UNIVERSITI PUTRA MALAYSIA

EVALUATION OF CORN COB-DERIVED MICROFIBRILLATED CELLULOSE FOR USE AS OIL/WATER EMULSION STABILIZER

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By

TANG TECK KIM

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Chairman : Lai Oi Ming, PhD
Institute : Bioscience

Environmental pollution has become a main concern in recent years. In developing countries, the main agricultural waste consisting of low value fiber/lignocellulose material are underutilized. Instead, burning of these materials for replanting purposes has become a norm in some countries, creating a huge air pollution almost every year. Hence, creating new usage and value-added compound from this lignocellulose material by employing a simple method yet environmental-friendly approach may indirectly increase the value of the fiber/lignocellulose material and reduce environmental problems. Corn cob, an agricultural waste has the potential to produce a type of nanocellulose called microfibrillated cellulose (MFC) which can be used as an emulsion stabilizer. Previously, such microfibrillated cellulose were prepared using wood pulp as main raw material and chemical pretreatment is the easiest way to pretreat the fiber. However, extensive usage of the chemicals for pretreatment creates environmental problems. Therefore, in the present study, a simple, milder and environmentally-friendly pretreatment process of corn cob was developed to convert corn cob into MFC prior to assessing and characterizing their ability to stabilize oil-in-water emulsion. In the approach, a lower dosage of sodium hydroxide pretreatment was successfully developed by response surface methodology before the treated corncob fibers were biobleached with xylanase. The color of the pretreated corn cob fiber/pulp was found to be enhanced and the use of enzyme-assisted biobleaching reduced the amount of chlorine-based bleach used. The treated pulp were then subjected to mild hydrolysis process using endoglucanase to facilitate the extraction of MFC through high pressure homogenization. It was found that the lowest concentration of endoglucanase was able to prevent blockage of the high pressure homogenizer unit and at the same time still preserved the intact structure of cellulose fiber. Subsequently, different cycles of high pressure homogenization were employed for the production of MFC. The water holding capacity, and resistance toward evaporation of MFC suspension were improved with the increased of high pressure homogenization cycle. MFC produced had increased shear viscosity and gelation properties due to its ability to form a network-like structure in suspension. These MFC were then used to test its emulsion stabilizing efficiency. All MFC-stabilized emulsion produced showed shear thinning effect and its shear viscosity increased with

homogenization cycle. The results also showed that MFC-stabilized emulsions were extremely stable under normal storage conditions at different temperatures from 5°C to 45°C. All the characteristics mentioned above indicated that MFC has high potential to be used as thickening agent or stabilizer in many food, cosmeceutical and pharmaceutical products.

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

PENILAIAN TERHADAP SELULOSA MIKROFIBRIL BERASAL DARI TONGKOL JAGUNG UNTUK DIGUNAKAN SEBAGAI PENSTABIL EMULSI MINYAK DALAM AIR

Oleh

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Pencemaran alam sekitar telah menjadi kebimbangan utama dalam beberapa tahun kebelakangan ini. Di negara membangun, sisa pertanian utama adalah terdiri daripada bahan serat /lignoselulosa bernilai rendah yang kurang digunakan. Pembakaran bahan-bahan ini untuk tujuan penanaman semula menjadi norma di beberapa negara yang menyebabkan pencemaran udara yang berleluasa hampir setiap tahun. Oleh itu, penghasilan penggunaan baru dan nilai tambah sebatian bahan lignoselulosa ini dengan menggunakan kaedah yang mudah dan pendekatan yang alam mesra secara tidak langsungnya dapat meningkatkan nilai bahan serat / lignoselulosa dan mengurangkan masalah alam sekitar. Tongkol jagung, sejenis sisa pertanian mempunyai potensi untuk menghasilkan sejenis nanoselulosa yang disebut selulosa mikrofibril (MFC) yang boleh digunakan sebagai penstabil emulsi. Sebelum ini, MFC biasa disediakan menggunakan pulpa kayu sebagai bahan mentah utama dan pra-rawatan kimia adalah cara termudah untuk merawat serat tersebut. Walau bagaimanapun, penggunaan bahan kimia yang banyak boleh menyebabkan masalah alam sekitar. Oleh itu, dalam kajian ini, proses pra-rawatan yang mudah, sederhana dan lebih mesra alam menggunakan tongkol jagung telah dibangunkan dan digunakan sebagai bahan mentah untuk menghasilkan MFC. Di samping itu, ciri dan kemampuan MFC untuk menstabilkan emulsi minyak dalam air telah dikaji. Dalam pendekatan ini, dos bahan kimia yang lebih rendah dalam pra-rawatan iaitu natrium hidroksida telah berjaya dibangunkan dengan kaedah gerak balas permukaan sebelum serat dirawat dengan xilanase sebagai bio-peluntur. Warna serat tongkol jagung/pulpa tongkol jagung didapati lebih baik dan penggunaan peluntur berasaskan klorin dapat dikurangkan dengan penggunaan bio-peluntur. Pulpa yang dirawat itu kemudiannya dirawat dengan proses hidrolisis ringan menggunakan endoglucanase untuk memudahkan pengekstrakan MFC melalui penghomogenan tekanan tinggi. Keputusan menunjukkan bahawa kepekatan endoglukanase yang paling rendah dapat mencegah penyumbatan unit penghomogenan tekanan tinggi dan pada masa yang sama masih dapat menggekalkan struktur utuh serat selulosa. Selanjutnya, kitaran berbeza penghomogenan tekanan tinggi digunakan untuk penghasilan MFC. Kapasiti memegang air, dan kerintangan terhadap penyejatan suspensi MFC telah dipertingkatkan dengan peningkatan kitaran penghomogenan tekanan tinggi. MFC yang

dihasilkan menunjukkan peningkatan dalam kelikatan ricih dan gelasi bertambah baik kerana keupayaannya membentuk rangkaian bertambah. MFC ini kemudiannya digunakan untuk menguji kecekapannya sebagai penstabilan emulsi. Kesemua emulsi dapat distabilkan oleh MFC dan menunjukkan kesan penipisan kelikatan ricih dan peningkatan kelikatan ricihnya dengan bertambahnya kitaran penghomogenan. Hasilnya juga menunjukkan bahawa emulsi MFC yang dihasil sangat stabil di bawah keadaan penyimpanan biasa pada suhu yang berbeza (5°C- 45°C). kesemua ciri-ciri yang dinyatakan di atas menunjukkan bahawa MFC mempunyai potensi tinggi untuk digunakan sebagai agen pemekatan atau penstabil dalam pelbagai produk makanan, kosmeseutikal dan farmaseutikal.

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LIST OF ABBREVIATIONS

TEMPO	2,2,6,6,-tetramethylpiperidine-1-oxyl
ANOVA	Analysis of variance
DP	Degree of polymerization
ECF	Elemental chlorine-free
FCCD	Face center composite design
HLB	Hydrophobic and hydrophilic balance
LVR	Linear viscoelastic range
MCC	Microcrystalline cellulose
NCC	Nanocrystalline cellulose
MFC	Microfibrillated cellulose
MWD	Molecular weight distribution
SEM	Scanning electron microscope
TCCD	Tetrachlorodibenzo-p-dioxin
$D_{4,3}$	Volume weighted diameter
$D_{3,2}$	Surface weighted diameter
WHC	Water holding capacity
ZP	Zeta potential

CHAPTER 1

INTRODUCTION

Corn, being one of the few important cereal crops after wheat and rice is utilized widely in the world as staple food. According to statistic obtained from the Department Agriculture, Malaysia (2015), there are around 10,030 Ha of area being used for corn crop plantation in Malaysia and this area generate an income of RM 174,888 million in 2015 alone. As corn is being used in various food applications, there are myriads of by-products that are eventually produced during corn processing such as corn cob. It was estimated that corn cob generated roughly 15% of waste out of total corn production which eventually resulted in the formation of large amount of waste (Gradinaru et al., 2018). Having said that, the by-product of corn cob has also found its applications in various areas. Often, the majority of the corn cobs which are low in nutrition content were either used as low quality food for ruminant or just burned/thrown away as a waste (Fang et al., 2017). When the latter is left unmanaged, it will certainly create environmental pollution particularly when it is thrown away in large quantities. Since the issue of food security is a major concern in today's world, it would be useful, if with the advancement in the science and technology, the underutilized corn cob can be transformed into a high-value food ingredients for the nation. With this, it will not only aid in managing the waste problem and sustainability issue but also to provide an alternative route to solve the food security issue.

Looking at the structure of the corn cob, it can be seen that corn cob contains mainly fiber that is made up of basically: cellulose, hemicellulose and lignin (Kartawiria et al., 2019) . Ideally, with its high amount of cellulose, low cost and relatively easy to source features, corn cob can be a good choice to be used a raw material for the production of cellulose-based products particularly those with nanosize dimension such as microfibrillated cellulose (MFC) that has received much attention lately. MFC is a type of nanocellulose having diameter that is in nanosize and length up to several microns (Larsson et al., 2019) . With such a unique structure, MFC can entangle among each other creating a three dimension network system that can entrap/stabilize materials (Lin et al., 2019). Currently, it is the trend for the food industries to opt for non-synthetic and healthier stabilizer or emulsifier. In line with the global demand, MFC can be a good choice to become a zero calorie stabilizer. Nevertheless, its properties to stabilize emulsion are not well explored at this moment. One of the setbacks that hinder the lack of exploration of MFC in this area is due to the difficulty in producing it in large scale as well as the high energy consumption of the processing method that makes it hard to be commercialized and used in the food systems.

MFC is commonly produced from wood sources. However, there are few challenges faced by the industries in producing MFC from wood sources. Firstly, the major raw materials for producing MFC generally come from woods or log. However, due to the restriction enforced on deforestation, these sources have become depleted. Therefore, it is imperative to look for alternative sources. Secondly, the major step in producing MFC from the start requires the pretreatment of raw materials to become pulp. This step usually involves the use of chemicals to treat the raw material such as chlorine bleaching which is highly toxic and pollutes to the environment (Mostafa et al., 2019). Instead, utilizing corn cob as the raw material for the preparation of MFC offers several advantages as compared to wood pulp. This is because corn cob has a loose structure and possess less pigmentation which requires lesser treatment to turn it into pulp which is important to reduce the energy require to produce MFC (Zhao et al., 2017). Thirdly, the final step of MFC production required the use of mechanical shearing which can't be replaced by chemical method. When chemical method is employed, it always end up producing cellulose nanocrystalline or nano whisker instead of the fibrillated cellulose. The mechanical shearing that utilized high pressure homogenizer to disintegrate fiber bundles (pulp) into MFC can be a challenging issue as the fiber bundles sometimes may block the valve in high pressure homogenizer due to the highly structural properties of the fiber bundles (Berto and Arantes, 2019). And this causes an obstacle in commercialization of MFC.

Therefore, in this study we aim to produce a versatile MFC that has potential to be used as rheological modifier, stabilizer, water holding agent, structurant and etc in many industries such as food, cosmeceutical and pharmaceutical. The production involved using underutilized corn cob by a simple and more environmental-friendly approach that require minimum amount of chemical and energy through the aid of enzymes before further investigating the stabilizing properties of MFC in oil in-water-emulsion under different storage conditions. The enzyme-assisted process of MFC production is more cost effective, especially when, with just a minute amount of enzyme endoglucanase, the structure of cellulose can be softened and further prevent the breakdown of the high pressure homogenizer during the defibrillation process. Thus, the objectives of the present study were as follows:

1) To optimize the alkaline pretreatment parameters (sodium hydroxide concentration, reaction time and reaction temperature) and biobleaching pretreatment parameters (concentration of Pulpzyme HC enzyme and reaction time for corn cob pulp preparation) for preparation of corn cob pulp using response surface methodology in order to enhance lignin removal and increase cellulose swelling during the pretreatment process.

2) To investigate the effect of endoglucanase enzyme and the number of cycles of high pressure homogenization on the physical properties of corn con-based MFC produced.

3) To characterize and investigate the stability of oil-in-water emulsion prepared using MFC produced from different cycles of homogenization and different concentration under normal gravitational and accelerated study (by centrifugation) at storage temperatures of 5°C, 25°C and 45°C.

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BIODATA OF STUDENT

Tang Teck Kim was born in a small village in Pontian, Johor. He enjoyed science subjects ever since his primary school days but was bad in languages. During his primary school days, he was introduced to a book called "Fountain of Life" which explained the importance of enzyme in our life and the book changed his life. His interest in science continued and he elected to take up a BSc. in Biotechnology and obtained a First Class Honours from UTAR in 2009. In 2014, he graduated with a MSc from UPM. In 2014, Teck Kim participated in the IFTSA Developing Solutions for Developing Countries (DSDC) competition held by Institute of Food Technologists in New Orleans USA where his team won the second place in the competition. It was also Teck Kim's first travel out of Malaysia. In 2018, he spent three months in Jinan University, Guangzhou, China for a research attachment.

LIST OF PUBLICATIONS

Tang, T. K., Lee, Y. Y., Phuah, E. T., Tan, C. P., Kanagaratnam, S., Wang, Y., Cheong, L.Z., Jamalullail, N.A., Lee, C. M., and Lai, O. M. Response surface methodology optimization study on corn cob pretreatment: reduction of sodium hydroxide usage and enhancement in pulpzyme HC biobleaching efficiency. *Food Research*-**Accepted**

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