteria, yeast, and anaerobic bacteria in the control increased to $5.4 \times 10^8$, $6.7 \times 10^8$, and $5.5 \times 10^8$ colony-forming units/g, and those in group B to $1.4 \times 10^9$, $8.3 \times 10^8$, and $7.5 \times 10^8$, respectively. Addition of the test materials decreased the three bacterial counts by a similar degree and was significantly effective on the suppression of microbial growth. Among the added materials, RFM was most effective in suppressing the growth of total bacteria ($5.3 \times 10^7$), yeast ($5.2 \times 10^7$), and anaerobic bacteria ($3.9 \times 10^7$).

3. Deodorant effects of the additives

The deodorant effects of the additives against odor of methylmercaptan were compared with that of perilla leaves. The results are shown in Fig. 3. Under the
Fig. 2. Effects of the additives on microbial growth in porcine small intestine in storage at 20°C for 1 day. Experimental conditions were the same as in Fig. 1 except that the data were obtained from separate experiments (Group A and B in the figure). Addition of the test materials decreased the counts of bacteria significantly (N=6, p<0.05). , total bacteria; , anaerobic bacteria; , yeast.

Fig. 3. Deodorant activity of the additives against methylmercaptan. Experimental procedures and a definition of the deodorant activity are described in the text. *1 Pretreatment was the same as in Fig. 1. *2 The mixture of green tea and distilled water, in the ratio 1:50 (w/v), was boiled for 10 min and filtered through a filter paper (No. 2). The residue was dried and used. *3 The mixture of green tea and distilled water, in the ratio 1:50 (w/v), was boiled for 10 min and filtered through a filter paper (No. 2). The filtrate (50 ml) was used. *4 Fresh leaves, cut into small pieces.

Experimental conditions employed, the deodorant activities of perilla leaves and dokudami reached 100%, and those of green tea leaves and RFM were 84 and 83%, respectively. Brown sugar was least effective (11%). The deodorant effects of dokudami, perilla leaves, and RFM in situ were investigated using homogenate of porcine small intestine, which had been stored at

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Fig. 4. Changes in odor score of the additives, as influenced by incubation time. Fresh porcine small intestine (50 g) was homogenized in 150 ml of distilled water. A portion (50 ml) of the homogenate was transferred into a capped 100 ml vial and stored at 20°C for 1 day. After the storage, the natural materials were added to the homogenates. Pretreatment with the additives was the same as Figs. 1 and 3. Immediately, or after incubation for 3 h at 20°C, the mixture was heated for 30 min at 60°C, and 2 ml of the head-space gas was withdrawn and injected directly into a gas chromatograph. A definition of the odor score is described in the text. _□_ , odor score immediately after adding the materials; _■_ , odor score 3 h after adding the materials.

20°C for 1 day before the test. Figure 4 shows the results of their odor scores. Odor scores were measured twice with each sample, immediately and 3 h after adding the tested material, as mentioned in the legends of the figure. When analyzed immediately after the addition of the test additives (1 g in dry weight), odor score of any additive was unexpectedly 100%. Three hours after the addition, dokudami and perilla leaves decreased the odor score to 24 and 55%, respectively, while RFM remained completely ineffective. Dokudami, the most active material in situ, showed a dose-dependent deodorant effect.

**DISCUSSION**

We previously reported (2) that many foodstuffs were effective in suppressing offensive odor development from porcine small intestine during storage. Brown sugar was effective in suppressing offensive odor development, in terms of sensory score, while soft sugar (white) was not. Since this observation suggests that a component of brown sugar other than sucrose takes part in the suppression of offensive odor, RFM was tested in the present experiment.

Dokudami has another name as “juyaku.” The word means “ten or more medicinal effects,” which include diuretic and sterilizing effects. Infusate of dokudami is taken as medicinal tea. Fresh leaves of dokudami are applied to

wounds. Moreover, dokudami is empirically used as a deodorizing agent.

In the intestine samples tested with those additives including green tea, dokudami, and RFM, offensive odor was least detectable and the evolution of ethanol and methylmercaptan was suppressed (Fig. 1). This suppressive effects of RFM and dokudami are hitherto not described. The antibacterial activities of tested additives (Fig. 2) appear to parallel with their suppressive effects on the evolution of volatiles (Fig. 1).

We determined water activities of the control sample and the experimental samples, which were mixed with dokudami, green tea, RFM, or brown sugar (5 g in dry weight), with the instrument (Novacina Co., HUMIDAT-RC). Their values were 0.997, 0.993, 0.997, 0.991, and 0.991, respectively, and scarcely changed after storage for 1 day. These facts indicate that the suppression in the microbial growth hardly resulted from the decrease in the water activity.

It is known that catechins (5, 6) in green tea and decanoyl-acetoaldehyde (7) in dokudami have inhibitory effects on the growth of some microorganisms. Decanoyl-acetoaldehyde is the main component of the characteristic smell which gives off from fresh leaves of dokudami (7), and is lost when heated (8). The suppressive effect of dokudami on microbial growth observed here may be the result of unknown components other than decanoyl-acetoaldehyde, since we used heat-dried, odorless dokudami in this experiment. Besides, the observation that RFM was more effective than brown sugar emphasizes the presence of an unknown suppressant against the growth of microorganisms. These factors remain undetermined in this experiment.

As shown in Fig. 3, the deodorant effects of dokudami and green tea against odor of methylmercaptan were as high as that of perilla leaves. We determined the deodorant activity according to the model by Tokita et al. (4). Yet the mechanism of deodorization cannot be precisely described at this stage of study. Deodorant effects appear to involve a variety of mechanisms, among which are physical adsorption of odor components, and masking or decomposition of odor components by substances contained in the test additives. It was reported that the deodorant effect of perilla leaves was very high (4) and that tea catechins showed remarkable effects on the elimination of methylmercaptan (9). The deodorant effect of dokudami has been reported against only trimethylamine (10). The deodorant effect of dokudami against the odor of methylmercaptan was first described in this report. Dokudami showed stronger activity than perilla leaves in situ. The question remained unsolved why the deodorant actions of perilla leaves and dokudami needed prolonged incubation in situ (Fig. 4) while their actions proceeded rapidly in vitro (Fig. 3). Further study to clarify the mechanism of deodorant action of dokudami or RFM is necessary.

Figure 5 shows the amounts of methylmercaptan that evolved from homogenates of porcine small intestine after 1 day incubation at 20°C in the presence of the additives. The results were similar to those shown in Fig. 1. Neither perilla leaves, one of the most active deodorizers in vitro, nor RFM, the most inhibitory on
microbial growth, was very effective in the restriction of methylmercaptan evolution. This indicates that the restriction of methylmercaptan evolution \textit{in situ} cannot be simply explained by either of deodorant or antibacterial action.

Some chemicals allowed to be used as food-additives, such as sodium dehydroacetate and sodium nitrite, retarded the evolution of offensive odor and bacterial growth (unpublished). But, the chemicals, added to allowed limits, could not effectively suppress the development of offensive odor, while their inhibitory effects on microbial growth were comparable to those additives tested in this study. Dokudami, green tea, and RFM were far more effective than those chemicals.

The results described in this paper suggest that the evolution of methylmercaptan \textit{in situ} is affected not only by suppressive effect of the additives on microbial growth, but also by their deodorant effects against the odor of methylmercaptan. Safety of food and health have recently become of general interest. The mechanisms of the deodorant and antibacterial actions of dokudami and RFM need to be elucidated before these materials are used in practice. It is expected that the natural materials investigated in this study can and will be used effectively and practically as deodorizers or antibacterial additives.

REFERENCES


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