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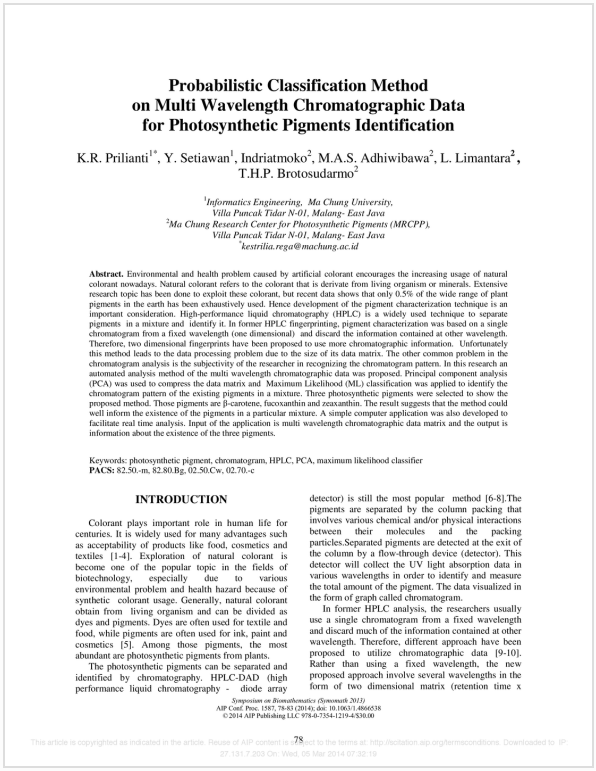

**Probabilistic Classification Method on Multi Wavelength Chromatographic Data for Photosynthetic Pigments Identification**

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**Abstract.** Environmental and health problem caused by artificial colorant encourages the increasing usage of natural colorant nowadays. Natural colorant refers to the colorant that is derivate from living organism or minerals. Extensive research topic has been done to exploit these colorant, but recent data shows that only 0.5% of the wide range of plant pigments in the earth has been exhaustively used. Hence development of the pigment characterization technique is an important consideration. High-performance liquid chromatography (HPLC) is a widely used technique to separate pigments in a mixture and identify it. In former HPLC fingerprinting, pigment characterization was based on a single chromatogram from a fixed wavelength (one dimensional) and discard the information contained at other wavelength. Therefore, two dimensional fingerprints have been proposed to use more chromatographic information. Unfortunately this method leads to the data processing problem due to the size of its data matrix. The other common problem in the chromatogram analysis is the subjectivity of the researcher in recognizing the chromatogram pattern. In this research an automated analysis method of the multi wavelength chromatographic data was proposed. Principal component analysis (PCA) was used to compress the data matrix and Maximum Likelihood (ML) classification was applied to identify the chromatogram pattern of the existing pigments in a mixture. Three photosynthetic pigments were selected to show the proposed method. Those pigments are β-carotene, facoxanthin and zeaxanthin. The result suggests that the method could well inform the existence of the pigments in a particular mixture. A simple computer application was also developed to facilitate real time analysis. Input of the application is multi wavelength chromatographic data matrix and the output is information about the existence of the three pigments.

Keywords: photosynthetic pigment, chromatogram, HPLC, PCA, maximum likelihood classifier
**PACS:** 82.50.-m, 82.80.Bg, 02.50.Cw, 02.70.-c

**INTRODUCTION**

Colorant plays important role in human life for centuries. It is widely used for many advantages such as acceptability of products like food, cosmetics and textiles [1-4]. Exploration of natural colorant is become one of the popular topic in the fields of biotechnology, especially due to various environmental problem and health hazard because of synthetic colorant usage. Generally, natural colorant obtain from living organism and can be divided as dyes and pigments. Dyes are often used for textile and food, while pigments are often used for ink, paint and cosmetics [5]. Among those pigments, the most abundant are photosynthetic pigments from plants.

The photosynthetic pigments can be separated and identified by chromatography. HPLC-DAD (high performance liquid chromatography - diode array detector) is still the most popular method [6-8].The pigments are separated by the column packing that involves various chemical and/or physical interactions between their molecules and the packing particles.Separated pigments are detected at the exit of the column by a flow-through device (detector). This detector will collect the UV light absorption data in various wavelengths in order to identify and measure the total amount of the pigment. The data visualized in the form of graph called chromatogram.

In former HPLC analysis, the researchers usually use a single chromatogram from a fixed wavelength and discard much of the information contained at other wavelength. Therefore, different approach have been proposed to utilize chromatographic data [9-10]. Rather than using a fixed wavelength, the new proposed approach involve several wavelengths in the form of two dimensional matrix (retention time x

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