UNIVERSITI TEKNOLOGI MARA

STUDY ON ANTHOCYANIN IN PIGMENTED PLANTS AND ITS APPLICATION IN MALAYSIAN STEAMED RICE CAKE *'APAM KUKUS'*

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Thesis submitted in fulfilment
of the requirements for the degree of
Doctor of Philosophy
(Food Science and Technology)

Faculty of Applied Science

October 2018

ABSTRACT

The application of anthocyanin extract in foods is gaining interest concurrent with the adverse effect of the synthetic colourants, though the instability of anthocyanin towards multiple food processing limits the application of anthocyanin especially in high-temperature food such as '*apam kukus*', a traditional Malaysian steamed rice cake. The main objectives of this study were to investigate the anthocyanin stability, colour properties, phenolic compositions and antioxidants activities of *Garcinia mangostana* peel, *Clitoria ternatea* flower, *Syzygium cumini* fruit and *Ardisia colorata* var *elliptica* fruit, as well as their potential application in '*apam kukus*'. Antioxidant assays employed in this study were ORAC, FRAP, ABTS and DPPH radical scavenging activity assays. The results revealed that *G. mangostana* exhibited higher in all antioxidant activities tested as well as a potent antimicrobial activity against *Aspergillus niger*, *Bacillus cereus*, *Bacillus subtilis*, and *Staphylococcus aureus*. *C. ternatea* exhibited moderate to low antioxidant activities but higher in colour density and indices of polymeric colour. Based on the HPLC results, the decreasing order of total phenolic acid was *G. mangostana* > *A. colorata* > *S. cumini* > *C. ternatea*; the decreasing order of total flavonoid was *G. mangostana* > *S. cumini* > *C. ternatea* > *A. colorata* and the decreasing order for total anthocyanin content was *G. mangostana* > *A. colorata* > *C. ternatea* > *S. cumini*. Based on the results of HPLC and LC-QTOF, cyanidin 3-glucoside, delphinidin 3-glucoside, and malvidin 3-galactoside is first time identified in *A. colorata*. The results showed that decreasing the extractant pH resulted in the increased in anthocyanin content and colour density (CD), however, decreased the antioxidant activities of the samples. Though a significant decreased in anthocyanin content and CD was noted at pH 4.5 and 5.0. Since *G. mangostana* and *C. ternatea* showed a significantly higher in antioxidant activities and colour stabilities, respectively, therefore were selected to be blended and studied for anthocyanin and colour stabilities. Result showed that mixture of 25% *G. mangostana* peel and 75% *C. ternatea* flower (25GMP:75CT) with the extractant pH 3.5 exhibited the most evident co-pigmentation, the highest synergistic effect and the lowest rate constant for colour and anthocyanin stabilities against 100°C, therefore, 25GMP:75CT was selected to be incorporated in '*apam kukus*'. Incorporation of 25GMP:75CT extract to '*apam kukus*' at 1% and 0.5% significantly ($p<0.05$) hardened the '*apam kukus*' compared to the control '*apam kukus*', though '*apam kukus*' with 0.5% 25GMP:75CT extract exhibited no significant differences in resilience and springiness compared to the control. Lightness value and hues of '*apam kukus*' incorporated with 0.5% and 1% 25GMP:75CT extracts are comparable with the commercial synthetic colourant, however, did not achieve the expected chroma as the synthetic colourants. The incorporation of 0.5% and 1% 25GMP:75CT extract in '*apam kukus*' markedly increased ($p<0.05$) the antioxidant activities of '*apam kukus*'. The incorporation of 25GMP:75CT extract in '*apam kukus*' at 0.5% and 0.25% showed significantly higher panellist score in "overall acceptability" than 1%, hence, it can be concluded that 0.5% 25GMP:75CT extract is suitable to be applied as a colourant in '*apam kukus*'.

ACKNOWLEDGEMENT

Assalamualaikum w.b.t.
Upon completion of this thesis, I would like to express my gratitude to many parties. First and foremost, grateful to the Almighty Allah for His merciful in giving me an opportunity to prepared and finished my PhD project.

My heartfelt thanks go to my supervisor, Prof Dr Noriham Abdullah who has patiently guided me, devoting her uttermost time and effort with generosity. I am indebted to all of her guidance, valuable supervised, comments and cooperation given by her since the beginning of my PhD. My special appreciation was also dedicated to my co-supervisor, Dr Nurhuda Manshoor for her advice, kindness and support throughout my project completion.

I would like to extend my appreciation to my intelligent discussion groups for all the comments, insightful suggestions, motivations and criticisms and for their help to accomplish my thesis. Special thanks and deepest appreciation goes to my parents and parents in law,

for the constant prayers, emotional encouragements, and supports. I would like to express my appreciation to my husband, Muhammad Shakir bin Mahamod who has been the constant source of support and encouragement during the challenges of my graduate school and life. Thanks to my daughters Fathiah Zaheera and Akifa Aulya, for being so understanding and for their smiles that brighten up my life that encouraged me to overcome the obstacles encountered in pursuit to finish the writing stage of my thesis.

Finally, I would like to express my gratitude to my families, friends and to those who have helped me to accomplish my PhD project. Words are powerless to express my appreciation and may Allah rewards you with good and return all your kindness.

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CHAPTER ONE
INTRODUCTION

1.1 Background of Study

In recent years natural colourants from plants are gaining interest due to the increasing reports of hyperactivity in children and allergic reaction to the synthetic additives and insect derived-colourants (Cox & Ebo, 2012; Greenhawt, McMorris, & Baldwin, 2009; Liippo & Lammintausta, 2009; McCann et al., 2007; Mpountoukas et al., 2010). In response to this issue, the United Kingdom Food Standard Agency (UK FSA) recommended the food manufacturers to find the alternatives for six artificial colourings named as *'The Southampton Six'* and these include Tartrazine, Ponceau 4R, Sunset Yellow, Carmoisine, Quinoline Yellow and Allura Red which are commonly found in sweets, biscuits, and soft drinks. Concurrent with the emerging of *'halal'* (Malay word) industry and increasing reports on the adverse effect of synthetic and animal-derived colourants on human, the applications of anthocyanin in food are gaining interest among the food researchers and manufactures in replace of synthetic and animal-derived colourants. Although the usage of synthetic colourants has gained critics globally, the awareness on the adverse effect of the synthetic colourants among Malaysian is lacking (Yatim, 2015). Indeed, the emerging of "rainbow foods" such as *'laksa pelangi'*, *'roti canai pelangi'* indicates that Malaysian are getting more enthusiastic on colouring their foods, and proved that the awareness on the toxicity of the synthetic colourants among Malaysian is lacking (Husain, 2014; Hussin, 2015; Samichu, 2016).

Anthocyanin is responsible for the attractive hue in plants ranging from scarlet, magenta, purple and blue colours. In fact, anthocyanin is one of the potential alternatives for Allura Red. Anthocyanin is a water-soluble pigment and non-toxic pigment which has been approved by World Health Organisation (WHO) as a colour additive (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009). However, the application of anthocyanin in bakery product is very limited due to their sensitivity towards heat, pH and food ingredients used in the formulation (Cavalcanti, Santos, & Meireles, 2011; Giusti & Wrolstad, 2003; Patras,