

CAPSAICIN LEVEL OF VARIOUS CAPSICUM FRUITS

IDA MUSFIROH*, MUTAKIN MUTAKIN, TREESYE ANGELINA, MUCHTARIDI MUCHTARIDI

Department of Pharmacochemistry, Faculty of Pharmacy, Padjadjaran University, Bandung, Indonesia. Email: idamusfiroh@unpad.ac.id

Received: 28 Oct 2012, Revised and Accepted: 29 Nov 2012

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 (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, 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.

INTRODUCTION

One of traditional plant that has so many pharmacology effects is chili fruit (*Capsicum sp*). Around the world is known five varieties of *Capsicum sp*, which are *C. annum*, *C. frutescens*, *C. chinense*, *C. baccatum*, *C. pubescens*, meanwhile in Indonesia only known 2 varieties, *C. annum* also known as red chili, paprika, gendot until curly chili, and *C. frutescens* (rawit) [1]. Shape and color variation of *C. annum* is similar with *C. frutescens* [2]. Yadiz and Olguvan (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 vanilamine 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 [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.

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

S. No.	Local Name	Scientific Name
1.	Green gendot chili	<i>Capsicum annum</i> L. var. Grossum
2.	Red gendot chili	<i>Capsicum annum</i> L. var. Grossum
3.	Green paprika	<i>Capsicum annum</i> L. var. Grossum
4.	Yellow paprika	<i>Capsicum annum</i> L. var. Grossum
5.	Red paprika	<i>Capsicum annum</i> L. var. Grossum
6.	Red chili	<i>Capsicum annum</i> L. var. Tanjung
7.	Tanjung chili	<i>Capsicum annum</i> L. var. Tanjung
8.	Green curly chili	<i>Capsicum annum</i> L. var. Tanjung
9.	Red curly chili	<i>Capsicum annum</i> L. var. Tanjung
10.	Green rawit chili (<i>cengek</i>)	<i>Capsicum frutescens</i> L.
11.	Red rawit chili (<i>cengek jawa</i>)	<i>Capsicum frutescens</i> L.
12.	Japlak rawit chili (<i>cengek japlak</i>)	<i>Capsicum frutescens</i> L.

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 (Ikaparmindo 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* SPD10A VP detector, Shim-Pack® VP-ODS 150x4.6 mm 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 280 nm. Retention time and area under curve of each sample was recorded.

Capsicum liq concentrate HC 149, 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 1 ml 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 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].

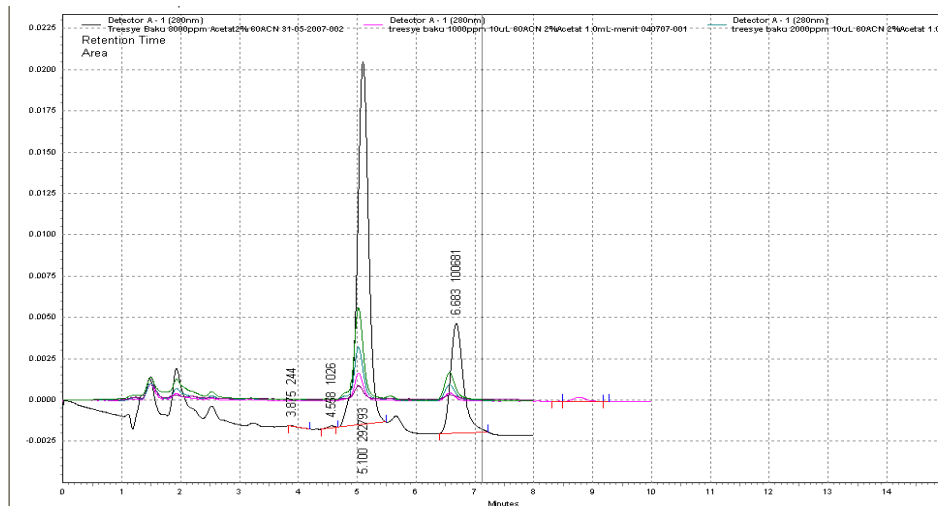


Fig. 1: Chromatogram of capsaicin in capsicum liq concentrate HC 14%, USP grade oleoresin Capsicum 8; 4; 2; 1 and 0,5 ml/L Standard Chromatogram using C18 column and Acetonitrile – CH₃COOH 2% (6:4) as mobile phase, flow rate 1,0 ml/minute, injection volume 10 µl, and UV detector at λ 280 nm.

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.

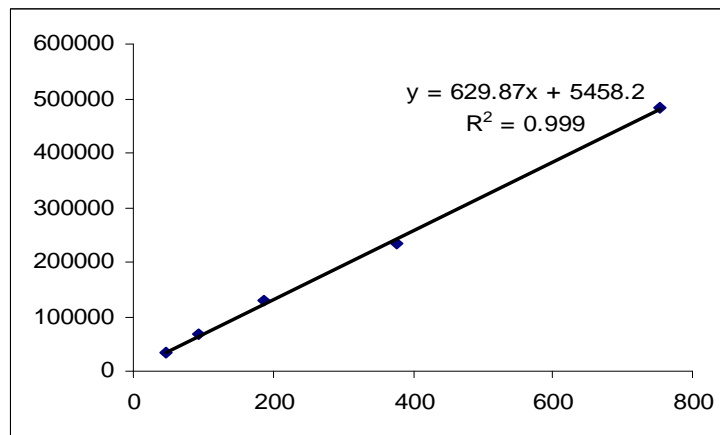


Fig. 2: Standard curve of capsaicin reference

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 capsicum fruit is 19.73 mg (1.97 %) in *C. frutescens* s from Nepal. Pruthi (2003) recorded that the highest of capasaicin in chili is obtained in Indian Chili (1.86 %) [2].

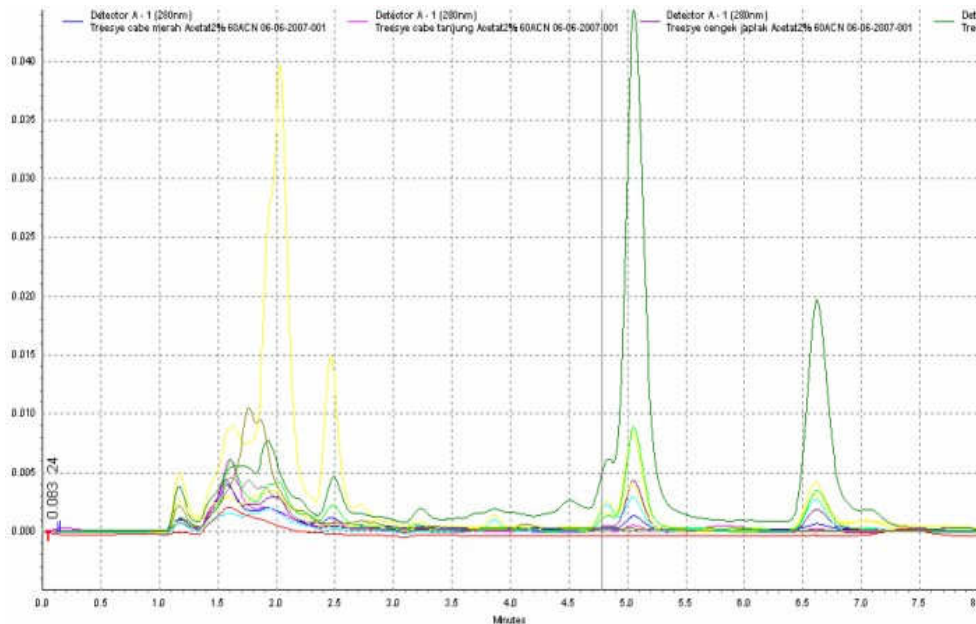


Fig. 3: Sample chromatogram using C18 column and Acetonitrile – CH₃COOH 2% (6:4) as mobile phase, flow rate 1.0 ml/minute, injection volume 10 µl, and UV detector at λ 280 nm. The color line coded shows in Figure 4.

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 annum, which means its capsaicin content is higher in *Capsicum frutescens* rather than in *Capsicum annum*. However, taste and capsaicin levels is not affected by the shape and size [3].

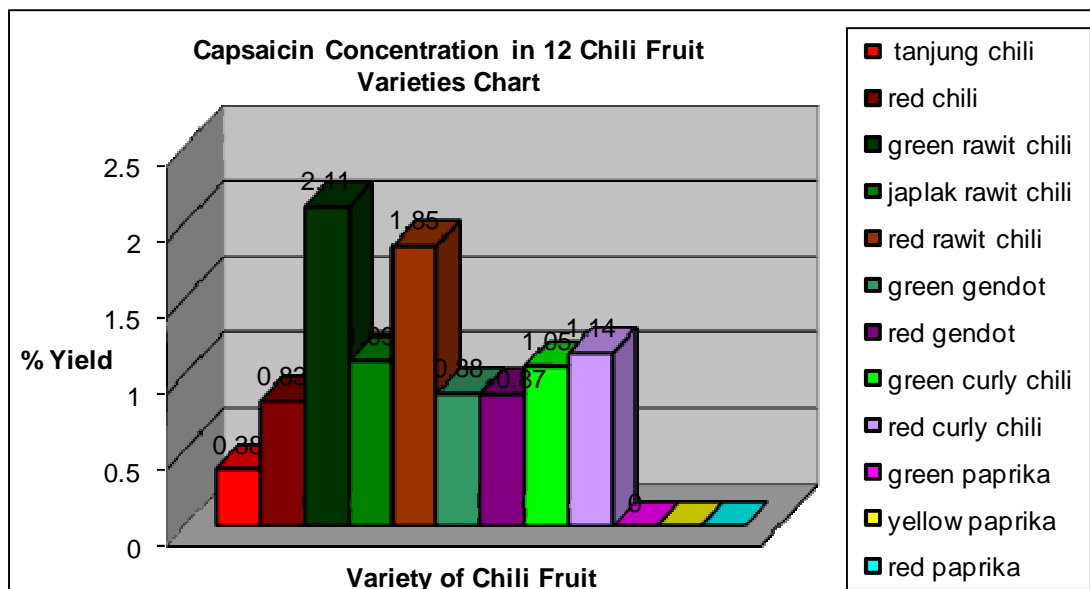


Fig. 4: Capsaicin level of various capsicum fruit from Bandung, Indonesia

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%; japlak rawit 1.09%; red curly chili 1.14%; red rawit chili 1.85%; and green rawit chili 2.11%.

ACKNOWLEDGEMENT

The authors are thankful to Prof. Sukrasno, School of Pharmacy ITB, for providing oleoresin capsicum for the research work

REFERENCES

1. Saikat Kumar B, Amit Krishna D. Historical and Botanical Perspectives. In: Capsicum: The Genus Capsicum, *In Capsicum* 2003, Edited by Amit Krishna D: CRC Pres. 1-15.
2. Pruthi J. Chemistry and quality control of Capsicums and Capsicum products. In: *Capsicum*: CRC Press 2003.
3. Yaldiz G, Ozguven M. A. Study of yield and yield components of different ornamental pepper (*Capsicum* sp.) species and lines in cukurova ecological conditions. *Pak J Biol Sci* 2011; 14: 273-281.
4. Final report on the safety assessment of capsicum annum extract, capsicum annum fruit extract, capsicum annum resin, capsicum annum fruit powder, capsicum frutescens fruit, capsicum frutescens fruit extract, capsicum frutescens resin, and capsaicin. *Int J Toxicol* 2007; 26 Suppl 1: 3-106.
5. Govindarajan VS. Capsicum--production, technology, chemistry, and quality. Part III. Chemistry of the color, aroma, and pungency stimuli. *Crit Rev Food Sci Nutr* 1986; 24: 245-355.
6. Aza-Gonzalez C, Nunez-Palenius HG, Ochoa-Alejo N. Molecular biology of capsaicinoid biosynthesis in chili pepper (*Capsicum* spp.). *Plant Cell Rep* 2011; 30: 695-706.
7. Sukrasno. Kapsaisin : Perkembangan Penelitian dan Pemanfaatan dalam Pengobatan. *Acta Pharmaceutical* 1994; 19:86-91.
8. Thapa B, Skalko-Basnet N, Takano A, Masuda K, Basnet P. High-performance liquid chromatography analysis of capsaicin content in 16 Capsicum fruits from Nepal. *J Med Food*. 2009; 12: 908-913.
9. Muchtaridi M, Musfiroh I. Off-Line SPE-GC/MS Analysis of Lead Compounds Aromatherapy in Blood Plasma of Mice of Essential Oils Materials from Indonesian Aromatic Plants. *Asian J. Chem* 2012; 24: 5124-5128.
10. Peng A, Ye H, Li X, Chen L. Preparative separation of capsaicin and dihydrocapsaicin from *Capsicum frutescens* by high-speed counter-current chromatography. *J Sep Sci* 2009; 32: 2967-2973.
11. Al Othman ZA, Ahmed YB, Habila MA, Ghafar AA. Determination of capsaicin and dihydrocapsaicin in Capsicum fruit samples using high performance liquid chromatography. *Molecules* 2011; 16: 8919-8929.
12. Kozukue N, Han J-S, Kozukue E, Lee S-J, Kim J-A, Lee K-R, et al. Analysis of Eight Capsaicinoids in Peppers and Pepper-Containing Foods by High-Performance Liquid Chromatography and Liquid Chromatography-Mass Spectrometry. *Journal of Agricultural and Food Chemistry* 2005; 53: 9172-9181.
13. Ajmera A, Deshpande Shrikalp, Patel P, Patel K, Solanki S, Rathod K. Reverse Phase High Performance Liquid Chromatographic (HPLC) Method for Simultaneous Determination of Atorvastatin, Torvastatin, Ezetimibe and Fenofibrate in Commercial Tablets, *Int J Pharm Pharm Sci* 2012; 4: 206-209.
14. Balan P, Carolin NI, Chundi S, Movva RV, Rajasekar S, A Validated RP-HPLC Method for Estimation of Drotaverine HCL In Pharmaceutical Tablet Dosage Form, *Int J Pharm Pharm Sci* 2012; 3: 77-80.