UNIVERSITI PUTRA MALAYSIA

RHEOLOGICAL, SPECTRAL AND THERMAL ANALYSES OF GELLAN/ DEXTRAN BLENDS AS GELATIN SUBSTITUTES

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IPPH 2014 9

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

November 2014

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

RHEOLOGICAL, SPECTRAL AND THERMAL ANALYSES OF GELLAN/ DEXTRAN BLENDS AS GELATIN SUBSTITUTES

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November 2014

Chair: Professor Shuhaimi Mustafa, PhD

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Microbial polysaccharides can serve as renewable sources of hydrocolloids that have great function as viscosifying, stabilizing, emulsifying or gelling agents which are important to improve texture and stability of food and pharmaceutical products. Similar functions are currently being offered by gelatin however the use of gelatin would give rise to health and religious concerns for particular group of consumers. Plant based polysaccharides have been utilized as gelatin substitute but plant requires longer harvest maturity and susceptible to geographical and seasonal changes. Viscosity and gel rigidity are important parameter to determine adequate food texture for mouth feel properties. Phase separation also could affect consumer perception on food products. The aims of this study are to investigate rheological properties that measure flow behaviour, viscosity and gel rigidity to be compared with gelatin as well as to predict molecular interaction between gellan (G) and dextran (D) in their binary blends (1:1, 1:2, 1:3 ratios) in varying concentration range of 0.5%, 1.5% and 3% (w/v) using spectral and thermal characteristics. For rheological measurements, all samples were subjected to steady shear and dynamic shear viscosity tests using rheometer. Spectral analysis and thermal analysis were performed using Fourier Transform Infrared (FTIR) and Differential Scanning Calorimetry (DSC) respectively. Rheological analyses revealed that during blending, dextran plays a significant role and transforms the solid-like characteristics to liquid-like behaviour of gellan with an increase in dextran concentration of G/D blends. The steady flow behaviour was well characterized by the Herschel-Bulkley model (standard error lower than 10) and G/D blends exhibited similar flow behaviour (shear thinning) as bovine and porcine gelatin at 1.5% and 3% concentration as compared to dilute concentration, 0.5% . For dynamic viscoelastic, G/D blends exhibit similar gel characteristic with both porcine and bovine gelatin at 3% as opposed to 0.5% and 1.5%. From spectral characteristic of the blend, a shift in the major

gellan band of carboxylic group at 1414 cm^{-1} and disappearance of two gellan bands at 1072 cm^{-1} and 1042 cm^{-1} as well as dextran band at 1080 cm^{-1} in glycosidic linkage were observed. DSC thermograms showed a single exothermic peak accompanied by a shifting to higher melting temperature for all blends in comparison of individual polysaccharide. These changes suggest a possible molecular interaction event between gellan and dextran. In conclusion, gellan and dextran blends exhibit desired viscosity for mouth feel properties and favorable molecular interaction depended on the amount of added dextran. This interaction is important to design food and pharmaceutical products with desired textural and stability attributes.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

ANALISIS REOLOGI, SPEKTRA DAN TERMA BAGI CAMPURAN GELLAN/ DEXTRAN SEBAGAI GANTIAN GELATIN

Oleh

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Polisakarida mikrob adalah sumber hidrokoloid yang berfungsi sebagai agen pemekat, penstabil, mengemulsi dan pelekat yang sangat penting untuk menambah baik tekstur dan kestabilan produk makanan dan farmaseutikal. Gelatin dari sumber haian mempunyai fungsi yang sama tetapi terdapat isu yang kritikal yang berkait rapat dengan isu keagamaan dan kesihatan pengguna. Hidrokoloid dari sumber tumbuhan digunakan untuk sebagai bahan ganti bagi gelatin tetapi tumbuhan memerlukan tempoh masa yang lama untuk mencapai tahap tuai matang dan terdedah kepada risiko perubahan geografi dan cuaca. Kelikatan dan ketegaran gel merupakan parameter yang penting untuk menentukan struktur makanan yang sesuai bagi perangsangan deria mulut. Fasa pengasingan di dalam makanan boleh persepsi pengguna kepada sesuatu produk makanan. Objektif penyelidikan ini adalah untuk mengkaji ciri-ciri reologi yang menentu ukur sifat aliran, kelikatan dan ketegaran gel untuk dibandingkan kepada ciri-ciri reologi yang ada pada gelatin serta mengkaji interaksi molekul antara gellan dan dextran pada nisbah 1:1, 1:2, 1:3 pada kepekatan 0.5%, 1.5% dan 3%. Analisa reologi iaitu *shear* stabil and dinamik dijalankan dengan menggunakan AR-G2 Rheometer. Analisa spektra dijalankan menggunakan inframerah peralihan-Fourier (FTIR) dan analisa terma menggunakan kalorimetri imbasan pembeza (DSC). Analisa reologi membuktikan bahawa dextran bertindak untuk mengubah sifat pepejal gellan kepada sifat cecair dengan peningkatan dextran di dalam campuran gellan dan dextran. Kestabilan sifat aliran juga diterangkan secara mendalam melalui model *Herschel-Bulkley* (ralat piawai di bawah 10) dan sifat aliran (*shear thinning*) campuran gellan dan dextran menunjukkan ciri yang sama pada gelatin pada kepekatan 1.5% dan 3% berbanding 0.5%. Bagi viskoelastik dinamik, campuran gellan dan dextran menunjukkan

karakter gel yang sama pada gelatin pada kepekatan 0.3% berbanding 0.5% dan 1.5%. Bagi analisa spektra, hasil eksperimen mendapati terdapat gellan mengalami perubahan pada kedudukan spektrum kumpulam karbosilik pada 1414 cm^{-1} dan kehilangan dua spektrum pada 1072 cm-1 dan 1042 cm^{-1} di dalam interaksi glikosida. Perubahan pada interaksi glikosida detxran dilihat pada 1080 cm^{-1}. Lingkaran DSC menunjukkan satu puncak *exothermic* diiringi dengan peningkatan suhu takat cair untuk kesemua campuran gellan/dextran berbanding gellan dan dextran secara tersendiri. Perubahan ini membawa kepada potensi interaksi molekular yang mungkin berlaku antara gellan dan dextran. Kesimpulannya, campuran gellan dan dextran mempunyai kelikatan yang sesuai bagi perangsangan deria mulut dan interaksi molekular yang padan bergantung kepada jumlah dextran. Interaski ini dapat memberi maklumat yang berguna sebelum menghasilkan sesuatu produk makanan dan farmaseutikal dengan tekstur yang dikehendaki dan lebih stabil.

ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious and the Most Merciful Allah Almighty, all praises to Allah for the strength, perseverance and good health to complete this thesis. My first and foremost thanks must go to my beloved husband, Muhammad Amsyar Jafri as well as my parents, Ahmad Wardi and Rosnani Hamim, for their endless encouragement and support. I would like to express my deepest gratitude to my supervisor, Professor Shuhaimi Mustafa for his incessant guidance for this research work. I bestow my earnest appreciation to Professor Madya Dzulkifly Mat Hashim for his endless assistance, precious remark, undying efforts and constructive opinion on this project. My special thanks must go to Professor Dato' Dr. Mohd Yazid Abd Manap, Dr. Ally El Shekar, Dr. Jasim Ahmed for their constant assistances throughout the course of my study. I would like to convey my remarkable recognition to my late supervisor, Professor Dato' Dr. Yaakob Che Man for being a great mentor during his presence. His full dedication and devotion to uplift research expertise in halal products, without doubt, is a significant contribution for the nation. To my dearest Nurrulhidayah, Nina Naquiah, Noorashikin, Hajaratul Najwa, Yanty, Aisyah, Dalila, Saliza and Atiqah thank you for being there through my joy and pain. I also would like to acknowledge Amran, Zhaffan, Rosmawati and Faizal for their technical assistances for this project. This scientific research project could not be achieved without the cooperation of all aforementioned people as well as numerous individuals who have directly or indirectly affected my research experience at UPM.

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

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LIST OF ABBREVIATION AND SYMBOLS

AOAC	Association of analytical chemist
ATR	Attenuated total reflectance
C-H	Methyl group
COO^-	Carboxylic acid
C=O	Carboxyl group
DSC	Differential scanning calorimetry
FTIR	Fourier Transform Infrared
G'	Elastic modulus
G''	Viscous modulus
G^*	Complex modulus
K	Consistency index
LVR	Linear viscoelastic region
OH	Hydroxyl group
R^2	Correlation coefficient
SE	Standard error
Tan δ	Tangent delta
T_c	Completion temperature
T_o	Onset temperature
T_m	Melting temperature
η^*	Complex viscosity
η_a	Apparent viscosity
ω	Frequency

$\dot{\gamma}$	Shear rate
τ	Shear stress
τ_0	Yield stres

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of the study

Hydrocolloids are polysaccharides that are dissolving in water and provide a wide range of purposes in food system, such as thickening, gelling, emulsifying, stabilizing, foaming and preventing crystal growth agent. Important sources of hydrocolloids primarily originated from plants (e.g. starch), seaweed (e.g. alginate), animal (e.g gelatin) and microbes (xanthan). Gelatin is marked as unique and extraordinary hydrocolloid because of its multipurpose functions in a wide range of applications in various industries. For Muslim consumers, the sources of gelatin become their main religious concerns because the two largest raw materials used to manufacture gelatin worldwide are pigskin (42%) as well as cattle bones and hides (55%). In addition, global gelatin production in 2011 was dominated by Europe (40%) followed by North America (23%), inferring that there is still a great market niche for halal gelatin (GIA, 2013). Gelatins also exhibit critical health risk in which cattle infected with bovine spongiform encephalopathy (BSE) or mad cow disease is unsafe for human consumption (Karim and Bhat, 2008). Plant and seaweed hydrocolloids have now been commercialized to accommodate religious and health concerns of gelatin. However, there are still several limitations in comparison to microbial hydrocolloids wherein plant and seaweed require longer harvest maturity prior polysaccharide production and also susceptible to geographical and seasonal damage. In contrast, microbial hydrocolloids pose fast yielding process because it is only takes several days for microbes to grow. Furthermore, microbial hydrocolloids can be produced under fully controlled environment via fermentation process (Donot et al., 2012).

Among microbial hydrocolloids that have great commercial potential includes gellan and dextran. Gellan is secreted by *Pseudomonas elodea*, consists of linear repeating tetrasaccharides: β-D-glucose (DGlc), L-rhamnose (L-Rha), and D-glucuronic acid (D-GlcA) in molar ratio of 2:1:1. Gellan is rigid and fragile gel with higher thermal stability in the deacylated form (Evageliou et al., 2010; Chaudhary et al., 2013). Dextran is highly water soluble polysaccharides produced by *Leuconostoc mesenteroides*, strain B-512F and contain a backbone of consecutive α-1,6 linkages, with the remaining being branched α-1,3 linkages (Tirtaatmadja et al., 2001). Gellan has been combined with other polysaccharides such as agarose and kappa carrageenan to form mixed biopolymers but these combinations resulted phase separation. Phase separation is not desired in food products because it will affect consumer perception on food texture.

Viscosity and gel rigidity of microbial polysaccharide are important parameter to determine food texture. Rheological test such as steady state flow and dynamic viscoelastic have been utilized to characterize viscosity and gel rigidity respectively, by correlating the relationship between applied stress acting on a given sample and the resulting characteristic, either more solid (elastic) or more fluid (viscous) under defined condition. Beside rheological properties, favorable interaction of mixed polysaccharides needs to be investigated to avoid phase separation. Spectral analysis performed by Fourier Transform Infrared (FTIR) is useful to determine functional groups changes involve when two polysaccharides are mixed together. The presence of new band accompanied by disappearance of band in the polysaccharide blend could be an indicator of chemical properties alteration. Shifting of specific bands in the polysaccharides blends in comparison to pure component is another way of looking at changes of molecular interaction. Further, thermal analysis performed by Differential Scanning Calorimetry (DSC) can also be used to detect interaction occur upon blending. Thermal transition such as melting point and enthalpy changes of polysaccharides blend as opposed to single polysaccharide can be observed as associative interaction if it is thermodynamically favorable and segregative if they are less favorable.

1.2 Objective of the study

In this study, gellan/dextran blends at different concentration and ratio were prepared as this polysaccharide combination has not previously studied based on literature. Therefore, the specific objectives of this study are:

1. To characterize rheological properties including flow characteristics and gel rigidity of gellan, dextran and gellan/dextran blends in different mixing ratios as compared to porcine and bovine gelatin.

2. To investigate the molecular interaction and phase transition using spectral and thermal analysis of gellan, dextran and their blends.

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