I declare that this thesis entitled "*Extraction and Analysis of Safranal and Crocin in Pure Crocus Sativus*" is the result of my own research except as cited in the references. This thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Name: Emma Suali

Date: 27 Nov 2006

EXTRATION AND ANALYSIS OF SAFRANAL AND CROCIN IN PURE SAFFRON (*CROCUS SATIVUS*)

EMMA SUALI

A thesis submitted in partial fulfillment of the

Requirements for the award of degree of

Bachelor of Chemical Engineering

Faculty of Chemical Engineering and Natural Resources

University College of Engineering and Technology Malaysia

NOVEMBER 2006

"I/We* hereby declare that I/we have read this thesis and in my/our* opinion this thesis is sufficient in terms of scope and quality for the award of the degree of...chemical engineering...."

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Date : .....

* *Delete as necessary*

To my beloved parent, Mr.Suali Sikir and Mdm.Soulin Banting.

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EXTRATION AND ANALYSIS OF SAFRANAL AND CROCIN IN PURE SAFFRON (*CROCUS SATIVUS*)

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ABSTRACT

A number of methods for the preparation, extraction and HPLC analysis of saffron were evaluated to determine a reliable method for quantifying safranal and crocin in *Crocus sativus L.* stigma tissues. Samples of saffron from Kashmir India have been chosen to use in this experiment. A simple, sensitive and specific High Performance Liquid Chromatography has been chosen to be analyzing the safranal and crocin in stigma of saffron. There are four different methods that have been used in this experiment. In method 1,sample were extracted by acetonitrile and water, in method 2,sample were extracted by ethanol and water, in method 3,sample were extracted acetonitrile and water but vaporized using rotary evaporator unit and in method 4,the sample have been extracted by ethanol and water followed evaporation by rotary evaporator unit. Preparation methods such as heating stigmas at 80°C for 30 min prior to extraction and followed by immediate HPLC analysis increased the concentration of safranal and crocin several fold compared to stigmas which had been prepared by freeze drying. Other equipments that have been used in this experiment are microcentrifuger to centrifuge the sample, water bath and oven to increase the level of saturation. Using different solvent to extract the stigma of saffron also effect the level of chemical composition in each sample.Safranal reacted more to acetonitrile compare to ethanol and crocin reacted more to ethanol compare to acetonitrile. The purpose of preparation of ethanol and water or acetonitrile stigma extracts by drying with a rotary evaporator prior to HPLC analysis were to find the effect of temperature to the stigma of saffron in which the boiling point of crocin is more 186 °C,safranal is 70 °C, ethanol is 78.4 °C and acetonitrile is 82 °C.

Keywords: *HPLC, Crocin, Safranal, saffron*

ABSTRAK

Kepelbagaian cara penyedian, cara pengekstrakan safranal dan crocin daripada saffron atau *crocus sativus* dijalankan untuk mengenalpasti cara yang bersesuaian untuk menguji kandungan safranal dan crocin dalam saffron dengan menggunakan HPLC. Dalam eksperimen ini, saffron yang digunakan sebagai bahan ujikaji adalah saffron dari Kashmir India. Terdapat empat cara yang berbeza dijalankan dalam eksperimen ini. Dalam cara yang pertama,bahan ujikaji telah dilarutkan dalam 'acetonitrile' dan air, dalam method yang kedua,bahan ujikaji dilarutkan dalam 'ethanol' dan air,dalam ujikaji yang ketiga, bahan ujikaji di larutkan dalam acetonitrile and air kemudian diwapkan dengan mengunakan alat 'rotary evaporator unit' dan dalam method yang keempat bahan ujikaji dilarutkan dalam 'ethanol' dan air kemudian diwapkan dengan menggunakan 'rotary evaporator unit'.Cara penyediaan seperti memanaskan saffron pada 80°C selama 30 minit diikuti dengan analisa sebaik sahaja pengekstrakan dilakukan dengan menggunakan HPLC meningkatkan kepekatan 'safranal' dan 'crocin' dalam saffron berbanding dengan saffron yang hanya dikeringkan alat pengering. Alat-alat lain yang digunakan dalam eksperimen ini adalah seperti 'microcentrifuger' untuk mempercepatkan keterlarutan 'safranal' dan 'crocin' di dalam sampel, 'water bath' dan 'oven'. Dengan mengunakan bahan pelarut yang berbeza untuk melarutkan saffron, cara-cara tersebut mempengaruhi tahap keterlarutan 'safranal' dan 'crocin' dalam saffron. Melalui eksperimen ini, 'safranal' lebih larut dalam 'acetonitrile' berbanding dengan 'crocin' dan 'crocin' lebih larut dalam 'ethanol' berbanding dengan 'acetonitrile'.Tujuan utama melarutkan sampel dengan air dan 'ethanol' kemudian dikeringkan dengan mengunakan 'rotary evaporator unit' adalah untuk meguji kesan suhu terhadap bahan ujikaji dimana takat didih 'crocin' adalah 186 °C, 'safranal' adalah 70 °C, 'ethanol' adalah 78.4 °C dan 'acetonitrile' adalah 82 °C.

Kata Kunci: *HPLC, Crocin, Safranal, saffron*

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Saffron is a spice derived from the flower of the saffron crocus (Crocus sativus), a species of crocus in the family Iridaceae. Saffron, which has for decades been the world's most expensive spice by weight, is native to Southwest Asia. Saffron a spice derived from the dried stigma of the saffron and saffron also known as *crocus sativus* among the chemist or simply its scientific name is *crocus sativus*. (Abdulalev et.al.2006) Saffron is native to Southwest Asia but first was cultivated in Greece. In late history of Egypt, they used a quarter cup of saffron to mix with warm water for bath because of its coloring and cosmetic properties.Egyptian used saffron as a treatment for all varieties of gastrointestinal ailments. Saffron was used by ancient Persian worshipers as a ritual offering to deities. It was also used as a brilliant yellow dye, a perfume, and a medicine. Various conflicting accounts exist that describe saffron's first arrival in South and East Asia. The first of these rely on historical accounts gleaned from Persian records. (Abdulalev et.al.2006) Saffron is dry, glossy and greasy to the touch when freshly dried, turning dull and brittle with age. It is easily bleached if not stored in the dark, and also stores better under conditions of low temperature and low relative humidity. (Rangahau Mana Kai.2003).The chemistry of saffron has been investigated in detail. The major pigment, a water-soluble carotenoid giving saffron its value as a dye, is crocin, a yellow-red pigment found at levels of up to 2%. Picrocrocin less than <4% is a bitter-tasting principle that hydrolyses to glucose and safranal less than, <4%, on drying. (Rangahau Mana Kai.2003) Chemists found this to be the most powerful contributor to saffron's fragrance despite its being present in a lesser quantity than safranal. Dry

saffron is highly sensitive to fluctuating pH levels, and rapidly breaks down chemically in the presence of light and oxidizing agents. It must therefore be stored away in air-tight containers in order to minimize contact with atmospheric oxygen. Saffron is somewhat more resistant to heat. (Abdulalev et. al.2006). It is the constituent primarily responsible for the aroma of saffron. It is believed that safranal is a degradation product of the carotenoid zeaxanthin via the intermediacy of picrocrocin. (Abdulalev et. al.2006) Safranal is the main component in saffron. (Maurizio D'Auria et.al.2002) Picrocrocin is a glycoside formed from glucose and safranal. It is found in the spice saffron, which comes from the crocus flower. Picrocrocin has a bitter taste and is the chemical most responsible for the taste of saffron. It is believed that picrocrocin is a degradation product of the carotenoid zeaxanthin.Its melting point is 154-156°C. (Abdulalev et. al.2006).In determine the quality of saffron tea, we based on three criteria that is its taste, odor and color. To determine the strength of color in saffron we based on ISO level. The stronger the color is the better more expensive. For saffron, absorbance is determined for the crocin-specific photon wavelength of 420 nm in a given dry sample of spice (Abdulalev et.al.2006) An International Standard for saffron is available that is ISO 3632-1:1993. Saffron in filaments is classified into four categories based on the content of floral waste and extraneous matter, with category 1 that is extra having a maximum of 0.5% floral waste and 0.1% extraneous matter. Category 1 has the highest bitterness. The quality of saffron is dependent on its coloring power, crocin concentration, and odor from safranal and taste from picrocrocin. The best quality saffron has high safranal content. (Rangahau Mana Kai.2003).Saffron contains in excess of 150 volatile and aroma-yielding compounds. It also has many nonvolatile active components, many of which are carotenoid, including zeaxanthin, leucopenia, and various α- and β-carotenes. However, saffron's golden yellow-orange color is primarily the result of α-crocin. This crocin is trans-crocheting di-(β-D-gentiobiosyl) ester or in systematic IUPAC name 8, 8-diapo-8, 8-carotenoic acid. It has insecticidal and pesticide properties, and may comprise up to 4% of dry saffron. Significantly, picrocrocin is a truncated version produced via oxidative cleavage of the carotenoid zeaxanthin and is the glycoside of the terpene aldehyde safranal. The reddish-colored zeaxanthin is, incidentally, one of the carotenoid naturally present within the retina of the human eye. (Subhuti Dharmananda.2006)

1.2 Objective

The objectives of this experiment were including to evaluating the improved procedures for extraction and analysis of safranal and crocin in saffron. Beside that, the objective of this experiment is to compare the result of different preparation method to extract sample of saffron and the effect of temperature to the samples of saffron.

1.3 Scope of Study

The scope of this project was including the extraction of chemical compounds in stigma saffron from Kashmir India. The chemical compounds that included in this experiment were safranal and crocin. Another scope of this experiment is analysis the chemical composition in saffron using High Performance Liquid Chromatography that is HPLC.

1.4 Problem Statement

1.4.1 General

Nowadays saffron become one of the most important and useful spice that have been used commercially and traditionally. Because of its healing power, an appropriate method should be developing so that peoples can fully take its benefits. saffron also facing challenging tasks such as the need of new manufacturing techniques, conservation of genetic resources, new chemical technologies to asses quality and to develop a wide range of saffron uses, particularly those related to human health. Because of inappropriate method in handling and manufacturing of saffron, it caused a lot of composition in saffron cannot deliver to people who need

REFERENCES

Abdulalev et.al. ((2006).*Crocin:* Wikipedia: The free encyclopedia.

Abdulalev et.al. (2006) *History of saffron:* Wikipedia: The free encyclopedia

Abdulalev ET. al. (2006) *Saffron:* Wikipedia: The free encyclopedia

A.V. Loskutov et.al. (1999). *Development of an improved procedure for extraction and quantitation of safranal in stigmas of Crocus sativus L. using High performance liquid chromatography:* Michigan: Michigan state University.

Bulvar Sajad, "Coloring Power."2006. *http://www.truesaffron.com:* April 2006.

Chiou GC et.al. (1999). *Effects of crocin analogs on ocular blood flow and retinal function. :* Ocul Pharmacol Therlan.

Hong Zhang et. al. (2004) *Semi-Preparative Isolation of Crocin from Saffron (Crocus Sativus L):* China: Sichuan University, Chengdu.

Heriberto et.al. (2005).*HPLC Quantification of Major Active Components From 11Different Saffron:* Mexico: University National Autonomy Mexico

Jose Antonio Fernandez. (2004) *Biology, Biotechnology and Biomedicine of Saffron:* India: University of Castilla-La Mancha..

Kwan.et.al (1999).*Crocin:* China: Chinese University of Hong Kong, Hong Kong.

Maurizio D'Auria et.al. (2002).*Volatile Organic Compounds From Saffron*: Italy: University Della Basilicata, Italy

Mohamad Zougagh et al. (2006) *Determination of total safranal by in situ acid hydrolysis in supercritical fluid media: Application to the quality control of commercial Saffron:* Spain: University of Cordoba

M.Carmona et.al. (2005*).Comparison of Different Extraction Procedures and HPLC Methods to Detect Crocin in Saffron:* Belgium: International Society for Horticultural Science Belgium.

Na Li et al. (1999). *Simultaneous quantification of five major biologically active ingredients of saffron by high-performance liquid chromatography.* China: The Chinese University of Hong Kong.

Olga Chfanou & Maria Tshldou (1995) *Evaluation of the colouriug strength of saffron spice by UV-Vis spectrometry:* Greece: Aristotle University, Thessaloniki

P. Lozano et.al.(2000) *A non-destructive method to determine the safranal content of saffron (Crocus sativus L.) by supercritical carbon dioxide extraction combined with high-performance liquid chromatography and gas chromatography*:Spain:Univeristy of Murcia.

Ramesh A et.al. (2001). *Inhibition of genotoxicity by saffron (Crocus sativus L.) in mice.* Drug Chem. Toxicol

Rangahau, Mana, Mana Kai. (2003) *Growing Saffron-the world's most expensive spice*: New Zealand: Institute for Crop & Food Research Limited.

Robinson A. (2001). *Notes on the saffron plant (Crocus sativus L.):* London: Harm Hist London.

Saito H. (2000). *Effects of saffron extract and its constituent crocin on learning behavior and long-term ostentation.* Phototherapy Res.

Subhuti Dharmananda. (2005) *Saffron: An-anti Depressant Herb*: Oregon: Institute for Traditional Medicine Portland.

Students of Kentucky University. "*HPLC*", 2006:http://Kerouac.pharm.Uky.edu.

Tarantilis et.al. (1994) *Separation of picrocrocin, cis-trans-crocins and safranal of saffron using high-performance liquid chromatography with photodiode-array detection:* Greece: Agricultural University of Athens, Greece.

Thomas Ombrello. (2006) *Saffron*: China: Union Country College