Suppressive Compounds from Japanese Pepper
(Zanthoxylum piperitum) against the Fishy Trimethylamine Odor

Yasumasa Yamada*

Faculty of Human Life and Science, Doshisha Women’s College of Liberal Arts,
Kyoto 602–0893, Japan

In the course of searching by the head-space gas method for compounds from edible plants that can suppress the unpleasant fishy trimethylamine odor, the flavonol glycoside, hyperin (I), from the fruit of “sanshou” Japanese pepper (Zanthoxylum piperitum) was identified by spectroscopic methods (H-NMR, 13C-NMR, FAB-MS) as being effective. The suppressive effect of hyperin was compared with that of the related flavonoids, rutin, quercetin 3-glucoside and hesperidin. Although the flavonol glycosides, rutin and quercetin 3-glucoside were effective, the flavanone glycoside, hesperidin showed only a weak effect.

(Received October 22, 2008; Accepted in revised form May 2, 2009)

Keywords: Japanese pepper, Zanthoxylum piperitum, trimethylamine, fishy odor, hyperin, rutin.

INTRODUCTION

Methods for suppressing the unpleasant fishy odor are used while cooking fish or during the industrial processing of seafood. Among edible plants, ginger (Zingiber officinalis), Japanese pepper (Zanthoxylum piperitum), and stoneleek (Allium fistulosum) are utilized in traditional Japanese dishes for this purpose.1) In the present study, Japanese pepper called “sanshou” was selected. Japanese pepper is known as a spice and a folk medicine in Japan, Korea, and China. The bioactivity of Japanese pepper has been found to originate from antioxidative,2) hepatic drug metabolizing,3) tyrosinase inhibitory,4) and antimicrobial5) compounds. Trimethylamine (TMA) is known as the major unpleasant amine odor from fish, and a suppressive compound against TMA was sought in extracts of Japanese pepper fruit.

MATERIALS AND METHODS

General

FAB-MS data were measured by a JEOL JMS HX 110 spectrometer, and the positive ions were detected by using glycerin as a matrix. 1H- and 13C-NMR spectra were obtained by a Varian Unity Inova 500 spectrometer in CD,OD, using TMS as an internal standard (H-NMR at 500 MHz, and 13C-NMR at 125 MHz). Rutin and hesperidin were purchased from Wako Pure Chemical Industries, and hesperidinase was presented by Tanabe Seiyaku Co. (Mitsubishi Tanabe Pharma Corporation).

GC analytical conditions for trimethylamine in the head-space gas

An FID-GC instrument equipped with a packed column (15% diglycerol +15% TEP +2% KOH, Uniport HP 60/80 mesh; 3 mm i.D. ×3 m) was used for analyzing TMA. The carrier gas was N2 at a flow rate of 50 ml/min. The injector, column, and detector temperatures were 70°C, 50°C, and 70°C, respectively.

Extraction and isolation

Japanese pepper (Z. piperitum) harvested in Kyoto was successively extracted three times each with n-hexane, acetone, methanol, and distilled water. The methanol extract was subsequently purified by polyamide column chromatography, eluting with water and methanol. Yellow powder was obtained from the methanol fraction. The isolated compound was identified as hyperin 6,7) by 1H-NMR, 13C-NMR and FAB-MS spectroscopic methods.

Hydrolysis of rutin by hesperidinase

An aqueous solution of rutin (550 mg) was incubated with hesperidinase (220 mg) at 40°C for 48 h. Quercetin 3-β-D-glucopyranoside was obtained (262
RESULTS AND DISCUSSION

Japanese pepper (Z. piperitum) was successively extracted with n-hexane, acetone, methanol, and distilled water. Among the extracts, that with n-hexane was excluded because it contained a favorable aromatic essential oil which might have masked the sensory perception of the unpleasant amine odor. We thought it important to search for those compounds could suppress the release of the volatile amine by the chemical reaction with TMA in an aqueous solution. A mixture of a 0.1% aqueous solution of TMA (1.0 ml) and each extract (4 mg) in a closed GC screw vial (5 ml) sealed with a silicone septum was incubated in a water bath at 70°C for 60 min. The head-space gas above the aqueous solution of the mixture was sampled with a gas-tight syringe without any loss of the TMA vapor, and then analyzed by FID-GC. The suppressive effect was evaluated by the decreased peak area (%) in the GC chromatogram in comparison with that of the control (a 0.1% aqueous solution of TMA only). The results of the GC analyses show the acidic fraction of the acetone extract and methanol extract to have reduced the peak area of TMA in the head-space gas. The effective compound from both extracts was purified by silica gel and Sephadex LH-20 gel chromatography, eluting with methanol, to yield a yellow powder. The compound was identified as quercetin 3-β-D-galactoside (hyperin) (1) by FAB-MS, 1H-NMR and 13C-NMR spectroscopic methods.

Hyperin (quercetin 3-β-D-galactopyranoside)

Positive FAB-MS (glycerin used as a matrix) m/z: 487[M+Na]+, 465[M+H]+; 1H-NMR (500 MHz, CD3OD) δ: 3.47 (1H, dd, J=6.0, 6.5 Hz, H-3'), 3.55 (2H, m, H-5', H-6a'), 3.64 (1H, dd, J=6.0, 8.5 Hz, H-6b'), 3.81(1H, dd, J=8.0, 8.8 Hz, H-2'), 3.85 (1H, brd, J=3.5 Hz, H-4'), 5.16 (1H, d, J=8.0 Hz, H-1'), 6.20 (1H, d, J=2.0 Hz, H-6), 6.39 (1H, d, J=2.0 Hz, H-8), 6.86 (1H, d, J=8.5 Hz, H-5'), 7.58 (1H, dd, J=2.0, 8.5 Hz, H-6'), 7.84 (1H, d, J=2.0 Hz, H-2'); 13C-NMR (125 MHz, CD3OD) δ: 61.9 (gal C-6'), 70.0 (C-2'), 73.2 (C-3'), 75.1 (C-5'), 77.2 (C-4'), 94.7 (C-8), 99.9 (C-6), 105.3 (C-1'), 105.6 (C-10), 116.1

Fig. 1. Structures of the four flavonoids
Suppressive Compounds from Japanese Pepper (Zanthoxylum piperitum) against the Fishy Trimethylamine Odor

Hydrolysate of rutin by hesperidinase, quercetin 3,β-D-glucopyranoside

FAB-MS: 487[M+Na]+, 465[M+H]+; 1H-NMR (500 MHz, CD3OD) δ: 3.21 (1H, m, H-5'), 3.37 (1H, m, H-4'), 3.42 (1H, dd, J=9.0, 9.0 Hz, H-3'), 3.48 (1H, dd, J= 8.0, 9.0 Hz, H-2'), 3.57 (1H, dd, J=5.0, 12 Hz, H-6a'), 3.70 (1H, dd, J=2.5, 12 Hz, H-6b'), 5.22 (1H, d, J=8.0 Hz, H-1'), 6.17 (1H, d, J=2.0 Hz), 6.35 (1H, d, J=2.0 Hz), 6.86 (1H, d, J=8.5 Hz), 7.57 (1H, dd, J=2.0, 8.5 Hz), 7.70 (1H, d, J=2.0 Hz); 13C-NMR (125 MHz, CD3OD) δ: 62.5 (glc C-6'), 71.1 (C-4'), 75.7 (C-2'), 78.1 (C-3'), 78.3 (C-5'), 95.0 (C-8), 100.4 (C-6), 104.4 (C-1'), 105.2 (C-10), 116.0 (C-5'), 117.5 (C-2'), 123.0 (C-6'), 123.2 (C-1'), 135.5 (C-3), 145.9 (C-3'), 149.9 (C-4'), 158.5 (C-2), 158.8 (C-9), 162.9 (C-5), 167.4 (C-7), 179.3 (C-4).

The suppressive effect of hyperin on the TMA odor was compared with that of the other two flavonol glycosides, namely rutin (2), and quercetin 3-glucoside (3) and with the flavanone glycoside, hesperidin (4) that are abundantly distributed in Citrus spp. (Rutaceae). Quercetin 3,β-D-glucoside was prepared from rutin as the substrate by specific hydrolysis between glucose and rhamnose by hesperidinase. The suppressive effects of these flavonoids were as follows: hyperin, 58.4±2.0%; quercetin 3,β-D-glucoside, 76.8±2.0%; rutin, 78.5±2.0%; and hesperidin, 22.0±12.0%. Although the former three flavonol glycosides were effective, hesperidin bearing the flavanone skeleton did not have a significant effect on suppressing the TMA odor. In addition to the flavone skeleton, it seems likely that the sugar moiety also contributed to the suppressive effect by its solubility in water. A study of the mechanism for the suppressive effect of flavonol glycosides on the fishy TMA odor will be continued in our laboratory.

The author is grateful to Ms. Mika Koyama, Chisato Takada, Chiharu Tokuda, and Ayako Yamazaki for their technical assistance.

REFERENCES

山椒（Zanthoxylum piperitum）に含まれる魚類のトリメチルアミン臭抑制化合物

山田 恭正
（同志社女子大学生活科学部）

原稿受付平成 20 年 10 月 22 日；原稿受理平成 21 年 5 月 2 日

食用植物から不快な魚類のトリメチルアミン臭を抑制する化合物をヘッドスペースガス法によって検索した。その過程において、山椒（Zanthoxylum piperitum）の果実から抑制効果を示したフラボノール配糖体 hyperin を 1H-NMR, 13C-NMR そして FAB-MS などの分光学的方法によって同定した。Hyperin と関連のあるフラボノイドである rutin, quercetin 3-glucoside, hesperidin と効果を比較した。その結果、フラボノール配糖体である rutin と quercetin 3-glucoside は効果的であったが、フラバノン配糖体である hesperidin は、弱い効果しか示さなかった。

キーワード：山椒，Zanthoxylum piperitum，トリメチルアミン，魚臭，hyperin，rutin.