SPRAY DRIED PRODIGIOSIN FROM *Serratia marcescens* AS A FOOD COLORANT

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To my beloved mother and father

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ABSTRACT

Pigments from microorganisms can serve as an alternative source to replace synthetic pigments used in the food industry. Natural pigments have some limitations including solubility, sensitivity and short stability upon exposure to light, pH and high temperature. Thus, encapsulation *via* spray drying can be employed to enhance the pigment's properties. In this study, spray-dried microcapsules containing red pigment (prodigiosin) extracted from *Serratia marcescens* was produced using κ-carrageenan and maltodextrin as encapsulation agents. The effect of spray-drying parameters on the encapsulation yield (EY), particle size, moisture content and colour intensity of the prodigiosin microcapsules at different ratios of prodigiosin/encapsulation agent were studied. The most intense colour was obtained for the 1:1 ratio (volume ratio of prodigiosin in ethyl acetate to κ-carrageenan solution) using 200^{o}C inlet temperature, feed flow rate of 60 m^{3}/h, air pressure of 1.5 bar and feed rate of 3 mL/min. The encapsulated pigment is most stable in powder form at 0°C when stored in the dark, and thus has superior stability compared to pigment in its free form. Characterization of spray-dried prodigiosin using FTIR and FESEM confirmed that the particles were properly coated with encapsulating agents. The morphology showed the particles were regular shaped spheres with mean diameters between 0.5μm and 5μm. Finally, the particles were successfully applied to milk, yogurt and carbonated drinks. The results suggest that the spray-dried prodigiosin can be useful as a food colorant under the above optimum operating conditions.

ABSTRAK

Pigmen yang diekstrak daripada mikroorganisma boleh dijadikan sebagai sumber alternatif bagi menggantikan pigmen tiruan dalam industri pemakanan. Pigmen semulajadi mempunyai beberapa kelemahan termasuk keterlarutan, kepekaan dan kestabilan yang rendah terhadap cahaya, pH dan suhu yang tinggi. Justeru itu, pengkapsulan melalui pengeringan sembur boleh digunakan bagi menambahbaik ciri pigmen tersebut. Dalam kajian ini, pemikrokapsulan secara pengeringan sembur bagi pigmen merah (prodigiosin) yang diekstrak daripada *Serratia marcescens* telah dihasilkan dengan menggunakan κ-karagenan dan maltodekstrin sebagai agen pengkapsulan. Kesan pengeringan sembur terhadap beberapa parameter seperti hasil pengkapsulan, saiz zarah, kandungan kelembapan dan keamatan warna prodigiosin yang telah dikapsulkan pada nisbah prodigiosin / agen pengkapsulan yang berbeza telah dikaji. Keamatan warna yang paling tinggi diperolehi pada nisbah 1:1 (nisbah isipadu prodigiosin di dalam etil asetat terhadap larutan κ-karagenan) dengan menggunakan suhu masukan 200°C, kadar aliran suapan 60 m^3 / h, tekanan udara 1.5 bar dan kadar suapan 3 mL / min. Prodigiosin yang telah dikapsulkan lebih stabil dalam bentuk serbuk pada 0°C apabila disimpan di tempat yang gelap dan kestabilannya lebih baik berbanding pigmen yang tidak dikapsulkan. Pencirian serbuk prodigiosin menggunakan FTIR dan FESEM mengesahkan bahawa zarah prodigiosin telah disalut sepenuhnya dengan agen pengkapsulan. Analisis morfologi menunjukkan partikel prodigiosin berbentuk sfera dan berdiameter di antara 0.5μm dan 5μm. Akhir sekali, serbuk prodigiosin tersebut telah berjaya diaplikasikan pada susu, yogurt dan minuman berkarbonat. Hasil kajian mendapati serbuk prodigiosin boleh digunakan sebagai pewarna makanan dengan menggunakan parameter optimum yang tersenarai di atas.

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LIST OF ABBREVIATIONS / SYMBOLS

[A]	-	Absorption
B.subtilis	-	*Bacillus subtilis*
Ch.violaceum	-	*Chromobacterium violaceum*
cm	-	Centimeter
DDW	-	Dionized Distilled Water
E.coli	-	*Escherichia coli*
EY	-	Encapsulation yield
FESEM	-	Field Emission Scanning Electron Microscope
FTIR	-	Fourier Transform Infrared Spectroscopy
g	-	gram
HCl	-	Hydrochloric acid
KBr	-	Potassium Bromide
kV	-	Kilo voltage
k	-	Degradation rate
κ-carrageenan	-	Kappa carrageenan
MC	-	Moisture content
mL/min	-	Milliliter per minute
m^3/h	-	Cubic meter per hour
mg	-	Milligram
NA	-	Nutrient Agar
NaoH	-	Sodium Hydroxide
nm	-	Nanometer
NB	-	Nutrient Broth
°C	-	degree Celsius
OD	-	Optical Density

OD_{540}	-	Optical Density at 540 nm
pH	-	$-\log[H^+]$
S.aureus	-	*Staphylococcus aureus*
rpm	-	Rotation per minute
$t_{1/2}$	-	Half life
UV-VIS	-	UV Visible spectroscopy
w/v	-	Weight per volume
µg	-	microgram

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Natural pigments have been used to replace synthetic dyes in recent decades. However, undesirable properties of natural pigments such as solubility and short term stability limit their application in the food industry (Tripathi *et al.,* 2007; Ersus and Yurdagel, 2007).

In order to make natural pigments widely applicable and comparable to synthetic pigments, some properties of natural colorants need to be modified. This is done *via* techniques such as encapsulation which will improve the properties of substances. Encapsulation is a technique for preservation of natural pigments by entrapping the ingredient in coating materials (Nedovic *et al*., 2011).

Among encapsulation methods, spray drying is the most common process employed in the food industry due to feasible and economic operation. By employing the spray drying method the replacement of natural pigments with synthetic colorants is possible in the food industry (Bono *et al*., 2012).

1.2 Problem Statement

Pigments from organisms have been shown to have biological activities and potential health benefits. Prodigiosin is a natural pigment responsible for the red

colour in *Serratia marcescens*. Characteristics such as antimicrobial, antitumor and antibiotic of prodigiosin make this natural pigment appropriate for medical applications. Besides unique characteristics of prodigiosin, this pigment is applicable as a colorant in foodstuffs.

However, prodigiosin have some disadvantages such as low solubility and rapid colour fading upon exposure to sunlight, high temperature and pH. Some physical methods *via* forming a barrier for sensitive substances may provide resistance toward factor that contribute to colour changes. Spray drying is a frequently applied encapsulation method in food industry. This method offers an effective procedure to cover an active compound with a protective wall material. Various advantages of spray-dried natural pigments make their properties sufficient to be used as food colorants.

1.3 Objectives of Study

The objectives of the research are:

1. To evaluate the use of encapsulating agents for spray drying of prodigiosin.
2. To evaluate the spray-dried pigment in terms of stability and storage.
3. To apply the encapsulated pigments in food (yogurt, milk and carbonated drink).

1.4 Scope of Study

Encapsulation of prodigiosin from *Serratia marcescens* using κ-carrageenan or maltodextrin as coating materials is one of the scopes of this study. The parameter optimization of spray dryer i.e. inlet and outlet temperature, feed rate, air flow rate and air pressure will be performed using several spray-drying runs. The encapsulated prodigiosin will be evaluated based on the Encapsulation Yield (EY), Moisture Content (MC) and intensity of the colour. This will be followed by characterization

of the encapsulated pigment using Field Emission Scanning Electron Microscopy (FESEM) and FTIR spectroscopy. Stability and optimum storage conditions of the encapsulated pigments will be tested upon exposure to different factors i.e. pH, temperature and light. The antimicrobial properties of the encapsulated pigments against gram negative and gram positive bacteria will also be studied using the spread plate method. Finally, the encapsulated pigment will be applied in food models such as milk, yogurt and carbonated drinks.

1.5 Significance of Study

The significance of this study is to achieve a more stable and environmentally friendly food grade pigment from microbial sources. Employing simple and cheap technique such as spray drying makes the pigment comparable to synthetic dyes in term of performance and price.

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