CAPSAICIN LEVEL OF VARIOUS CAPSICUM FRUITS

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ABSTRACT

The capsaicin level of the fruits is lead in hot taste of various type of Capsicum sp for seasoning or pharmaceutical purposes. The determination of capsaicin content of several of capsicum fruit has been conducted. The samples were collected from different area in Indonesia which consisted of twelve types of edible Capsicum. The fruits were extracted and analyzed using high performance liquid chromatography. The optimum condition of analysis was attained using a reversed phase system, with a mobile phase of acetonitrile – acetate acid 2% (6:4), a flow rate of 1.0 ml/minute and a detection wavelength (ºA) of 280 nm using UV detector. The result of analysis showed that green paprika, yellow paprika, and red paprika contained no capsaicin, while chili tanjung, red chili, red gendot, green gendot, green curly, japlak rawit, red curly, red rawit and green rawit (cayenne) were 0.38; 0.83; 0.87; 0.88; 1.05; 1.09; 1.14; 1.85 and 2.11% (w/w), respectively.

Keywords: Capsaicin analysis, Extract ethanol, Chili fruits.

MATERIAL AND METHODS

Chili fruits used in this experiment were chili fruits on common harvest age and marketed in Caringin Central Market.

Table 1: Local chili fruit in traditional market of Bandung, Indonesia

<table>
<thead>
<tr>
<th>No.</th>
<th>Local Name</th>
<th>Scientific Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Green gendot chili</td>
<td>Capsicum annuum L. var. Grossum</td>
</tr>
<tr>
<td>2.</td>
<td>Red gendot chili</td>
<td>Capsicum annuum L. var. Grossum</td>
</tr>
<tr>
<td>3.</td>
<td>Green paprika</td>
<td>Capsicum annuum L. var. Grossum</td>
</tr>
<tr>
<td>4.</td>
<td>Yellow paprika</td>
<td>Capsicum annuum L. var. Grossum</td>
</tr>
<tr>
<td>5.</td>
<td>Red paprika</td>
<td>Capsicum annuum L. var. Grossum</td>
</tr>
<tr>
<td>6.</td>
<td>Red chili</td>
<td>Capsicum annuum L. var. Tanjung</td>
</tr>
<tr>
<td>7.</td>
<td>Tanjung chili</td>
<td>Capsicum annuum L. var. Tanjung</td>
</tr>
<tr>
<td>8.</td>
<td>Green curly chili</td>
<td>Capsicum annuum L. var. Tanjung</td>
</tr>
<tr>
<td>9.</td>
<td>Red curly chili</td>
<td>Capsicum annuum L. var. Tanjung</td>
</tr>
<tr>
<td>10.</td>
<td>Green rawit chili (cengek)</td>
<td>Capsicum frutescens L.</td>
</tr>
<tr>
<td>11.</td>
<td>Red rawit chili (cengek japlak)</td>
<td>Capsicum frutescens L.</td>
</tr>
<tr>
<td>12.</td>
<td>Japlak rawit chili (japlak cengek)</td>
<td>Capsicum frutescens L.</td>
</tr>
</tbody>
</table>

There are twelve chili fruit samples as listed in Table 1 Voucher specimen was stored at Plant Taxonomy Laboratory, Departement of Biology, Mathematic and Natural Science Faculty, Padjadjaran University, and then it was dried and chopped. For 100 g chopped chili was macerated with 1 L ethanol 95% for 24 hours. Liquid extract then evaporated at 40°C to eliminate the solvent.

Chemical materials used in this experiment were Capsicum liq concentrate HC 149, USP Grade Oleoresin Capsicum; aqua bidistillate (Ikapharmindo Putramas); acetonitrile (JT.Baker); ethanol 95% (re-distillate); glacial acetic acid (Merck).

HPLC method was applied for analysis capsaicin in chili by some publication. Thapa et al. (2009) is employed HPLC-UV detector to analysis capsaicin in 16 capsicum fruit in Nepal [8]. However, the preparation sample for analysis capsaicin using solid phase extraction has been yet published. Solid Phase Extraction is a very important technique in Chromatography sample preparation [9]. This method can reduce contaminant disturbing capsaicin analytical process. The current study, high-speed countercurrent chromatography is applied to rapidly separate capsaicin in Capsicum sp [10].

In this study, capsaicin of Capsicum sp cultivar from Bandung, West Java (Indonesia) was analyzed to give scientific information about capsaicin content quantitative data on ethanol extract that can be used to determine the best chili fruit variety as raw material of capsaicin.

INTRODUCTION

One of traditional plant that has so many pharmacology effects is Capsicum (Capsaicin) as known five varieties of Capsicum sp, which are C. annuum, C. frutescens, C. chinense, C. baccatum, C. pubescens, meanwhile in Indonesia only known 2 varieties, C. annuum also known as red chili, paprika, gendot until curly chili, and C. frutescens (rawit) [1]. Shape and color variation of C. annuum is similar with C. frutescens [2]. Yadiz and Olgunve (2011) deeply studies in focusing yield component of different various Capsicum sp. and its effect on the surrounding ecology chili plant growth [3], even the report of safety of capsaicin and Capsicum has been listed in Int.J. Toxicology [4].

Chili fruit has a hot taste. This comes from capsaicinoid compounds that are amide acids from vanillic acid and fatty acid chain branched at C9 and C11. Capsaicinoid consists of capsinic, dihydrocapsaicin, homocapsaicin, and homohydrocapsaicin. 6% of Capsaicinoid is capsaicin, which is a marker compound and has an effect on stimulating hair growth [5]. Capsaicin is lead in bitterness chili fruit, thus red chili more hot taste than green chili, because the content of capsaicin of red chili two or three fold more than green chili fruits [3, 6].

In modern ways, capsaicin has been made in oleoresin (ethanol or acetone extract) form and has effect as carminative, neuron stimulant and counterirritant for lumbago, neuralgia and rheumatoid [7].

Capsicum sp. marker compound analysis has been carried out so many times before because of its pharmacological effects. Experiment of capsaicin quantitative analysis on ethanol extract of several Capsicum sp. varieties needs to be carried out to determine the best chili fruit variety as raw material to produce capsaicin. Ethanol extract has a mixture of capsaicinoid compounds. One of many methods that can separate and identify compound in a mixture is High Performance Liquid Chromatography method.

HPLC method was applied for analysis capsaicin in chili by some publication. Thapa et al. (2009) is employed HPLC-UV detector to analysis capsaicin in 16 capsicum fruit in Nepal [8]. However, the preparation sample for analysis capsaicin using solid phase extraction has been yet published. Solid Phase Extraction is a very important technique in Chromatography sample preparation. [9]. This method can reduce contaminant disturbing capsaicin analytical process. The current study, high-speed countercurrent chromatography is applied to rapidly separate capsaicin in Capsicum sp [10].

In this study, capsaicin of Capsicum sp cultivar from Bandung, West Java (Indonesia) was analyzed to give scientific information about capsaicin content quantitative data on ethanol extract that can be used to determine the best chili fruit variety as raw material of capsaicin.
then, each standard solution was passed through SPE after conditioning steps as follow: passed 5 ml of acetonitrile and then 5 ml of aqua bidistillate. After 1 ml sample was passed through, capsaicin will be trapped in SPE and to get the capsaicin, 4 ml acetonitrile and 1 ml mixture of 1% acetic acid in acetonitrile were passed through SPE.

These were collected and injected into column with chosen condition, peak area was written and calculate its correlation coefficient \( r \) equation on linear regression equation \( Y = a + b \times X \). Linear regression equation achieved by plotting measurement result peak area to capsaicin concentration in injected oleoresin Capsicum. Capsaicin concentration obtained by converting oleoresin Capsicum (Capsicum liq concentrate HC 149, USP Grade Oleoresin Capsicum) concentration into contained capsaicin.

Thick extract of Capsicum sp was weight for about 160 mg to be dissolved with acetonitrile in 5.0 ml volumetric flask. Solution was sonicated to help analyte solubility, and then it was homogenized. Each sample solution was passed through SPE with equal procedure as standard solution. After SPE step, collected results were analyzed under chosen conditions.

Peak area obtained from the analysis was calculated for capsaicin content using calibration curve. Then, sample's capsaicin concentration was measured, and put into a chart.

RESULTS AND DISCUSSION

HPLC method used in this experiment was chosen because capsaicin is soluble in organic solvent (acetonitrile) used as mobile phase. Besides, HPLC is a separation method with a short-time analysis and high efficiency, a simple, rapid, accurate and precise analytical method [11,12]. HPLC can separate a component from mixture and identify it based on retention time [13].

SPE process can reduce capsaicinoid compound except capsaicin. Before samples were passed through SPE column, it was conditioned first. And then 1 ml sample was passed through. SPE conditioning made capsaicin and dihydrocapsaicin trapped in the column. To obtain it, column was eluted by 4 ml acetonitrile and 1 ml 1% acetic acid solution in acetonitrile. Eluent was collected and analyzed by chosen HPLC condition.

Standard and samples analysis showed that there are two chromatogram peaks as shown Figure 1. At 5 minutes retention time, shows a peak that always higher than another peak at 6.7 minutes retention time. It has been mentioned before that capsaicin is the major capsaicinoid compound, so that we can conclude that the higher peak was capsaicin. The other study shows that capsaicin appears at over 10 minutes with using solvent acetonitrile : formic acid (5.5: 4.5 until 4.5: 5.5) [14].

Linear regression equation was obtained from measuring capsaicin area under curve of peak standard to capsaicin concentration in standard derived from capsaicin content conversion in Capsicum liq concentrate HC 149, USP Grade Oleoresin Capsicum. Capsaicinoid amount in Capsicum liq concentrate HC 149, USP Grade Oleoresin Capsicum was 14%, while capsaicin amount was 9.4%. Linear equation was showed in Graph of Figure 2.
In this study, we found capsaicin content in Capsicum fruit as to be as high as 2.10 % (green rawit chili) from *Capsicum frutescens*. The range capsaicin content among all samples was from 0 mg (no detection in sample to 21.1 mg (2.11 % in 1 mL sample) of dried fruit as shown in Figure 4. These results were in accordance with the previous study that the highest content of capsaicin fruit is 19.73 mg (1.97 %) in *C. frutescens* from Nepal. Pruthi (2003) recorded that the highest of capsaicin in chili is obtained in Indian Chili (1.86 %) [2].

![Figure 4: Sample chromatogram using C18 column and Acetonitrile – CH₃COOH 2% (6:4) as mobile phase, flow rate 1.0 ml/minute, injection volume 10 μl, and UV detector at λ 280 nm. The color line coded shows in Figure 4.

Highest capsaicin concentration was obtained in green rawit chili and followed by red rawit chili. This both chilies were a *Capsicum frutescens* species. Physically, *Capsicum frutescens* has [8] taste than *Capsicum annuum*, which means its capsaicin content is higher in *Capsicum frutescens* rather than in *Capsicum annuum*. However, taste and capsaicin levels is not affected by the shape and size [3].

![Fig. 4: Capsaicin level of various capsicum fruit from Bandung, Indonesia](image)

There were three samples that were not giving peak in chromatogram, that are yellow paprika, green paprika and red paprika. This was shown by its physical properties which are a big chili and it doesn't have smell or taste like other chili. It doesn't even have a hot taste, instead sweet taste. Because of that, paprika oleoresin Capsicum was only used as colorant in cosmetics and foods. Capsaicin concentration percentage in samples (% yield) can be shown in Figure 4.

**CONCLUSION**

Based on capsaicin marker compound analysis on ethanol extract of several varieties of *Capsicum sp.*, we can conclude that capsaicin content on green paprika, yellow paprika and red paprika were 0.0%; tanjung chili 0.38%; red chili 0.83%; red gendot chili 0.87%; green gendot chili 0.88%; green curly chili 1.05%; japak rawit 1.09% red curly chili 1.14%; red rawit chili 1.85%; and green rawit chili 2.11%.
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REFERENCES