UNIVERSITI PUTRA MALAYSIA

EXTRACTION AND PHYSICO-CHEMICAL AND FUNCTIONAL CHARACTERISATION OF HYDROCOLLOIDS FROM TAMARILLO (Solanum betaceum Cav.) FRUIT

SRI PUVANESVARI GANNASIN

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By

SRI PUVANESVARI GANNASIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATIONS

I would like to dedicate my thesis to my heavenly father Mr Gannasin Kalimuthu and my beloved family members for always being a part of my endeavours.

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

EXTRACTION AND PHYSICO-CHEMICAL AND FUNCTIONAL CHARACTERISATION OF HYDROCOLLOIDS FROM TAMARILLO (*Solanum betaceum* Cav.) FRUIT

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SRI PUVANESVARI GANNASIN

June 2015

Chair: Associate Professor Sharifah Kharidah Syed Muhammad, PhD
Faculty: Food Science and Technology

Amongst the fruits, only red tamarillo (*Solanum betaceum* Cav.) contains both polar (anthocyanins) and non-polar (carotenoids) pigments in its mesocarp. The ability to retain both polar and non-polar pigments in the mesocarp could be related to the unique properties of its hydrocolloids. To understand the hydrocolloid-pigment interaction in the fruit, information on physico-chemical and functional properties of the hydrocolloids is required. Therefore, hydrocolloids from the anthocyanin-rich seed mucilage fraction of the tamarillo and its carotenoid-rich pulp fraction were extracted and characterised. Water and 1% citric acid were able to extract the seed mucilage hydrocolloid from tamarillo puree while pulp hydrocolloid was extracted from the puree using 72% ethanol and 20 mM HEPES buffer. The solubility of seed mucilage hydrocolloid in water was almost 90% compared to that of pulp hydrocolloid (28%). Hydrophilic seed mucilage hydrocolloid was mainly composed of uronic acids (> 65%) apart from the major neutral sugars arabinose, galactose and rhamnose. Meanwhile, the less polar pulp hydrocolloid was predominantly constituted of glucose, galactose, arabinose and xylose and approximately 20-30% of uronic acids. Molecular weight and radius of gyration of seed mucilage hydrocolloid (~ 2×10^5 g/mol; ~ 50 nm) were lower than that of pulp hydrocolloid (~ 2×10^7 g/mol; ~ 120 nm). Based on physico-chemical properties of the hydrocolloids, it was deduced that seed mucilage hydrocolloid was primarily composed of arabinogalactan protein-associated low methoxyl pectin whereas the pulp hydrocolloid was composed of hemicellulosic polysaccharides. Rheological and functional characterisations of the hydrocolloids were also performed. Oscillation frequency sweep test of 2% (w/v) seed mucilage hydrocolloid revealed its liquid-like behaviour in water while the pulp hydrocolloid exhibited gel-like behaviour at 2% (w/v) in water. The water-holding capacity of pulp hydrocolloid (~ 25 g water/g sample) was almost five folds higher than that of seed mucilage hydrocolloid while oil-holding capacity of pulp hydrocolloid was double the value of seed mucilage hydrocolloid (~ 2 g oil/g sample). The emulsifying activity and emulsion stability of pulp hydrocolloid were 90% and 95%, respectively, which were exceptional in comparison to that of other commercial hydrocolloids studied. Meanwhile, the foaming capacity of seed mucilage hydrocolloid (~35%) was higher than that of thirteen commercial hydrocolloids studied except for bovine gelatine (62%). Nevertheless, the seed mucilage hydrocolloid had better foam stability (80%)

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than bovine gelatine (11%) after 2 h of foam formation. In addition to good technological functionalities, 2% (w/v) of tamarillo pulp hydrocolloid possessed higher bile acid-binding capacity (38%) than 2% (w/v) of commercial oat fibre (27%). *In vitro* fermentation of the hydrocolloids using gut microbiota showed that seed mucilage hydrocolloid increased the number of lactobacilli and bifidobacteria more than the pulp hydrocolloid. Short chain fatty acids (acetate, propionate and butyrate) formation reduced the pH in the batch culture fermentation units which further indicated the prebiotic activity of the hydrocolloids. In conclusion, red tamarillo mesocarp contains two types of hydrocolloids with unique properties: (i) hydrophilic arabinogalactan protein-associated low methoxyl pectin in the anthocyanin-rich seed mucilage and (ii) less polar hemicellulosic polysaccharides in the carotenoid-rich pulp.

Keywords: Extraction; physico-chemical; functional; characterisation; tamarillo; hydrocolloid

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGEKSTRAKAN DAN PENCIRIAN FIZIKO-KIMIA DAN KEFUNGSIAN HIDROKOLOID DARIPADA BUAH TAMARILLO (*Solanum betaceum* Cav.)

Oleh

SRI PUVANESVARI GANNASIN

Jun 2015

Pengerusi: Profesor Madya Sharifah Kharidah Syed Muhammad, PhD
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Antara buah-buahan yang wujud, hanya mesokarpa buah tamarillo merah mengandungi dua jenis pigmen yang berlainan kutub iaitu antosianin dan karotenoid. Keupayaan untuk mengekalkan kedua-dua pigmen yang berlainan kutub dalam satu mesokarpa boleh dikaitkan dengan sifat unik hidrokoloidnya. Untuk memahami interaksi pigmen-hidrokoloid dalam mesokarpa tersebut, maklumat mengenai ciri-ciri fiziko-kimia dan kefungsian hidrokoloid diperlukan. Oleh itu, hidrokoloid daripada musilaj benih tamarillo yang kaya dengan antosianin dan pulpa tamarillo yang kaya dengan karotenoid diekstrak dan pencirian hidrokoloid dilakukan. Air dan 1% asid sitrik digunakan untuk mengekstrak hidrokoloid daripada musilaj benih manakala 72% etanol dan penimbal 20 mM HEPES digunakan untuk pengekstrakan hidrokoloid pulpa. Kebolehlarutan dalam air adalah hampir 90% bagi hidrokoloid daripada musilaj benih berbanding dengan hidrokoloid daripada pulpa (28%). Hidrokoloid daripada musilaj benih yang hidrofilik terdiri terutamanya daripada asid uronik (> 65%) selain daripada monosakarida neutral seperti arabinosa, galaktosa dan ramnosa. Sementara itu, hidrokoloid daripada pulpa terdiri daripada glukosa, galaktosa, arabinosa, xylosa dan kira-kira 20-30% asid uronik. Jisim molekular dan jejari legaran hidrokoloid daripada musilaj benih (~ 2 x 10^5 g/mol; ~ 50 nm) adalah lebih rendah daripada hidrokoloid daripada pulpa (~ 2 x 10^7 g/mol; ~ 120 nm). Pencirian fiziko-kimia menunjukkan bahawa hidrokoloid daripada musilaj benih adalah pektin bermetoksil rendah yang berhubung dengan arabinogalaktan-protein manakala hidrokoloid daripada pulpa terdiri terutamanya daripada hemiselulosa. Pencirian reologi dan kefungsian hidrokoloid juga telah dijalankan. Ujian ayunan kekerapan sapu menunjukkan bahawa pada kepekatan hidrokoloid 2% dalam air, hidrokoloid daripada musilaj benih mendedahkan sifat seperti cecair manakala hidrokoloid daripada pulpa mempamerkan sifat seperti gel. Kapasiti pemerangkapan air oleh hidrokoloid daripada pulpa (~ 25 g air/g sampel) adalah hampir lima kali ganda lebih tinggi daripada hidrokoloid musilaj benih manakala kapasiti pemerangkapan minyak oleh hidrokoloid daripada pulpa adalah dua kali ganda nilai hidrokoloid daripada musilaj benih (~ 2 g minyak/g sampel). Aktiviti pengemulsian dan kestabilan emulsi hidrokoloid daripada pulpa adalah masing-masing 90% dan 95%. Sementara itu, kapasiti pembusaan hidrokoloid daripada musilaj benih (~ 35%) adalah lebih tinggi daripada tiga belas hidrokoloid komersial yang telah dikaji kecuali gelatin lembu (62%). Walau bagaimanapun, hidrokoloid daripada musilaj benih mempunyai kestabilan pembusaan (80%) yang

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lebih baik daripada gelatin lembu (11%) selepas 2 jam pembentukan busa. Selain kefungsian teknologi yang baik, pada kepekatan hidrokoloid 2%, kapasiti pengikatan asid hempedu oleh hidrokoloid daripada pulpa (38%) adalah lebih tinggi daripada serat oat komersial (27%). Fermentasi "*in vitro*" menggunakan hidrokoloid yang telah diekstrak dan mikroorganisma dalam usus menunjukkan bahawa hidrokoloid daripada musilaj benih meningkatkan bilangan lactobasili dan bifidobakteria lebih daripada hidrokoloid daripada pulpa. Pembentukan asid lemak berantai pendek (asid asetik, asid propionik dan asid butirik) menyebabkan nilai pH medium dalam unit fermentasi berkurangan yang merupakan penunjuk utama aktiviti prebiotik oleh hidrokoloid. Pada kesimpulannya, mesokarpa buah tamarillo merah mengandungi dua jenis hidrokoloid dengan ciri-ciri yang unik: (i) pektin bermetoksil rendah yang berhubung dengan arabinogalaktan-protein yang bersifat hidrofilik dalam musilaj benih yang kaya dengan antosianin dan (ii) hemiselulosa yang kurang hidrofilik dalam pulpa yang kaya dengan karotenoid.

Kata kunci: Pengekstrakan; fiziko-kimia; kefungsian; pencirian; tamarillo; hidrokoloid

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I certify that a Thesis Examination Committee has met on 17 June 2015 to conduct the final examination of Sri Puvanesvari Gannasin on her thesis entitled "Extraction and Physico-Chemical and Functional Characterisation of Hydrocolloids from Tamarillo (*Solanum betaceum* Cav.) Fruit" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF EQUATIONS

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LIST OF ABBREVIATIONS

a	Mark-Houwink-Sakurada empirical constant
AGPs	Arabinogalactan proteins
BABC	Bile acid-binding capacity
CDTA	Cyclohexanediaminetetraacetate
CP	Citrus pectin
CTAB	Hexadecyltrimethylammonium bromide
Da	Dalton
DAPI	4′, 6-diamidino-2-phenylindole
DE	Degree of esterification
DMAC	N, N-dimethylacetamide
dn/dc	Specific refractive index increment
DW	Dry weight
EA	Emulsifying activity
EDTA	Ethylenediaminetetraacetate
ES	Emulsion stability
FC	Foaming capacity
FISH	Fluorescent in situ hybridisation
FMOC	9-fluorenyl-methyl chloroformate
FS	Foam stability
FT-IR	Fourier transform infrared
FW	Fresh weight
G’	Storage modulus/elastic component
G”	Loss modulus/viscous component
GA	Gum arabic
h	Hours
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HG	Homogalacturonan
HNO_3	Nitric acid
HPSEC	High performance size exclusion chromatography
HSD	Honest significant difference
KOH	Potassium hydroxide
LiCl	Lithium chloride
LMP	Low methoxyl pectin
LS	Light scattering
min	Minutes
M_w	Molecular weight
Na_2CO_3	Sodium carbonate
$NaBH_4$	Sodium borohydride
NaCl	Sodium chloride
NaN_3	Sodium azide
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
η_w	Instrinsic viscosity
OF	Oat fibre
OHC	Oil holding capacity
OPA	*o*-phthaldialdehyde

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PDI	Polydispersity index
PHE	Pulp tamarillo hydrocolloid extracted with 72% ethanol
PHH	Pulp tamarillo hydrocolloid extracted with 20 mM HEPES buffer
PR	Previous residue
RG-I	Rhamnogalacturonan I
RG-II	Rhamnogalacturonan II
RI	Refractive index
RP-HPLC	Reversed phase-high performance liquid chromatography
SCFAs	Short chain fatty acids
SEM	Scanning electron microscopy
SHC	Seed mucilage tamarillo hydrocolloid extracted with 1% citric acid
SHW	Seed mucilage tamarillo hydrocolloid extracted with water
THC	Tamarillo puree hydrocolloid extracted with 1% citric acid
THE	Tamarillo puree hydrocolloid extracted with 72% ethanol
THH	Tamarillo puree hydrocolloid extracted with 20 mM HEPES buffer
THW	Tamarillo puree hydrocolloid extracted with water
tr	Traces
UV	Ultraviolet
VS	Viscometer
WHC	Water holding capacity
XGA	Xylogalacturonan
Y	Hydrocolloid yield on dry weight basis
Y_f	Hydrocolloid yield on fresh weight basis

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CHAPTER 1

INTRODUCTION

Fruits are widely consumed as a part of healthy diet due to the fact that they contain good proportions of macro- and micro-components that can prevent various chronic diseases. Fresh fruits are usually composed of 70-95% moisture and dietary fibre as the major macro-component apart from some small amounts of protein, starch and lipid. In addition, fruits are rich in natural pigments, vitamins and minerals (Jacob et al., 2012).

In common, most fruit mesocarps (the middle layer/major edible part of most fleshyfruits) contain one type of pigment. However, to the best of our knowledge, only red tamarillo contains both polar (anthocyanins) and non-polar (carotenoids) pigments in two different zones of the mesocarp. The tamarillo or tree tomato (*Solanum betaceum* Cav.) is a subtropical fruit native to the Ecuadorian-Peruvian Andes (Vasco et al., 2009). There are three types of tamarillos namely red, yellow/gold, and purple/dark red (Prohens & Nuez, 2001). The variety being cultivated in Malaysia (Cameron Highlands) is the red type which is egg-shaped with a thin reddish-brown skin, orange pulp, and dark red seed mucilage coating the seeds.

Anthocyanins are present in the tamarillo seed mucilage fraction while carotenoids are found in the pulp fraction of the fruit. Delphinidin 3-rutinoside and pelargonidin 3-rutinoside are the major anthocyanins while β-cryptoxanthin and β-carotene are the main carotenoids in the fruit (De Rosso & Mercadante, 2007; Mertz et al., 2009). The ability to retain both polar and non-polar pigments in the tamarillo mesocarp could be related to the unique properties of its hydrocolloids. Recently, few studies have shown that pigment stability was affected by hydrocolloid-pigment interaction (Buchweitz et al., 2012, 2013; Fernandes et al., 2014; Goncalves et al., 2012; Tachibana et al., 2014).

Various interesting findings have also been reported on the versatile technological and health benefits of fruit hydrocolloids and pigments. Pectin is the predominant hydrocolloid in fruits that possess good gelling, emulsifying and stabilising properties besides showing potential ability in the prevention of colon cancer, hyperglycemia and hypercholesterolemia (Wicker et al., 2014; Williams & Phillips, 2009a). Meanwhile, fruit pigments which include betalains, chlorophylls, anthocyanins and carotenoids are broadly used as natural colourants in foods and beverages. Based on their antioxidative properties, natural pigments are also often linked with the prevention of chronic diseases (Rajendran et al., 2014).

Red tamarillo which has both anthocyanins and carotenoids in its mesocarp can be used as a natural model system to understand the hydrocolloid-pigment interaction. To date, tamarillo pigments were well studied but not the properties of its hydrocolloids. Therefore, this study was undertaken to extract and characterise the hydrocolloids from red tamarillo to prove the hypothesis that fruits with non-polar and polar pigments

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would contain two types of hydrocolloids with different physico-chemical and functional properties.

The specific objectives of this research were:

1. To extract hydrocolloids from different fractions of tamarillo fruit
2. To characterise the physico-chemical properties and technological functions of hydrocolloids from tamarillo puree
3. To evaluate the bile acid-binding capacity and prebiotic activity of hydrocolloids from tamarillo puree

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