Analyses of Glycolipids in Clove, Red Pepper, and Nutmeg by High-Performance Liquid Chromatography

H. SUZUKI, W-K. PARK, AND S-Y. LIM

ABSTRACT: To determine the existence of glycolipids (neutral glycosphingolipid and glycoglycerolipid) in clove, red pepper, and nutmeg, we performed silica gel chromatography and high-performance liquid chromatography (HPLC) using an Aquasil-SS column and a C8-reversed-phase silica gel column. HPLC (Aquasil-SS column) with a UV absorption detector was used to analyze neutral glycosphingolipid. These chromatograms showed two typical peaks in clove lipids. UV-HPLC (C8-reversed phase silica gel column) was also used to analyze glycoglycerolipid. The chromatograms indicated a small peak in clove lipids. Moreover, we observed the same two peaks in the glycolipid fraction of clove lipid when we used HPLC (Aquasil-SS column) with a differential refractometer detector. These results suggest that clove may contain new and plural neutral glycosphingolipids.

Key Words: glycolipids, HPLC analyses, spices

Introduction

Glycosphingolipid is composed of hydrophobic ceramide (spingosine and fatty acids) and hydrophilic oligosaccharide chains. Several functional studies of glycosphingolipid in the fields of cell biology and hematology have recently been reported (Nakamura 1997). Glycosphingolipid contributes to the function of the carbohydrate-rich glycolipid and thus affects the surface properties of cells. A recent growing interest in the physiological roles of glycosphingolipid has generated a need to separate glycolipid from a variety of species and tissues. High-performance liquid chromatography (HPLC) is a rapid and sensitive analytical method commonly used to separate glycolipids (Kaye and Ullman 1984; McCluer and Gross 1985; Ullman and McCluer 1987).

Spices have been utilized in numerous fields. They are used to season many kinds of foods either alone or as a part of complex seasoning. They add flavor, pungency, and color characteristics to foods, and give antioxidant, antimicrobial, pharmaceutical, and nutritional properties simultaneously. The basic actions of clove, red pepper, and nutmeg consist of masking or deodorizing, pungency, and flavoring, respectively. The mortality rate for mites from the essential oil of clove and eugenol, the components of clove, has been reported to exceed 97%. Clove is a most effective anthelmintic spice, and it exhibits antimicrobial activities (Hiras and Takemasa 1998). Furthermore, eugenol is used in dental treatment as an anti-inflammatory agent (Yamahara and Takemasa 1998). Moreover, these spices and their unidentified components may exhibit many other functions. However, there is little information on the existence of glycolipids as one of the functional compounds in spices.

In this study, we extracted glycolipids from clove, red pepper, and nutmeg by silica gel chromatography. We then analyzed glycolipid classes (neutral glycosphingolipid and glycoglycerolipid) by HPLC with ultraviolet (UV) absorption and differential refractometer (RI) detectors to determine the existence of glycolipids and to separate different types of glycolipids.

Materials and Methods

Chloroform, acetone, methanol, 2-propanol, n-hexane, and distilled water of HPLC grade were obtained from Wako Pure Chemicals (Osaka, Japan) and were used without further purification. Anhydrous sodium sulfate (Na2SO4) and silica gel (C-300; 45–75 μm, for silica gel column chromatography) were also purchased from Wako Pure Chemicals.

Materials

The powders of clove (Syzygium aromaticum L.) and nutmeg (Myristica fragrans Hort.) were purchased from Yashima Corp. (Tokyo, Japan). The clove and nutmeg were harvested from Zanzibar, United Republic of Tanzania and East India, respectively. The powder of red pepper (Capsicum annuum L.), harvested from Korea, was provided by Ottogi Corp. (Anyang, Kyeonggi, Korea). All of the materials were kept at 5 °C and used within 1 week.

Sample preparations

Lipids were extracted from the powders of clove, red pepper, and nutmeg according to the method of Bligh and Dyer (1959). Each sample (30 g) was combined with 60 ml of chloroform and 120 ml of methanol and homogenized for 2 min in a Waring blender (Nihon Seiki Seisakusho Co., Tokyo, Japan). Sixty milliliters of chloroform and 60 ml of distilled water were added to each mixture and stirred for 30 s. Each mixture was then filtered through a Whatman No. 2 filter paper using a Buchner funnel into a 500 ml sidearm flask under vacuum. The filtrates were transferred to a 250 ml separatory funnel and partitioned into organic solvents by vigorously shaking the solution for 20 to 30 s. Sufficient time was allowed for the layers to separate, and the chloroform layer was transferred to a round-bottom flask after standing over anhydrous Na2SO4 for 10 min at room temperature. The extracts were evaporated to dryness using a rotary vacuum evaporator (Tokyo Rikakikai Co., Tokyo, Japan) with the water bath heated at 40 °C.

Fractionation by silica gel chromatography

Silica gel chromatography was performed according to the method of Privett and others (1973). Each crude lipid extract...
Analyses of Glycolipids in Specific Spices . . .

(10 g) was applied to a column (25 × 300 mm) packed with silica gel in chloroform. The glycolipid fraction was eluted with 250 ml of acetone after the triglyceride fraction was removed by eluting with 300 ml of chloroform. The phospholipid fraction was eluted with 300 ml of methanol. After the solvent was reduced by evaporation, each fraction was weighed, and the glycolipid fraction was subjected to HPLC analyses (Fig. 1).

**HPLC analyses**

HPLC analyses were conducted using a model 880 HPLC pump, a model 860-CO column oven, and a syringe-loading sample injector with a 20 μl loop (Japan Spectroscopic Co., Ltd., Tokyo, Japan). Detection was made with a model 870 UV detector (208 nm; Japan Spectroscopic Co., Ltd.). Neutral glycosphingolipids in the spices were separated on an Aquasil-SS column (200 mm × 8 mm i.d.; particle size, 50 Å; Senshu, Co., Tokyo, Japan) using a solvent system of 2-propanol/n-hexane/distilled water. The composition was eluted linearly with 20:80:0 to 55:45:0 for 20 min, then eluted with 55:40:5 for 10 min, at a flow rate of 1.2 ml/min and a column temperature of 40 °C (Suzuki and others 1990). The injection volume was 20 μl. Furthermore, we performed differential RI-HPLC analyses to detect sugars of neutral glycosphingolipids in the glycolipid fraction of clove. The glycolipid fraction was eluted with 2-propanol/n-hexane/distilled water (55:40:5) for 30 min (flow rate, 1.2 ml/min; column temperature, 40 °C; injection volume, 20 μl). Glycoglycerolipids in the spices were measured using a C4-reversed-phase silica gel column (250 mm × 4.6 mm i.d.; particle size, 120 Å; Senshu, Co.) according to the method of Smith and others (1985). The glycoglycerolipids were separated with methanol/distilled water (96:4) and detected by UV (205 nm) under slightly different conditions from before (flow rate, 0.8 ml/min; column temperature, 40 °C; injection volume, 20 μl).

**Statistics**

The weight percentages of lipids and each lipid fraction are expressed as the means of five replicates ± SD. The significant difference in the values between samples was analyzed by one-way analysis of variance (Duncan's pairwise comparisons) using the SIGMSAT statistical program package (Jandel Corp., Erkrath, Germany).

**Results and Discussion**

A COMPARATIVE STUDY WAS CONDUCTED to determine the differences in the amounts of total lipids, and the triglyceride, glycolipid, and phospholipid fractions of the lipids contained in clove, red pepper, and nutmeg. The results are shown in Table 1. As can be seen, the lipid content in spices was ranked in the following order: clove lipid (3.47%), red pepper (1.28%) > nutmeg (0.88%) > red pepper (0.79%).

HPLC analyses using a UV detector were conducted to separate neutral glycosphingolipids and glycoglycerolipids. The UV absorption detector is clearly the most sensitive detector for compounds that have double bonds (Munk 1970). HPLC chromatograms of the lipids in the glycolipid fraction obtained from neutral glycosphingolipid analysis are shown in Figure 2. Chromatogram (a) shows a typical peak in the glycolipid fraction of clove (retention time = 19 and 21 min). (b) and (c) exhibit small peaks in the fraction of red pepper and nutmeg, respectively. These results suggest that there may be different types of neutral glycosphingolipids in clove. Figure 3 shows HPLC chromatograms of the lipids in glycolipid fraction obtained from glycoglycerolipid analysis. Chromatogram (a) exhibits a small peak in the fraction of clove (retention time = 16 min). No marked peaks of the glycolipid fraction of red pepper and nutmeg lipids are observed in chromatograms of (b) and (c). These results suggest that glycolipids may contain a small amount of glycoglycerolipids.

We performed HPLC analysis using an RI detector to determine sugars of glycolipids in clove. Figure 4 presents UV-HPLC and RI-HPLC chromatograms of the

---

**Table 1—Lipid content (g/100g) and distribution (%) in each fraction eluted by silica gel column chromatography**

<table>
<thead>
<tr>
<th>Spices</th>
<th>Lipid content</th>
<th>Fraction A (Triglycerides)</th>
<th>Fraction B (Glycolipids)</th>
<th>Fraction C (Phospholipids)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clove</td>
<td>16.1 ± 0.2#, a</td>
<td>71.4 ± 1.0#, a</td>
<td>7.95 ± 0.71#, a</td>
<td>3.47 ± 0.67#, a</td>
</tr>
<tr>
<td>Red Pepper</td>
<td>17.1 ± 0.4b</td>
<td>86.8 ± 1.3b</td>
<td>4.61 ± 0.70b</td>
<td>2.67 ± 0.10b</td>
</tr>
<tr>
<td>Nutmeg</td>
<td>34.0 ± 0.4c</td>
<td>84.1 ± 2.9c</td>
<td>2.59 ± 0.34c</td>
<td>1.72 ± 0.24c</td>
</tr>
</tbody>
</table>

*Values are means of five replicates ± SD. Values for each sample with different superscript roman letters in the same fraction are significantly different at p < 0.05 by Duncan's pairwise comparisons.

---
glycolipid fraction in the lipids. The chromatograms show two typical peaks of the same retention times (10 and 11 min) under the conditions of neutral glycosphingolipid analysis using RI-HPLC. The RI detector detects the presence of glycolipids on the basis of the difference of the refractive index of the solution (Huber 1969). A UV detector is not as effective for sugars, which do not possess specific UV-absorbing chromophores, because of the poor selectivity over many of the other components encountered in foods (Garcia and Palmer 1980). Thus, the RI detector is the most commonly used instrument in carbohydrate analysis (Macrae 1985). The above results suggest that these peaks in the glycolipid fraction of clove lipids may be neutral glycosphingolipids, and clove may contain plural glycolipids.

We have recently reported that typical peaks (retention times = 9, 11, 14 min) are found in the glycolipid fraction of different fish, shellfish, and sea snake by HPLC under the same condition (neutral glycosphingolipid analysis) as this study (Lim and others 1999). From the differences in the retention times of peaks between clove (retention times = 19, 21 min) and sea food lipids, there seem to be different types of neutral glycosphingolipids in clove. The present study is only a preliminary experiment on separating glycolipids from spices and must be extended by further work to isolate other glycosphingolipid classes and to determine the specific oligosaccharide structure of glycolipid.

Conclusions

Our results revealed that the percentage of glycolipid fraction was higher in the lipids of clove than that in the lipids of red pepper and nutmeg. Moreover, we observed typical peaks in the glycolipid fraction of clove by HPLC under the conditions of neutral glycosphingolipid analysis. We also detected typical peaks by an RI detector in the glycolipid fraction of clove lipids. The results suggest that clove may contain a small amount of neutral glycosphingolipids.

References


Authors Suzuki and Lim are with the National Food Research Institute, 2-1-2, Kannondai, Tsukuba, Ibaraki 305-8642, Japan. Author Park is with the Ottogi Corporation, 160, Pyeongchon, Dongan, Anyang, Kyeonggi, Korea. Address inquiries to Dr. Hiramitsu Suzuki, National Food Research Institute, 2-1-2, Kannondai, Tsukuba, Ibaraki 305-8642, Japan. (E-mail: hirasuzu@nfriAFFRC.go.jp)