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EXTRACTION AND SPRAY-DRYING OF ANTHOCYANIN FROM SEEDS OF TWO SPECIES OF KUNDANG, *Bouea macrophylla* Griffith AND *B. oppositifolia* (Roxb.) Adelb

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By

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EXTRACTION AND SPRAY-DRYING OF ANTHOCYANIN FROM SEEDS OF TWO SPECIES OF *KUNDANG*, *Bouea macrophylla* Griffith AND *B. oppositifolia* (Roxb.) Adelb

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Anthocyanin gain attention recently due to its potential health benefits and as an alternative source to synthetic colourants. Pinkish-purple seed of Kundang (*Bouea macrophylla*) can be a good source of natural colourant. Since information on the extraction of anthocyanin from Kundang seeds is scarce, therefore the objectives of this research were (i) to evaluate the effects of different solvents on the extraction of anthocyanin from Kundang seeds and (ii) to optimise spray-drying condition of *B. macrophylla* seeds extract. In the first part of this study, five different solvents [distilled water, absolute ethanol, aqueous ethanol (70%), acidified aqueous ethanol with acetic acid (pH 1), and acidified aqueous ethanol with hydrochloric acid (pH 1)] were used to extract anthocyanin from two different species of Kundang (*Bouea macrophylla* and *Bouea oppositifolia*) to explore its potential as natural colourant with anthocyanin and antioxidant properties. Proximate compositions of the Kundang seeds were identified prior to the extraction, where the moisture, fat, protein, ash, and carbohydrate content were in the ranged of 9.82-14.87%, 0.67-1.15%, 5.13-5.74%, 1.19-1.50%, and 77.82-82.10%, respectively. The results obtained indicated that type of solvents used tend to influence the extractability of anthocyanin and antioxidant compounds of the Kundang seeds. However, acidified solvents, regardless of the acid type, showed the most prominent results for both species of Kundang. For *B. macrophylla* seeds, aqueous ethanol with HCl produced extract with the highest extraction yield (17.5%), *a* (66.30) and *b* (17.51) values, total monomeric anthocyanin (TMA, 1.2 mg cyanidin-3-O-glucoside/g extract), total flavonoid content (TFC, 6.58 mg RE/g extract), and DPPH scavenging activity (82.22%) compared to other solvents ($p<0.05$). Besides, the *B. macrophylla* seeds contained cyanidin-3-O-glucoside, malvidin-3-O-glucoside, and peonidin-3-O-glucoside. This finding could provide insight into *B. macrophylla* seeds as a novel source of natural anthocyanin food colourants with antioxidant benefits.

Since anthocyanins are unstable during processing, the study on spray-drying of *B. macrophylla* seeds extract was conducted and the sensory evaluation acceptability of 'agar-agar' with the spray-dried seeds extract powder was determined in the second part of this study. The central composite design (CCD) of the response surface methodology (RSM) was performed to optimise the spray-drying of *B. macrophylla* seeds extract and to analyse the effect of inlet air temperature (160-180 °C) and maltodextrin (DE10) concentration (3-17%, w/w) on the powder characteristics. Significant response surface models with high coefficients values (R^2>0.910) for the solubility (0.979) was obtained from the experimental data. Sensory analysis on the acceptability of 'agar-agar' that contained spray-dried powder obtained at 170 °C of the inlet air temperature and maltodextrin (DE10) concentration at 10% (w/w) was carried out by 30 untrained panellists. A non-significant difference ($p>0.05$) was observed in terms of the overall aroma, texture, flavour, and acceptability except for the overall colour, whereby commercial colouring powder (CCP) had higher acceptance in colour compared to spray-dried *B. macrophylla* seeds extract powder (SSP, $p<0.05$). As the conclusion, *B. macrophylla* seeds extract powder potent to be a substitute to the synthetic food colouring.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGEKSTRAKAN DAN SEMBUR KERING ANTOSIANIN DARIPADA DUA SPESIES BIJI KUNDANG, *Bouea macrophylla* Griffith DAN *B. oppositifolia* (Roxb.) Adelb

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Antosianin mendapat perhatian baru-baru ini kerana manfaat kesihatan mereka yang berpotensi sebagai sumber alternatif kepada pewarna sintetik. Biji Kundang (*Bouea macrophylla*) yang berwarna ungu merah jambu boleh dijadikan sebagai sumber pewarna semulajadi. Maklumat mengenai antosianin dari biji Kundang amatlah terhad. Oleh itu, objektif bagi kajian ini ialah (i) untuk mengkaji kesan pelarut yang berbeza terhadap penghasilan antosianin dari biji Kundang dan (ii) untuk mengoptimakan keadaan pengering sembur dalam penghasilan serbuk ekstrak biji *B.macrophylla*. Bahagian pertama dalam kajian ini, lima pelarut yang berbeza [air suling, etanol, etanol (70%), etanol (70%) dengan asid asetik (pH 1), dan etanol (70%) dengan asid hidroklorik (pH 1) digunakan untuk mengekstrak dua jenis spesies biji Kundang (*Bouea macrophylla* dan *Bouea oppositifolia*) untuk mencari potensi sebagai pewarna semulajadi yang mengandungi antosianin dan sifat antioksidan. Komposisi biji Kundang telah dikaji sebelum pengekstrakan, di mana kandungan kelembapan, lemak, protein, abu, dan karbohidrat masing-masing berada di antara julat 9.82-14.87%, 0.67-1.15%, 5.13-5.74%, 1.19-1.50%, dan 77.82-82.10%. Keputusan yang diperoleh dengan jelas menunjukkan bahawa jenis pelarut cenderung mempengaruhi pengekstrakan antosianin dan antioksidan daripada biji Kundang. Walau bagaimanapun, pelarut berasid tanpa mengira jenis asid menunjukkan keputusan yang paling menonjol untuk kedua-dua spesies Kundang. Bagi biji *B. macrophylla*, etanol (70%) dengan HCl menghasilkan ekstrak dengan hasil ekstraksi (17.5%), nilai *a* (66.30) dan *b* (17.51), jumlah kandungan antosianin monomerik (TMA) (1.2 mg sianidin-3-O-glukosida/g ekstrak), jumlah kandungan flavonoid (TFC) (6.58 mg RE/g ekstrak), dan DPPH (82.22%) yang tertinggi berbanding dengan pelarut-pelarut yang lain ($p<0.05$). Selain itu, biji *B. macrophylla* mempunyaii sianidin-3-O-glukosida, malvidin-3-O-glukosida, dan peonidin-3-O-glukosida. Dapatan ini menjelaskan bahawa biji *B. macrophylla* merupakan sumber baru antosianin semulajadi yang mengandungi bahan antioksidan.

Kajian mengenai pengeringan sembur ekstrak biji *B. macrophylla* telah dilakukan dan penerimaan penilaian sensori aga-agar yang mengandungi serbuk kering semburan ekstrak biji Kundang ditentukan memandangkan antosianin adalah tidak stabil semasa pemprosesan. Reka bentuk pusat komposit (CCD) daripada kaedah gerak balas permukaan (RSM) dilakukan untuk mengoptimumkan pengeringan semburan ekstrak biji *B. macrophylla* dan untuk menganalisis kesan suhu udara masuk (160-180 °C) dan kandungan maltodekstrin (DE10) (3-17%, w/w) terhadap sifat serbuk. Model permukaan tindak balas yang ketara ($p<0.05$) dengan nilai koefisien tinggi ($R^2>0.910$) hanya untuk kelarutan (0.979) yang didapati dari data eksperimen. Analisis deria terhadap penerimaan agar-agar mengandungi serbuk kering semburan yang diperoleh daripada suhu udara masuk pada 170 °C dan kandungan maltodekstrin (DE10) pada 10% (w/w) telah dilakukan oleh 30 ahli panel yang tidak terlatih. Perbezaan yang tidak ketara ($p>0.05$) diperhatikan dari segi aroma, tekstur, rasa, dan penerimaan keseluruhan kecuali warna keseluruhan dimana serbuk pewarna komersil (CCP) mempunyai warna yang lebih diterima oleh ahli panel berbanding dengan serbuk semburan ekstrak biji *B. macrophylla* (SSP, $p<0.05$). Sebagai rumusan, serbuk ekstrak biji Kundang (*B. macrophylla*) berpotensi untuk menggantikan pewarna makanan sintetik.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

%	Percentage
°C	Degree celsius
°	Degree
µL	Microlitre
µm	Micrometre
β	Beta
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
θ	Theta
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BC	Before Christ
CCP	Commercial Colouring Powder
cm	Centimetre
FAMA	Federal Agricultural Marketing Authority
FCC	Food Chemicals Codex
g	Gram
h	Hour
M	Molarity
m	Metre
mg	Miligram
mL	Mililitre
mm	Milimetre
MPa	Milipascal
MT	Metric Ton
Mw	Molecular weight

N	Normality
nm	Nanometre
Pa	Pascal
R^2	Regression coefficient
rpm	Revolutions per minute
s	Seconds
SSP	Spray-Dried Seeds Powder
USD	United States Dollar

CHAPTER 1

INTRODUCTION

The colourant is an additive presents in food products to provide food with an attractive and durable appearance. Synthetic colourants have been widely used since the first 'coal tar' colourant was synthesised in 1856, to change the colour of foods (Lakshmi, 2014). However, the safety of synthetic colourant questionable, leading to a reduction of permitted food colourants (Chandrasekhar, Madhusudhan, & Raghavarao, 2012). In the United States, only seven synthetic colourants are permitted, while eight synthetic colourants in specified food commodities are permitted in India (Fiszman, Giboreau, & Spence, 2012). Meanwhile, in Malaysia, 14 synthetic colourants are permitted to be used as colouring substances in food as stated in Food Act 1983 (Act 281) & Regulations. Hence, the replacement of synthetic with natural colourants has attracted significant attention among manufacturers over the last few years. For example, the grape extract has been used as a colourant in beverages or soft drinks in the United States (Junka, Rattanamechaiskul, Wongs-Aree, & Kanlayanarat, 2017). The purple colour obtained from purple maize cob, which was a by-product of the maize industry could be used as a food colourant (Yang, Fan, Gu, Han, & Chen, 2008). Several coloured plants, such as red radish, beetroot, carrot, and other extracts could replace synthetic colourants (Chethana, Chetan, & Raghavarao, 2007).

Natural colourants can be obtained from various sources including plants, insects, and minerals (Shahid, Shahidul, & Mohammad, 2013) and well known for their biodegradability, non-toxicity, and non-carcinogenic properties (Mirjalili, Nazarpoor, & Karimi, 2011). There have been many advances in developing natural colourants with respect to the extraction processes, purification, identification of new sources, and stability. However, there is still a need for developing new natural colourants with high stability and good colouring strength that have wide industrial applications (Shahid, Shahidul, & Mohammad, 2013). Currently, several natural colourants are obtained from vegetable matrices and many natural colourants have been applied in commercial foods including carotenoids, anthocyanins, chlorophyll, and betalains which providing pigments and perform functional activities in the human body. However, there is no enough production to support the demand of the food industry for natural colourants. Thus, investment in research and development of natural colourants is needed in order to find abundant sources of natural colourants which make its application technical and economically feasible (Nathia-Neves & Meireles, 2018).

Anthocyanin is one of the most important pigments found in nature and has been used widely as an alternative to the synthetic colourants in the food industry (Assous, Abdel-Hady, & Medany, 2014). It is known to be responsible for the purple, blue, and red colours found in many plant tissues, which depends on its structure, the acidity of the environment, and the presence of co-pigments (Oancea, Stoia, & Coman, 2012). In addition to its colouring effects, anthocyanin exhibited health benefit including antioxidant, anti-inflammatory as well as anticancer properties (Santos, Albarelli, Beppu, & Meireles, 2013). The natural sources of anthocyanin pigments are abundant

especially in grapes, berries, red cabbage, apples, radishes, orchids, and black rice (Kang, Jung, & Lee, 2014). The high demand for natural anthocyanin leads to a continuous search for various sources of anthocyanin.

Kundang or scientifically known as *Bouea macrophylla* (generally known as plum mango), is a tropical fruit that grows in the ASEAN region including Malaysia, Thailand, and Indonesia. The fruit looks like mango but much smaller in size, yellowish in colour when matured, and contain brightly coloured purple seeds. Ripe fruits are consumed fresh or cooked into syrup, while unripe fruits are consumed as an ingredient of 'sambal' (a chilli-based condiment), as pickles or as fruit and vegetable salad. Kundang has been claimed traditionally and scientifically to contain a significant amount of nutrients and able to protect against colon, breast, leukaemia, and prostate cancers (Rajan, Bhat, & Karim, 2014). The seed is bitter and unutilised, even though it is edible (Lim, 2012). The purple colour of the seed has the potential to be used as a natural anthocyanin and become one of the sources of natural colourants. Besides, the extract of Kundang seeds was proven to possess promising anticancer activity and causes decreased cancer cell viability by activating the apoptotic process (Arapoc, Zaffar Ali, Zainah, Rosniza, & Shafii, 2016).

Like other natural colourants, anthocyanin is an unstable compound resulting from the effects of pH, heat, enzymes, light, freezing, and formation of co-pigments during processing and storage (De Souza, Thomazini, Baileiro, & Favaro-Trindade, 2015). Therefore, an alternative processing method is introduces to overcome this problem during spray-drying. Spray-drying is a conventional method that protects the ingredients that are sensitive to light, oxygen, and free radical degradation (Mahdavi, Jafari, Ghorbani, & Assadpoor, 2014).

Therefore, the objectives of this study were;

1. To investigate the effect of different types of solvent on the properties of anthocyanin extracted from different species of Kundang (*B. macrophylla* and *B. oppositifolia*) seeds.

2. To determine the optimum condition of the spray-drying process for production of anthocyanin powder from *B. macrophylla* seeds extract.

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BIODATA OF STUDENT

Nur Hafezah was born on 9th August 1974 in Pantai Medical Center, Kuala Lumpur. She went to Kolej Islam Sultan Alam Shah, KISAS to further her secondary education. In 1994, she enrolled in University Putra Malaysia for a degree in Bachelor of Food Science and Technology. After graduated, she has been working as a teacher at Sekolah Kebangsaan Bandar Enstek, Negeri Sembilan to this day before she started her study in Master of Science in February 2015 under Hadiah Latihan Persekutuan (HLP) by Malaysia Education Ministry. She has 6 children and her husband is working as an engineer at Air Asia X.

PUBLICATION

Scientific Journal Publications:

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