

AN ANALYSIS OF CAPSAICIN LEVEL OF VARIOUS CAPSICUM FRUITS FROM BANDUNG INDONESIA*

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The capsaicin level of the fruits determines the hot taste of various type of *Capsicum* sp for seasoning or pharmaceutical purposes. An analysis of capsaicin been carried out. The samples were collected from different area in Indonesia which consisted of 12 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 wave length (λ) 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, cabai keriting hijau, rawit japlak, cabai keriting merah, rawit merah, and cayenne were 0.38; 0.83; 0.87; 0.88; 1.05; 1.09; 1.14; 1.85 and 2.11%(w/w) respectively. The data showed that cayenne has the highest capsaicin concentration among the other type of capsaicin and must be the most prospective type for seasoning or pharmaceutical purposes.

Keywords : capsaicin analysis, extract ethanol, chilli fruits.

I. Introduction

One of traditional plant that has so many pharmacology effects is chili fruit (*Capsicum* sp). Around the world was known 5 variety 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 chili). Shape and color variation of *C. annuum* is more than *C. frutescens* (Sukrasno, 1997).

Chili fruit has a hot taste. This comes from capsaicinoid compounds that are amide acids from vanilinamine and fatty acid chain branched at C₉ and C₁₁. Capsaicinoid consists of capsaicin, dihydrocapsaicin, homocapsaicin, and homodihydro-capsaicin.

69% of capsaicinoid is capsaicin, which is a marker compound and has an affect on stimulating hair growth. 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 (Sukrasno, 1994).

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 is an analytical method of compound in a mixture with high sensitivity and selectivity that can analyze rapidly, with good separation (Gritter, 1991). HPLC success in quantitative chemical analysis of certain compound is very depending on compounds separation in column and detection process in UV spectrophotometer detector (Robertson, 1984).

Sample preparation for HPLC is due to get a homogenic sample solution, free of contaminant, indestructible to the column and appropriate with chosen method. *Solid Phase Extraction* is a very important technique in HPLC sample preparation. This method can reduce contaminant disturbing capsaicin analytical process.

Results of this experiment was expected to gave scientific information about capsaicin content quantitative data on

ethanol extract of several variety of *Capsicum sp.* that can be used to determine the best chili fruit variety as raw material of capsaicin.

Experiment was carried out through many steps as well:

1. Material collection and determination
2. Extraction of *Capsicum sp.* several varieties.
3. Optimum condition estimation
4. Data analysis.

II. Method

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

There are twelve chili fruit samples. Samples determined 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 maserate with 1 L ethanol 95% for 24 hours. Liquid extract then evaporated at 40⁰ C to eliminate the solvent.

Chemical materials used in this experiment were *Capsicum liq concentrate HC 14%, USP Grade Oleoresin Capsicum*; aqua bidistillate (Ikapharmindo Putramas); acetonitrile (J.T.Baker); ethanol 95% (re-distillate); glacial acetic acid (Merck).

Instruments used in this experiment were HPLC (Shimadzu[®]) model SCL-10A VP equipped with ultraviolet *visible* SPD-10A VP detector, Shim-Pack[®] VP-ODS 150x4.6mm column, ultrasonic stirrer (NEY[®]), *rotary evaporator* (Buchi[®]), digital balance (Sartorius[®]), *Solid Phase Extraction HLB* (OASIS[®]), micropipette 20-200 μ l (Finnpipette[®]), and micropipette 1000 μ l (Finnpipette[®]).

Capsaicin qualitative and quantitative analysis was carried out by reversed phase HPLC method, with C₁₈ column and Acetonitrile – CH₃COOH 2% (6:4) as mobile phase, flow rate 1,0 ml/minute, injection volume 10 μ l and using UV detector at λ 280nm. Retention time and area under curve of each sample was recorded.

Capsicum liq concentrate HC 14%, USP Grade Oleoresin Capsicum standard solution then was made at 8; 4; 2; 1; and 0,5

ml/L. And 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 1ml sample was 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 $Y = a + b x$. 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 14%, 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.

III. Results and Discussion

HPLC method used in this experiment was chose 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. HPLC can separate a component from mixture and identify it based on retention time.

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% CH₃COOH solution in acetonitrile. Eluent was collected and analyzed by chosen HPLC condition.

Standard and samples analysis shows that there are two chromatogram peaks. 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.

Linear regression equation was made from measured capsaicin standard peak area to capsaicin concentration in standard derived from capsaicin content conversion in *Capsicum liq concentrate HC 14%, USP Grade Oleoresin Capsicum*. Capsaicinoid amount in *Capsicum liq concentrate HC 14%, USP Grade Oleoresin Capsicum* was 14%, while capsaicin amount was 9.4%. Linear equation was:
 $y = 629.87x + 5458.2$.

Table 3.1 Capsaicin Standard Solution Measurement at Various Concentrations

Oleoresin concentration (ppm)	capsaicin concentration (ppm)	Peak area
500	6.58	33235
1000	13.16	68341
2000	26.32	127959
4000	52.64	233155
8000	105.28	482320

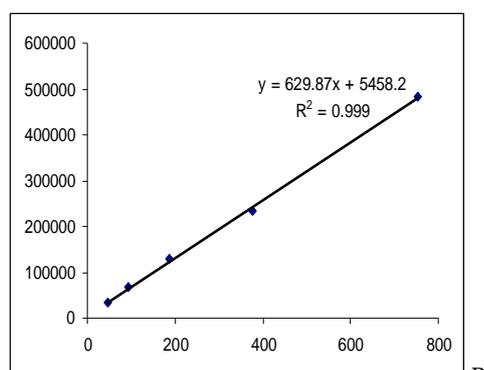
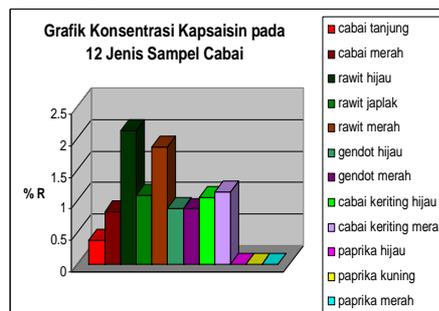


Figure 3.1 Capsaicin Standard Calibration Curve

Capsaicin concentration percentage in samples (% R) can be made into chart as

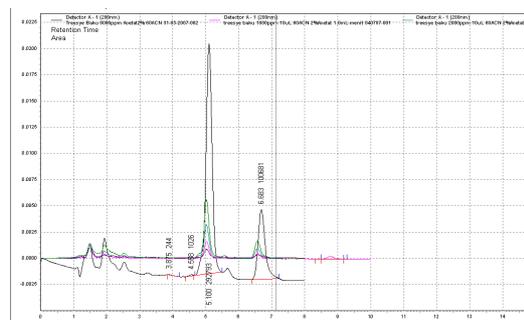
followed:



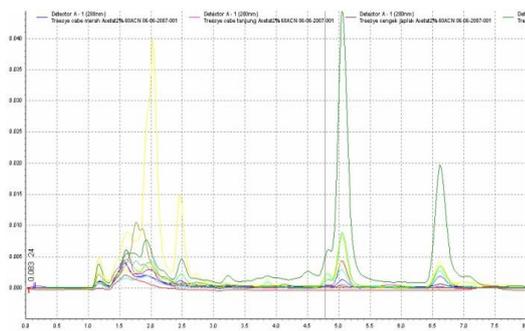
Picture 3.2 Capsaicin Concentration in Samples Chart

Highest capsaicin concentration was measured in green rawit chili and followed by red rawit chili. This both chilies were a *Capsicum frutescens* species. Physically, *Capsicum frutescens* has hotter taste than *Capsicum annuum*, which means its capsaicin content is higher in *Capsicum frutescens* rather than in *Capsicum annuum*.

There are 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.



Picture 3.3 Capsaicin Standard Chromatogram



Picture 3.4 Extract ethanol Sample Chromatogram

IV. Conclusion and Comment

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 0,87%; green gendot 0,88%; green curly chili 1,05%; japlak rawit 1,09%; red curly chili 1,14%; red rawit 1,85%; and green rawit 2,11%.

Structure analysis of each chromatogram's peak using NMR method should be carrying out to confirm molecular structure of compound for each chromatogram.

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