CHROMATICITY, ANTIOXIDANT, AND ANTIMICROBIAL ACTIVITY OF LUTEIN AND β-CAROTENE FROM LOCAL PUMPKIN AND SWEET POTATO

BY

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ABSTRACT

Carotenoids are coloured pigments that found in nature which have their specific colour range of yellow, orange and red colours. Colour plays an important role to make sure the products are attractive to the consumers. The natural colorant is colouring that can be obtained from natural sources such as plants, vegetables, animals and minerals. This study established that the working scheme via open column chromatography (OCC) established from pumpkin and sweet potato demonstrated that isolation of the β-carotene and lutein pigment were successful with 97.2% and 95.46% purity with the coefficient of correlation of 0.9961 and 0.9959 respectively. The β-carotene extract has presented strong activity on DPPH scavenging radicals with the percentage of inhibition at 99.45% in 0.008 mg/ml concentration as compared to lutein, which contributed 65.6% at 0.5mg/ml. As for antimicrobial activity, a positive result for both β-carotene and lutein pigment for bacterial and fungal species except for *Tricho* sp. In this study, the results from chromaticity stability of PLA-β-carotene and lutein coating from 1mg/ml up to 3mg/ml treated with different pH value from 3.0 to 11.0, as well as the time of exposure from day 1 until day 15 revealed that there is no stable relationship between pH, yellow colour concentration and period of time in response to light. Marked differences were observed for PLA-β-carotene coating treated with different concentration of NaCl for 1 and 2 mg/ml concentration of carotenoid pigment for a period of time of 10 days and PLA-lutein coating treated with different temperature from -25°C to 50°C when exposed for 6 hours. In term of light exposure, PLA-β-carotene coating with lower concentration and PLA-lutein with higher concentration were found to slow the degradation process. In conclusion, this data suggests that although different carotenoid pigment concentrations had influenced chromaticity stability, light and time also affected pigment colour difference.

خلاصة البحث

الكاروتينات هي أصباغ ملونة موجودة في الطبيعة والتي لها مجموعة ألوان محددة الأصفر والبرتقالي والأحمر. يلعب اللون دورًا مهمًا في التأكد من أن المنتجات جذابة للمستهلكين. التلوين الطبيعي هو التلوين الذي يمكن الحصول عليه من المصادر الطبيعية مثل النباتات والخضروات والحيوانات والمعادن. أثبتت هذه الدراسة أن مخطط العمل باستخدام كروماتوجرافيا العمود المفتوح (OCC) الذي تم إنشاؤه من القرع والبطاطا الحلوة أظهر أن عزل صبغة بيتا كاروتين ولوتين نجحت بنسبة 97.2 ٪ و 95.46 ٪ مع معامل الارتباط بين 0.9961 و 0.9959 على التوالي . قدم مستخلص كاروتين نشاطًا قويًا على جذور النحل DPPH مع نسبة تثبيط بنسبة 99.45٪ بتركيز 0.008 ملغم / مل مقارنةً باللوتين ، مما ساهم بنسبة 65.6٪ عند تركيز 0.5 ملجم / مل. بالنسبة للنشاط المضاد للميكروبات ، نتيجة إيجابية لكل من أصباغ بيتا كاروتين واللوتين للأنواع البكتيرية والفطرية باستثناء .*Tricho* sp . في هذه الدراسة ، تمت معالجة النتائج الناتجة عن ثبات اللونية لطلاء PLA- بيتا كاروتين واللوتين من 1 ملجم / مل إلى 3 ملجم / مل مع قيم مختلفة من الرقم الهيدروجيني من 3.0 إلى 11.0 ، وكذلك وقت التعرض من اليوم الأول حتى اليوم 15 ، كشفت أنه لا توجد علاقة قوية بين درجة الحموضة وتركيز اللون الأصفر والفترة الزمنية استجابة للضوء. لوحظت فروق واضحة بالنسبة لطلاء PLA- بيتا كاروتبن المعالج بتركيزات مختلفة من كلوريد الصوديوم NaCl بتركيز 1 و 2 ملجم / مل من تركيز صبغة الكاروتينويد لفترة زمنية مدتها 10 أيام ولوحة PLA-lutein المعالجة بدرجات حرارة مختلفة من -25 درجة مئوية إلى 50 درجة مئوية عند التعرض لمدة 6 ساعات. أما من حيث التعرض للضوء ، فقد تم العثور على طلاء PLA بيتا - كاروتين بتركيز أقل و PLA- لوتين بتركيز أعلى لإبطاء عملية التحلل. في الختام ، تشير هذه البيانات إلى أنه على الرغم من أن تركيزات صبغات كاروتينويد المختلفة قد أثرت على استقرار اللونية ، إلا أن الضوء والفترة الزمنية كان لهما أيضًا تأثير على اختلاف لون الصبغة.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Halal Industry Science).

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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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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

°C	Degree Celsius
µg/g	Microgram per gram
µL	Microlitre
ANOVA	Analysis of variance
cm	Centimetre
g	Gram
g/mol	Gram per mole
HCL	Hydrochloric acid
HPLC	High performance liquid chromatography
mg/mL	Milligram per mililiter
mL	Millilitre
mm	Milimitre
NaCl	Sodium chloride
UV	Ultra-violet
UV-Vis	Ultraviolet-visible
β	Beta
Ea	Ethyl acetate
EF	Edible film
EC	Edible coating

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND STUDY

Colorants are mainly used in food, medicine, cosmetics, supplements and others. Colours play an important role as to attract the consumers to buy the products. With today's sophisticated formulations and transparent packaging, it is important to improve and maintain the product's key visual and sensorial appearance, due to consumers are preferred colour to a specific item (Chrisosto et al., 2003). By applying the colour to the products, it will help the consumers to differentiate the products easily by making different colours on the different products. It can help in imparting the colour to the variety of materials which can described as a substrate by enhancing the highly colorant on the products (Sivakumar et al., 2011). By doing that, the consumers are eager to know the real products inside the colourful packaging or the colour of the products itself. By choosing high quality colours to the products, it is one of the basis factor for successfully communicating from the brands of the product to the consumers. Colours as a means of behaviour-centred communication (Gorn et al., 2004).

For food sector, the colours play a huge role in making the attraction of food. It will influence the appetite of the consumers and so onto the choice of the foods. Recently, the foods that we take, must be attractive as to attract consumers appealing to buy the foods and achieve one of the goal in manufacturing food industry by increasing the safety and quality of the foods as to enhance the marketability of the food products (Tan et al., 2014). Due to this, the manufacturers are tending to use the various food additives including colours and flavours to improve the visual

appearance of the foods and increase the taste of the foodstuffs. (Tan et al., 2014). The primary reasons of choosing the colours to the foods include; with the proper use of colour to the products, it can influence the choices from the people itself, as influenced to the fact that, the first 90 seconds are needed by the people to make up their minds as to choose in buying the products (Singh, 2006). It also reported that, in making the choices of the foods, the environmental stimuli can be influenced by the consumer's perception itself, such as temperature, smell, distractions, wait time and the colour of the food (Stroebele, 2004). The colour of the foods play a vital role for the consumers when the foods are ready prepared for them, whether they are interested in consuming or not. Consumers are now demanded on different foods with different colours. It will result in different perspectives to the consumer's preference due to the colours being chosen to the foods, towards the food's quality (Williams, 2007).

For the pharmaceutical sector, colorants are mainly used to convey a distinctive appearance of pharmaceutical dosage forms. We also can say that, in the pharmaceutical sector, the appearance of the medicines are the cosmetics to the pharmaceutical preparations. It covers the attraction aesthetic appearance of the dosage forms and can be boost by using suitable and attractive colorants. The primary of dosage forms that are coloured are: tablets (coating or the core), soft and hard gelatin capsules (the capsule shell or coated beads), topical creams, toothpastes, ointments, and oral liquids (Allam & Kumar, 2011). The elegance and attractive products of pharmaceutical can easily attract especially for the children who often used to treat with the syrups, tablets, or capsules as to avoid injection and can be treated at home. Pharmaceutical preparations are made for this reasons: The unattractive medicine can be attractive if the colour are chosen correctly and the

preparation of the batch to batch variation can be make differently as to increase and smooth the production. This will help for the patients to describe and alert on the colour of their own medicines. This will help in contributing the patience compliance (Allam & Kumar, 2011).

1.2 PROBLEM STATEMENT

Non-Halal, synthetic colorant and food additives are widely being used, especially in our country and has being affected to the consumers itself, especially in health. Non-halal colorants can be considered as a product that are not applying the cleanliness based on Shariah perspective and might use the non-halal ingredients for food colorants. For the synthetic colorant, the impact towards the health has being overly attached and can caused a severe damage to the body, especially when it comes to the oral application. This is due to the color can make magnificent attraction of foodstuffs, which can change the preference influences, the eating desires and selection towards the food that need to consume (Delgado-Vargas & Paredes-Lopez, 2003; Shim et al., 2011). For the food additives used, it has been banned in several countries due to the side effects and high toxicity which can cause to short-term and long- term effects. From the organization itself, Food and Drug Administration (FDA) and European Food and Safety Authority (EFSA), they have promoted the health issues due to protect the quality and safety of the food products as to make sure the human health being preserved (Amchova et al., 2015; Carocho et al., 2014).

1.3 RESEARCH OBJECTIVES

In order to achieve the research aims, the following objectives have been formulated:

1. To isolate β-carotene and lutein from local pumpkin and sweet potato
2. To determine antioxidant and antimicrobial activity of β-carotene and lutein obtained from objective 1.
3. To determine chromaticity and stability of β-carotene and lutein obtained from objective 1.

1.4 RESEARCH QUESTIONS

1. Which natural plant resources can be used as biomass for mass production of beta carotene and lutein?
2. What are the best concentration having high activities of antioxidant and antimicrobial?
3. How stable β-carotene and lutein pigments towards extreme microclimate?

1.5 RESEARCH HYPOTHESIS

β-carotene and lutein are potential to be used as food colorant and coating agent for food industry or others related in halal industry.

1.6 SIGNIFICANCE OF THE STUDY

The findings from this study will be benefited to halal food and beverages, pharmaceutical, cosmeceutical and nutraceutical industries. Natural colorants especially from plant pigment are the best and safe to consume.

1.7 RESEARCH SCOPE

Only β-carotene and lutein will be extracted from two natural plant resources namely sweet potato and pumpkin. Stability test will be conducted only at certain parameters namely heat, pH, salinity and light intensity for four weeks' period of time. Only food borne microbes will be tested for antimicrobial activities and DPPH assay for antioxidant properties.

CHAPTER TWO

LITERATURE REVIEW

2.1 PROPERTIES AND FUNCTIONS OF CAROTENOIDS

Carotenoids form one of the most important classes of plant pigments and play a crucial role in defining the quality parameters of fruit and vegetables (Van den Berg et al., 2000). Carotenoids are responsible for many of the red, orange, and yellow hues of plant leaves, fruits, and flowers, as well as the colours of some birds, insects, fish, and crustaceans. Carotenoids are considered as a natural coloured pigment which play a vital role towards the photosynthesis of plants, photosynthetic bacteria and algae. It can also be found in some of the non-photosynthetic bacteria, yeasts and crustaceans (Chandrika, 2009). The colorant pigment from the animals can be produced in a small scale due to the animals cannot be synthesize their own carotenoids. In addition, the familiar examples of carotenoid coloration come from the oranges of carrots, citrus fruits, the pinks from flamingos and salmon, and the reds of peppers and tomatoes (Pfander, 1992; Omayma & Abdel, 2013). The colorant from animal's carotenoids are allowed as a colorant.

Carotenoids can be divided into two parts, which is hydrocarbon carotenes (contain carbon and oxygen) and oxygenated xanthophylls (contain carbon, oxygen, and hydrocarbon). Carotenoids have a large value of applications especially as anti-oxidants in dietary intake supplements, as potential colours in foods and beverages and also popular in poultry and fish sector which work as pigments. Over 600 carotenoids have been found in nature, 40 are present in a typical human diet itself and only 14 from them considered as metabolites that have been identified in blood and tissues (Bendich A. 1993; Omayma & Abdel, 2013).

2.1.1 Carotenoids Role as Pro-Vitamin A

Vitamin A (retinol) is an essential nutrient needed in small amounts by humans for the normal functioning of the visual system; growth and development; and maintenance of epithelial cellular integrity, immune function, and reproduction. It will help in maintaining the eye health and promotes growth and development. If the intake of vitamin A would not sufficient to the body itself, it will cause the body systems not functioning well. Other than that, it will help in maintaining healthy bones and teeth. Furthermore, it will enhance the protection and regeneration of cells and mucous membrane. On top of that, vitamin A will maintains healthy respiratory and intestinal tracts which can prevent the constipation. For hair, nails, and skin, it will maintain if the intake of vitamin A in a balanced diet is sufficient.

Pro-vitamin A is one of the established function of carotenoids in the human diet that is related to β- carotene. Apart from that, α-carotene and β- cryptoxanthin also play their own role of carotenoids, but less than β-carotene (Van den Berg et al., 2000). β-carotene also give the best health benefits part in the dietary carotenoids, which it is come up with the strong dietary precursor of vitamin A, and it is one of the recognizable in human and animal nutrition (Van den Berg et al., 2000; Bramley, 2002). By consuming natural sources of vitamin A, it will not be risking in any toxicity.

2.1.2 Carotenoids as Antioxidant

Carotenoids work best in antioxidants, which from the plants that shows their interesting parallels as one of the potential antioxidants in humans and foods (Van Den Berg et al., 2000). It shows when, the singlet oxygen quenching and their ability to trap peroxyl radicals that make up as their best documented properties (Stahl &

Sies, 1996; Omayma & Abdel, 2013). The activity of quenching from the carotenoids are mainly concerned from the conjugated double bonds of the molecules which is influenced to a lesser extent by carotenoid end groups (cyclic or acyclic) or towards the nature of substituents in carotenoids that have cyclic end in the groups (Krinsky, 1998; Omayma & Abdel, 2013). For the carotenoid pigments, they are very widely distributed in nature, and play an important role in protecting cells and organisms, as to against the effects of light, air, and sensitizer pigments. This process is occurred in bacteria, algae, plants, animals, and also humans. Antioxidant can be both in enzymatic and non-enzymatic system, due to the complex network of the organism. For the antioxidant property of carotenoids, it is formed from the double carbon-carbon bonds which is connecting to each other through conjugation and causing electrons to move freely in the molecule.

In addition, many reports have mentioned that, carotenoids can retain some anti-carcinogenic properties due to their ability to interact and quench various radical species that can be generated within the cells. Carotenoids also one of the pigments that play a major role in the plants, which it helps in the protection of plants against photo-oxidative processes, due to the efficient antioxidants scavenging singlet molecular oxygen and peroxyl radicals. Other than that, for human organism, carotenoids are part of the antioxidant defence system, which they can interact symbiotically with other antioxidants considering the mixtures of carotenoids are more effective as compared to the single compounds.

2.1.3 Carotenoids in the Macular Region of the Retina

One of the most sensory organ that need to be taken care is the eye which can promote a healthy and encourage the productive lifestyle. Based on the previous studies, the