



STUDY ON LIPOPHILIC (C₄₀) AND HYDROPHILIC
(C₅₀) CAROTENOIDS: PRODUCTION FROM SHRIMP
WASTE AND SAFFRON AS POTENTIAL *HALAL*
PIGMENTS

BY

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A thesis submitted in fulfilment of the requirement for the
degree of Doctor of Philosophy in Halal Industry

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JUNE 2019

ABSTRACT

A perceptible visual aesthetics in terms of colour is one of the important aspects for product marketability and acceptability. This is due to colour itself being inherently ubiquitous and capable of conveying valuable messages of the products. Nevertheless, various controversial issues regarding the safety of synthetic colourant have drawn a great deal of attention from consumers. Carotenoids are an excellent source of colouring agent that is acknowledged for its bioactive compounds ability and pro vitamin A and antioxidant activity. It is believed to aid in enhancing one's health while also capable of creating a visually appealing image to consumers. Astaxanthin is a reddish lipophilic carotenoid (C₄₀) has been reported to have greater antioxidant activities by ten-fold compared to other carotenoids, and 100 times better than α -tocopherol. In contrast, crocin is known as one of the unique hydrophilic carotenoid (C₅₀) that produces yellowish red colour due to its water solubility properties. Thus, this research has aimed to explore lipophilic and hydrophilic carotenoids from natural resources as potential halal biocolourants, specifically from shrimp waste and saffron. Astaxanthin extraction from shrimp waste has been undertaken using different extraction methods, such as chemicals extraction, high pressure processing (HPP) and supercritical fluid extraction (SFE) technologies. These methods have yielded the highest amount of total astaxanthin content at 5701.99, 308.58, and 198.27 $\mu\text{g/g}$ DW respectively. Although the total astaxanthin content produced by SFE was lower than via chemical and HPP extraction, it has produced high purity extracts enriched with astaxanthin. Furthermore, the application of such environmentally-friendly technology is a cost-effective and swift operation concomitantly. It is also capable of producing quality product without generating toxic substances, and causes minimal degradation only of the active compounds. Meanwhile, maceration extraction has been done to extract hydrophilic carotenoids in saffron from different localities (i.e. Iran, Turkey, Kashmir) as another potential *halal* biocolourant. The highest amount of crocin content has been detected in Iranian saffron at 11414.67 $\mu\text{g/g}$ DW. Next, a screening assessment of the antioxidant, antibacterial, and antifungal in crocin and astaxanthin has also been performed. Their respective extracts have displayed strong activity on DPPH scavenging radicals, which is determined by IC₅₀ values at 283.918 and 589.628 $\mu\text{g/ml}$ respectively. Moreover, with regards of the antimicrobial assay performed, crocin has shown potential as an antimicrobial agent specifically against *Staphylococcus epidermidis*. Besides, colour stabilities of astaxanthin and crocin have also been evaluated across different period via salinity, pH, and UV and heat stability tests. Astaxanthin was observed to be unstable at the highest concentration of NaCl, whereas crocin is stable at different NaCl concentrations. They are both unstable towards acidic condition. Then, astaxanthin is more stable at a higher temperature and under UV irradiation compared to crocin. Finally, astaxanthin-PLA and crocin-PVA coatings stability have also been evaluated using salinity and pH stability test, with astaxanthin-PLA being stable when treated with different salinities and pH. However, the presence of NaCl and alkaline pH has decreased the crocin-PVA colour stability. Additionally, the presence of light has also been determined to be a major factor influencing the colour variations and stability of the coating.

خلاصة البحث

تعد الجماليات المرئية المحسوسة المبنية على الألوان أحد الجوانب المهمة لتسويق وتقبل المنتجات، ويرجع ذلك إلى كون الألوان بطبيعتها موجودة في كل مكان وهي قادرة على إيصال رسائل ذات قيمة للمنتجات. ومع ذلك فقد جذبت العديد من القضايا المثيرة للجدل المتعلقة بسلامة الألوان الصناعية الكثير من الاهتمام من قبل المستهلكين. تعتبر الكاروتينات مصدرًا ممتازًا لعوامل التلوين، وهي معروفة بقدرات مركباتها النشطة بيولوجيًا وخصائصها المساندة للفيتامين أ ومضادات الأكسدة. يُعتقد بأن الكاروتينات تساعد في تحسين صحة الشخص وهي قادرة على إنشاء صورة جذابة بصريا للمستهلكين. أظهرت التقارير امتلاك مركب أستازانتين، وهو كاروتينويد محمر أليف للدهون (C₄₀)، لأنشطة مضادة للأكسدة بقوة أكبر بعشرة أضعاف مقارنة بالكاروتينات الأخرى، وبجودة أفضل بمئة مرة مقارنة بألفا توكوفيرول. في المقابل يُعرف الكروسين بأنه أحد الكاروتينويدات الفريدة الأليفة للماء (C₅₀) والتي تنتج اللون الأحمر المصفر بسبب خصائصه القابلة للذوبان في الماء. ولذلك هدف هذا البحث إلى استكشاف كاروتينات أليفة للدهون وأليفة للماء من موارد الطبيعية كمواد بيولوجية محتملة مصنفة كحلال، وتحديدًا من الزعفران وفضلات الروبيان. تم إجراء عملية استخلاص الأستازانتين من فضلات الروبيان باستخدام طرق استخلاص مختلفة، وهي تقنيات استخلاص المواد الكيميائية، والمعالجة بالضغط العالي، واستخلاص السوائل فوق النقطة الحرجة. أسفرت هذه الطرق عن أعلى كمية من إجمالي محتوى أستازانتين بمعدل 5701.99، 308.58، و 198.27 ميكروغرام/غرام من الوزن الجاف على التوالي. على الرغم من أن إجمالي محتوى الأستازانتين الذي أنتجته تقنية استخلاص السوائل فوق النقطة الحرجة كان أقل من الاستخلاص الكيميائي والمعالجة بالضغط العالي، إلا أنه أنتج مستخلصات عالية النقاء غنية بالأستازانتين. علاوة على ذلك فإن تطبيق هذه التكنولوجيا الصديقة للبيئة كانت عملية فعالة من حيث التكلفة وسرعة العمليات بشكل متزامن، كما أنها قادرة على إنتاج منتجات ذات جودة عالية دون توليد مواد سامة، ولم تسبب سوى تدهور ضئيل فقط للمركبات النشطة. وفي الوقت نفسه تم بطريقة التنقيح استخلاص الكاروتينات الأليفة للماء في الزعفران المزروع في مواقع مختلفة (مثل إيران وتركيا وكشمير) باعتباره ملونا حيويًا آخر محتملاً بتصنيف حلال. تم اكتشاف أن أكبر محتوى للكروسين كان في الزعفران الإيراني بمعدل 11414.67 ميكروغرام/غرام. بعد ذلك تم التقييم للكشف عن خصائص المركب النشطة حيويًا مثل النشاط المضاد للأكسدة والمضاد للجراثيم والفطريات في الكروسين والأستازانتين. أظهرت المستخلصات نشاطًا قويًا على جذور DPPH الكاسحة، والتي حددت بـ IC₅₀ بمعدل 283.918

و 589.628 ميكروغرام/ملغ، على التوالي. بالإضافة إلى ذلك، فيما يتعلق بفحص مضادات الميكروبات التي تم إجراؤها، أظهر الكروسين إمكانية كعامل مضاد للميكروبات، وعلى وجه التحديد بكتيريا العنقوديات البشرية. بالإضافة إلى ذلك تم تقييم الثبات اللوني للأستازانتين والكروسين على فترات مختلفة عن طريق اختبارات الملوحة، والحموضة، والأشعة فوق البنفسجية، وثبات الحرارة. لوحظ أن الأستازانتين كان غير مستقرا عند أعلى تركيز لكلوريد الصوديوم، في حين أن الكروسين كان مستقرا على تراكيز مختلفة لكلوريد الصوديوم. كلاهما كانا غير مستقرين تجاه الوضع الحمضي وكان الأستازانتين أكثر ثباتاً عند درجة حرارة عالية وتحت الإشعاع فوق بنفسجي مقارنة بالكروسين. وفي النهاية تم تقييم ثبات الأستازانتين-PLA والكروسين-PVA باستخدام اختبار الملوحة والاستقرار الحمضي، حيث كان الأستازانتين-PLA مستقرا على أوضاع ملوحة وحموضة مختلفة، أما مع الكروسين-PVA فقد قلل وجود كلوريد الصوديوم الوضع الحمضي من استقرار اللون. تم أيضا في هذا البحث إثبات التعرض للضوء كعامل رئيسي في التأثير على تباين الألوان وثبات الصبغة.

APPROVAL PAGE

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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CAROTENOIDS: PRODUCTION FROM SHRIMP WASTE AND
SAFFRON AS POTENTIAL *HALAL* PIGMENTS**

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ACKNOWLEDGEMENTS

In the Name of Allah, the Most Compassionate, the Most Merciful. All praise and thanks be to Allah, the Lord of the Worlds. May the peace and blessings of Allah be upon Prophet Muhammad, his family and his companions. Thanks to Almighty Allah for giving me strength and the ability to complete this PhD thesis.

First of all, I owe my deepest gratitude to my supervisor, Assoc. Prof. Dr. Rashidi Othman, for his patient guidance and constructive recommendations for this research work. He always been there listening and giving countless words of encouragement throughout this journey. May Allah give His Blessing and rewards him for all valuable time, advices, and knowledge given. My grateful thanks are also extended to my co-supervisor, Asst. Prof. Dr. Norazian Mohammad Hassan, for her guidance and thoughtful knowledge of this research. Without both of you, this study would hardly be completed. Special thank goes to the Ministry of Higher Education (MOHE) for the sponsorship given in order to fulfill the *amanah* in seeking for knowledge *fisabilillah*. I hope the completion of this research will give a contribution to our *Ummah* and nation.

I wish to thank many individuals who help me for the completion of this work. I am highly indebted to Asst. Prof. Dr. Muhamad Shirwan Abdullah Sani who helped me in handling instruments in INHART Laboratory; Asst. Prof. Dr. Noorasikin Samat who providing polymer material for coating; Hannan Corporation Sdn. Bhd. and their committed staff, Sister Nuratika Hambal for providing the raw materials (shrimp waste) for this research; Sister Cicy Irna who taught me in handling HPP; Brother Deni Subara for his assistance in doing experimental design for optimization stage; Brother Akram Abdurasid who helped me in doing antimicrobial assays; for her assistance given during my lab work; and all INHART staff in assisting me throughout my study. Not to forget, my beloved friends, Sister Razanah Ramya, Sister Nurul Azlen Hanifah, Sister Nur Fadhlina Mohd Noor and Sister Alifah Md Amin for giving me hands during sample preparation. My special appreciation also goes to all my lab mates for supporting me and sharing great moments during the journey. Dear colleagues, every path have its own puddle. I pray all of you will reach the finish line, no matter what you have gone through.

I wish to thank my family, especially my beloved parents, Mr. Mohd Hatta Yahya, Ms. Robitoh Hassan, Mr. Hamzah Said and Ms. Nor Hashimah Alias for their endless love and encouragement throughout my study. The invaluable encouragement and love of these wonderful people continues motivating me to excel. Finally, I also would like to express my deep gratitude and love to my the other half, Muhammad Ammar Hamzah, who always showed his patience and supporting me during the hard time and never letting me give up. Dear husband, may Allah grant you Jannah for all your sacrifice for me. To my beloved kids, Munaa 'Umairah and Muhammad Fawwaz, this work is dedicated to you and I hope it will inspire you to become a successful *Khalifah* in the future. My deepest grateful to Allah, for giving me these wonderful kids accompanied and cheered me up along my journey. Their endless love is my motivation to complete this work.

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