ANTIOXIDANT, ANTIMICROBIAL AND COLOUR STABILITY OF CURCUMIN-RICH *CURCUMA LONGA* EXTRACT IN FREE STATE AND IN POLY LACTIC ACID POLYMERS

BY

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ABSTRACT

Turmeric has been a popular spice throughout South Eastern Asia from earlier times which even until now are used in culinary as spice, traditional medicine, and even as dye. Its iconic yellow colour is caused by a compound called curcumin, which is known to have anti-inflammatory, antioxidant, and antimicrobial properties aside from various other health benefits. This research sought to investigate the potential of curcumin, which was extracted from turmeric (*Curcuma longa*) rhizome as a '*Halalan Toyyiban'* alternative for synthetic colourant while studying its antioxidant and antimicrobial properties. The curcumin was extracted by using two extraction method, which then characterised and quantified using HPLC analysis. The extracted curcumin was tested for antimicrobial activity using the disc diffusion test, and tested for antioxidant property using the DPPH radical scavenging assay. The extracted curcumin, in both free state and in PLA polymer, was also tested for chromaticity stability where its colour changes was observed and analysed using the CIELab colour space system after it was exposed to different environmental factors as an indicator of its stability. The results showed that alkaline extraction produced higher curcumin yield compared to chemical based extraction. The extracted curcumin showed positive antimicrobial activity towards most of the tested microbes and the same inhibition activity for antioxidant test as shown from previous studies. The stability test using CIELab colour system showed that curcumin in both free state and in PLA polymer is very vulnerable towards degradation under the exposure of light, compared to any other environmental factor it was exposed to in this research. These results showed that curcumin retained, if not improved, its antimicrobial and antioxidant properties after being extracted from rhizome of *Curcuma longa*, and was easily degraded under the exposure of light.

ملخص البحث

كان الكركم التوابل مشهورة في جميع أنحاء جنوب شرق آسيا من العصور السابقة والذي نستخدمه حتى الآن في المطبخي كما التوابل والطب التقليدي، وأيضا الصبغة. ولونه الأصفر الايقوني هو ينتج عن مركب يسمى الكركمين، المعروف بأنه مضاد للالتهاب، ومضادات الأكسدة، والخصائص المضادة للميكروبات جانبا من مختلف الفوائد الصحية الأخرى. وسعى هذا البحث للتحقيق في إمكانات الكركمين، الذي تم استخلاصه من الكركم (كركم لونجا) رهيزومي كبديل "حلالا طيّبًا" للألوان الاصطناعية أثناء دراسة خصائصه المضادة للأكسدة والمضادة للميكروبات. وتم استخراج الكركمين باستخدام طريقتين للإستخراج، والتي تتميز وكميا باستخدام تحليل HPLC. وبالإضافة إلى ذلك، قام تماما إختبار الكركمين المستخرج للنشاط المضاد للميكروبات باستخدام الإختبار إنتشار القرص، وإختبارها لملكية مضادة للأكسدة باستخدام DPPH الكسح الجذور مقايسة. الكركمين المستخرج في كلتا الحالتين الحرة وأيضا في بوليمر PLA ، لاستقرار اللونية حيث لوحظ تغير لونه وتحليله باستخدام نظام الفضاء الملون CIELab بعد تعرضه لعوامل بيئية مختلفة كمؤشر لاستقراره. وأظهرت النتائج أن استخراج القلوية تؤدي إلى أعلى إنتاجية الكوركمين مقارنة بالإستخلاص الكيميائي. أثبت الكركمين المستخرج نشاطًا إيجابيًا مضادًا للميكروبات تجاه معظم الميكروبات التي تم اختبارها ونفس النشاط المضاد لإختبار مضادات الأكسدة كما هو موضح من الدراسات السابقة. وأبرز إختبار الاستقرار باستخدام نظام الألوان CIELab أن الكركمين في كل من الحالة الحرة وفي بوليمر PLA معرض بشدة للتدهور تحت التعرض للضوء، مقارنة بأي عامل بيئي الآخر تعرض له في هذا البحث. وأظهرت هذه النتائج أن الكركمين هو الاحتفاظ، إذا لم يكن تحسين، خصائصه المضادة للميكروبات ومضادات الأكسدة بعد استخراجها من رهيزومي الكركم لونغا (*curcuma longa*) ، وكان يتحلل بسهولة تحت التعرض للضوء.

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 in Science (Halal Industry Science)

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Turmeric (*Curcuma longa*) is a popular spice which is used extensively in most Asian countries as food preservatives, colouring, and even used as traditional medicine to treat and cure various illnesses. It is known to have anti-inflammatory, antimicrobial, and anti-cancer properties (Chattopadhyay *et al.*, 2004). Turmeric plant is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae. It is a native plant of the Southern Asia which requires a large amount of annual rainfall to thrive. The optimum temperature range for turmeric plant growth is between 20ºC and 30ºC (Govindarajan & Stahl, 2009). Turmeric has always been an important ingredient in Asian culinary until today. It is used as spice and it gives yellowish to orangey hue or colour to food. In the medical field, the usage of turmeric is popular in Ayurvedic practices, an ancient Indian health practice. Even in common traditional medicine, turmeric is applied on skin to relieve inflammation due to its anti-inflammatory effects. Other than that, turmeric is also used as a dye (Shameem & Prathapan, 2014). There are a lot of compounds found in turmeric that are beneficial for both consumption and industrial purposes. Among them is lectin, a special group of sugar-binding proteins with antimicrobial properties, where it was found in a study that it has the potential for lectin-mediated drug delivery (Biswas & Chattopadhyaya, 2014). This research, however, would focus on the principle curcuminoid inside turmeric which gives turmeric its colour-curcumin.

1.2 PROBLEM STATEMENT

Recent toxicology studies in European countries have raised people's awareness and concern in regard to the uses of synthetic colourant in food. The main concern is that it might have been related to the cause of attention-deficit hyperactivity disorder (ADHD) in children (Amchova *et al*., 2015). Some of the most commonly used synthetic food colourants are even suspected to be carcinogenic (Martelli *et al*., 2014) which can possibly lead to other detrimental health problems to health-sensitive people (Vazhangat & Thoppil, 2016). In Islamic teachings, this part obviously reflects the lack of *toyyiban* element in the usage of the synthetic colourant.

Apart from the food itself, there are also issues with the packaging they come with another problem related to synthetic colourant is the general development of food technologies. Large-scale production of food-related industries had widen the opportunity to mix and include doubtful ingredients into the production lines. To tackle this problem, halal food industries are advised to aim for the *toyyiban* aspect of food production to remove uncertainty and enhance the quality of Muslim products (Kamali, 2013).

On the other hand, natural colourants are seen to be good alternatives for the synthetic ones. Despite of being abundant in nature (Ngamwonglumlert *et al*., 2015) and having a lot of health benefits, natural colourant application is limited in term of its instability, possible interactions with food ingredients, and hues inconsistencies (Sigurdson *et al*., 2017). Moreover, further research is still needed to develop a better method of extraction that is cost-effective, so the natural colourants can be produced in large-scale and widely applied (Shahid *et al*., 2013).

1.3 MAIN OBJECTIVE

To assess the potential of curcumin properties as natural polymer coating agent towards environmental stability, microbial activities and antioxidant activities.

1.4 RESEARCH OBJECTIVES

The study aimed to achieve the following objectives:

1- To produce a curcumin-rich extract from rhizome of *Curcuma longa*;

2- To determine antioxidant and antimicrobial of extract from objective 1; and

3- To determine chromaticity stability of free curcumin-rich extract incorporated into polylactic acid polymers

1.5 RESEARCH QUESTIONS

1. How much curcumin can be extracted from raw turmeric using chemical extraction method?
2. What are the environmental key factors that influence the curcumin stability?
3. What are the best formulations to integrate curcumin as natural polymer coating agent?

1.6 RESEARCH HYPOTHESIS

Curcumin can be used as Halal natural biocolourant and can be integrated into polymers with the right formulation as stable coating agent.

1.7 SIGNIFICANCE OF THE STUDY

This study would show the potential of natural colourant, specifically curcumin, as an excellent substitute of the synthetic colourant which has been claimed to cause various detrimental health effects on the consumers.

The study would also demonstrate the possible yield of the extraction of curcumin from raw turmeric by using different methods of extraction using total carotenoid calculation. It would also emphasise on the antimicrobial and antioxidant properties of curcumin which are a very attractive added value to a colourant especially to the food industry as it could increase the shelf life of food products. Furthermore, this study will show the chromaticity stability of curcumin under a range of environmental factors which results could be used to show the best condition for curcumin to be used as colourant.

1.8 SCOPE OF THE STUDY

This research would only be focusing on curcumin extracted from turmeric through chemical-based and alkaline extraction. As for the antimicrobial activities, they would only focus on food borne microbes including some common fungi species, and stability test would only be tested on one type of polymer which was polylactic acid (PLA). Only four environmental variables which were used in this research, which were heat, pH, salinity, and ultraviolet (UV) light exposure.

1.9 CHAPTER SUMMARY

This chapter has presented and discussed the background of the study. It was also explained why it is crucial to find an alternatives for synthetic colourant and why natural colourant is the best candidate for that. Additionally, the statement of the problem was

discussed where some of the previous studies related to the bad effects of synthetic colourant on both human and environment were mentioned. This was followed by the discussion of the need for an alternative in accordance to the Islamic perspective and justification. The research questions, hypothesis and objectives were also listed and specified. It was followed by the significant of the study which highlighted on how this study would fill the gap in research literature in relation to natural colourant (curcumin) and its chromaticity stability when applied as colourant. Finally, the scope of the study was mentioned.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

Since the study aimed to assess the potential of curcumin properties as an alternative for synthetic colourant, it is important to know the application of colourants in industries, the comparison between two main types of colourants which are synthetic and natural colourants, and also their effects - both good and bad. This review would also discuss the issue of synthetic colourant from the Islamic perspective, emphasising on the *Halal* and *Toyyiban* aspects. Furthermore, the review would also focus on the properties of curcumin that exhibited its potential to be an alternative for synthetic colourant.

2.2 SYNTHETIC COLOURANT

Synthetic colourants are laboratory made copies of vegetables, animals and mineral-based colourants. These dye that are derived from petroleum are also commonly known as coal tar dye. They are used extensively in food, textile, cosmetics, and personal care products. They are used in food to restore the original appearances of processed food that have lost their natural colour during processing treatment (Carocho *et al*., 2014). In effect of the recent toxicology studies in Europe, the popularity of synthetic dye is slowly declining as they are suspected to have detrimental effects on children's behaviour such as hyperactivity (Amchova *et al*., 2015). Synthetic colourants are even suspected to be carcinogenic (Abd El-Wahab & Moram, 2013). One of the toxicology concern is the finding that several colourants have the ability to bind to human serum albumin (Amchova *et al*., 2015). Hassan (2010) had conducted a study that showed the

effect of tartazine and chocolate brown synthetic food colourant on the gene of rats. The results showed that both colourants caused DNA liver and kidney damages as detected by comet assay. A similar test was carried out using another range of colourants namely carmoisine, trans-anethole, tartrazine and vanillin. The results also showed a significant decrease in body weight, haemoglobin concentration and red blood cell count on colourant-added food diet (Abd El-Wahab & Moram, 2013). Synthetic dye is also a threat to the environment. In the year 2004, it was estimated that over 10,000 tons of dye waste was produced every year. The number has increased to over 700,000 tons per year in 2011, where 10%-15% of them were discharged in wastewater (Sun *et al*., 2011). They also caused a significant environmental pollution in addition of having the potential to be a health-risk (Forgacs *et al*., 2004). Due to these concerns, there are a lot of research being done to find a way to remove these contamination from water bodies. One of the solutions found from the studies was the usage of enzymes to decolorise and detoxify textile and other synthetic dye (Husain, 2006), and magnetic/reduced graphene oxide nanocomposites (Sun *et al*., 2011).

2.3 NATURAL COLOURANT

Due to the concerns over the health effects caused from the usage of synthetic colourants, natural colourants have seen a rise in popularity. Not only that they are abundant in nature, they are also varied and can be found from different sources such as plants, insects and even microbes. Moreover, they have a lot of potential health benefits which is the reason why more and more studies are being conducted on them (Rodriguez-Amaya, 2015). In one study alone, plant pigments were extracted, quantified and characterised from 24 different species of traditional vegetables (*ulam*) in Malaysia. Those pigments were identified - using HPLC as neoxanthin, violaxanthin,

lutein, zeaxanthin, β- cryptoxanthin, α-carotene and β-carotene, all from a group of compound called carotenoids (Othman *et al*., 2015). However, natural colourants do have their own disadvantages, especially on its extraction and application aspects. On application, natural colourants were shown to have low stability, weak colour strength and difficulty in expressing the desired hues (Sigurdson *et al*., 2017). The conventional method to extract natural colourant include Soxhlet extraction, maceration and hydrodistillation. Several non-conventional extraction methods have also been developed which include supercritical fluid extraction (SFE), ultrasound-assisted extraction, microwave-assisted extraction, pulsed-electric field extraction, enzyme-assisted extraction, pressurised liquid extraction, and ultrasound-sound extraction (Ngamwonglumlert *et al*., 2015). Natural pigments can also be used as dye in textile industries. The best attribute it would have against synthetic dye would be its biodegradable trait and it is environmentally safe as waste (Yi & Cho, 2007).

2.4 ANTIOXIDANT

Antioxidants are compounds that are able to delay or inhibit the processes of oxidation which occur with the presence of atmospheric oxygen or reactive oxygen species. Apart from playing an important role in the defense mechanism of organisms against the danger of free radicals and its pathological association, antioxidants are also applied in various commercial purposes, namely in stabilization of polymeric products which includes petrochemicals, cosmetics, pharmaceuticals, and even foodstuffs (Pisoschi & Negulescu, 2011).

2.5 CAROTENOID

Carotenoids are groups of compound comprising over 600 plant pigments which contribute to the various natural colours we see in them, such as the red in tomatoes, and orange in carrots (Krinsky & Johnson, 2005). They are well known for their provitamin A properties, specifically β-carotene that are mostly found in carrots, and a lot of other health benefits (Carvalho *et al.,* 2012). Structurally speaking, carotenoids are essentially derived by base structure modification through end group cyclisation, and oxygen functions introduction which gives them their unique colours along with their antioxidant properties (Rao & Rao, 2007).Carotenoids are also well known to be antioxidant, and some are popular choices in industrial applications such as ß-carotene, lycopene, lutein, astaxanthin and zeaxanthin (Anunciato and Filho 2011). This property has made carotenoids to be popular not only in food, but also cosmetic industries. One of the reasons behind the said popularity is that carotenoids can protect against the oxidative effect of UV radiation, a property called photoprotective (Pandel *et al.,* 2013). A study conducted by Scarmo *et al.* (2010) emphasised the ability of lycopene and ß-carotene to act as natural skin photoprotection, which if compared to lutein and zeaxanthin, they have a higher level of presence or amount in human skin of the population of the United States although both lutein and zeaxanthin have been proven through clinical trials to contribute in skin photoprotection, skin elasticity, and skin lipid peroxidation (Palombo *et al.* (2007). The consumption and physical application showed the highest antioxidant protection for lutein and zeaxanthin. Another study by Saba (2016) had stated that the consumption of ß-carotene at adequate level not only provides glow to skin, but can also prevent hair loss and dandruff problems.

2.6 CAROTENOID POTENTIAL AS *HALALAN TOYYIBAN* BIOCOLOURANT

Halal in Islamic teaching basically means permissible and the term is usually mentioned in regard to food. There are actually only a few things that are prohibited or '*Haram*' food in Islam. It is stated in the Qur'an:

> *"He hath only forbidden you dead meat, and blood, and the flesh of swine, and that on which any other name hath been invoked besides that of Allah…"*
>
> (Surah al-Baqarah: 173)

> *"Forbidden to you (for food) are: dead meat, blood, the flesh of swine, and that on which hath been invoked the name of other than Allah. that which hath been killed by strangling, or by a violent blow, or by a headlong fall, or by being gored to death; that which hath been (partly) eaten by a wild animal; unless ye are able to slaughter it (in due form); that which is sacrificed on stone (altars); (forbidden) also is the division (of meat) by raffling with arrows: that is impiety…"*
>
> (Surah al-Ma'idah: 3)

Allah had made everything permissible or *Halal* to be consumed, except those that are prohibited in the holy Qur'an and through tradition of the prophet Muhammad peace and blessings be upon him (P.B.U.H.). As mentioned in the Qur'an:

> *"This day, all things good and pure have been made lawful for you"*
>
> (Surah al-Ma'idah: 6)

> *"O ye people! Eat of what is on earth, Lawful and good;"*
>
> (Surah al-Baqarah: 168)

In the above translation, *toyyiban* is translated as good. In another word, Allah has commanded us to find food that are wholesome, not just permissible (*halal*).

According to the Islamic perspective, synthetic colourants lack the *toyyiban* aspect. We can see in a verse in the Qur'an which stated:

> *"...and make not your own hands contribute to (your) destruction; but do good; for Allah loveth those who do good."*
>
> (Surah al-Baqarah: 195)

The verse clearly stated that human should not in any way harm him/herself. Consuming synthetic colourants even after knowing all of its detrimental effects would go against this command. Therefore, a need for a better and healthier alternative is required to replace the commercially available synthetic dye to preserve the well-being of the *ummah*.

2.7 *CURCUMA LONGA*

4.1.1 Botany

The turmeric plant, or its scientific name, *Curcuma longa*, consists mainly of its shoots and roots. The roots are fleshy and usually bear ellipsoid tubers. The leafy shoots, with the total height of 1 to 2 metre, bear a group of leaves surrounded by bladeless sheathes which form a pseudostem. The leaf blades are often relatively erect (Holttum, 1950).

The rhizome of turmeric comprises of two parts. The central part is the bulb and its lateral or the pear-shaped "mother rhizome". The other part is its axillary branches which are also known as the "fingers". There is usually only one main axis which will develop into the aerial leafy shoots. The seed rhizome or the planting unit, comprises of either a whole finger or a bulb and usually produces only one main axis. The main axis' base will enlarge and become the first formed part of the rhizome, the bulb or mother rhizome. The following development of the axillary buds from the main axis' lower nodes will trigger the bulb to undergo branching. These axillary buds on growth will

produce the first-order branches, often named the primary fingers. The amount of primary fingers can often varies from 2 to 5. The primary branches will develop to some length and then either grow into an aerial shoot or halt any further growth. The primary fingers, which grow in different directions, can sometimes grow up to the ground level with a single, a few, or even without any foliage leaves. The secondary branches which grow at higher nodes of the primary branches are diageotropic (Shah and Raju, 1975). Some primary branches do not form any aerial shoot after reaching the ground level, but show positive geotropic growth instead. The primary fingers go through further branching, generating secondary and tertiary branches, and these branches do not grow aerial shoots. Most of them show positive geotropic growth or diagonally downward growth.

4.1.2 History

There are over 100 species in the turmeric genus, but the species *Curcuma longa* (turmeric) of the family Zingiberaceae, also commonly known as "*Haldi*" or "*Holud*", is renowned for being the most studied species of the genus (GRIN Database, 2005; Curcuma list, 2005). The name of the genus Curcuma which is now used to define turmeric originally meant "saffron" in Arabic. Most of the species of this genus requires hot and moist climate with a fairly light soil are perennials and grow well in tropics and subtropics. Turmeric can be found and easily grown in South Eastern Asia and are cultivated extensively in Bengal (Bangladesh and India), Sri Lanka, China, Taiwan, Peru, Indonesia, Australia, and the West Indies. However, India is the world's main producer of the commercially most essential species, *C. longa*.

The establishment of the genus Curcuma was by Linnaeus (1753). The generic nickname is derived from the Arabic word for yellow, "*karkum*", referring to the yellow