ISOLATION OF NANOCRYSTALLINE CELLULOSE FROM *LEUCAENA LEUCOCEPHALA* PODS AND ITS APPLICATION AS A FAT REPLACER IN PRODUCTION OF LOW-FAT MAYONNAISE

By

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

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ABSTRACT

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

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July 2022

Chair: Professor Dr Ir. Yus Aniza Binti Yusof, PhD

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This study investigated the properties of nanocrystalline cellulose (NCC) isolated from *L. leucocephala* mature pods. Four different bleaching agents were used during the bleaching process, followed by sulphuric acid hydrolysis. Physical and chemical properties, crystallinity index, water binding capacity, and thermal behaviour of obtained NCC were determined by using transmission electron microscopy (TEM), Fourier transforms infrared (FTIR), X-ray diffraction (XRD) and thermogravimetric analysis (TGA), respectively. The results showed individual fibres of rod-shaped particles with a nano-sized average diameter (17 to 49 nm) and length (133 to 239 nm) in all NCC produced. The FTIR spectra indicated that the peaks attributed to lignin and hemicellulose were absent after chemical and bleaching treatment. Both components were completely removed from the samples after acid hydrolysis. The XRD analysis showed that crystallinity increased after acid hydrolysis, indicating the isolated NCC's crystalline nature for all samples. NCC treated with 7% sodium hypochlorite shows the highest crystallinity, 71.1%, even though cellulose degradation occurs in the bleaching stage. TGA analysis displayed that degradation of NCC

occurred at 143 ℃, and the T_{max} was at 188 ℃ with 25.9% residue at 600 ℃. A three-region viscosity profile in liquid crystalline systems was observed in sonicated samples. Besides that, from the rheological analysis, it can be concluded that NCC suspension behaves as a shear-thinning material. Further work was carried out to produce low-fat mayonnaise with isolated NCC as a fat replacer. The mayonnaise with 30% oil reduction was prepared and incorporated with NCC, MCC, and MCC+NCC (1:1). Both control and mayonnaise with fat replacer appeared stable even after one month of storage. The oil reduction in mayonnaise does affect the colour as all fat-reduced mayonnaise had greater brightness (L*) than the control sample. Besides that, when NCC replaced fat, a* increased significantly, whereas b* decreased after storage. The whiteness index of all fat-reduced mayonnaise was in the range of 68%. All samples showed stable emulsion even after one month of storage at room temperature. The particle size of oil emulsion for all mayonnaise was within the range of theory, between 200 to 500 nm. The rheological properties of mayonnaise produced showed the shear thinning behaviour. From all obtained results, the isolated NCC has the potential to be used as a fat-replacer in mayonnaise as it showed the best stability in emulsion and rheological analysis.

ABSTRAK

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

PENGASINGAN SELULOSA NANOKRISTAL DARIPADA POD *LEUCAENA LEUCOCEPHALA* DAN PENGGUNAANNYA SEBAGAI PENGGANTI LEMAK DALAM PENGHASILAN MAYONIS RENDAH LEMAK

Oleh

AIDA SAFINA BINTI ARIDI

Julai 2022

Pengerusi: Professor Dr. Ir. Yus Aniza Binti Yusof, PhD

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Kajian ini bertujuan untuk mengkaji sifat-sifat sellulosa nanokristal (NCC) yang diekstrak daripada pod matang petai belalang (*Leucaena leucocephala)*. Empat agen peluntur yang berbeza telah digunakan semasa proses pelunturan, diikuti oleh hidrolisis asid sulfurik. Sifat fizikal dan kimia, indeks penghabluran nanokristal selulosa, kapasiti mengikat air, dan sifat termal NCC yang diperolehi ditentukan dengan menggunakan penghantaran electron mikroskop (TEM), inframerah transformasi Fourier (FTIR), pembelauan sinar-X (XRD) dan analisis termogravimetrik (TGA), masing-masing. Keputusan menunjukkan gentian individu zarah berbentuk rod dengan diameter purata bersaiz nano (17 hingga 49 nm) dan panjang (133 hingga 239 nm) dalam semua NCC yang dihasilkan. Spektrum FTIR

menunjukkan bahawa puncak yang dikaitkan dengan lignin dan hemiselulosa tidak hadir selepas rawatan kimia dan pelunturan dan nampaknya kedua-dua komponen telah dikeluarkan sepenuhnya daripada sampel selepas hidrolisis asid. Analisis XRD menunjukkan bahawa kehabluran meningkat selepas hidrolisis asid yang menunjukkan sifat kristal NCC terpencil untuk semua sampel. NCC dirawat dengan 7% natrium hipoklorit menunjukkan kehabluran tertinggi iaitu 71.1% walaupun degradasi selulosa berlaku pada peringkat pelunturan. Analisis TGA menunjukkan bahawa degradasi NCC berlaku pada 143°C, dan T_{max} berada pada 188°C dengan 25.9% residu pada 600°C. Profil kelikatan tiga wilayah yang dilihat dalam sistem kristal cecair diperhatikan dalam sampel yang disonikasi. Selain itu, berdasarkan analisa sifat reologi, boleh disimpulkan bahawa NCC berkelakuan sebagai bahan penipisan ricih. Kerja lanjut telah dijalankan untuk menghasilkan mayonis rendah lemak dengan NCC digunakan sebagai pengganti lemak. Mayonis dengan 30% pengurangan minyak telah disediakan dan digabungkan dengan NCC, MCC dan MCC+NCC (1:1). Kedua-dua kawalan dan mayonis dengan penggantian lemak kelihatan stabil walaupun selepas satu bulan disimpan di suhu bilik. Pengurangan minyak dalam mayonis memang mempengaruhi warna kerana semua mayonis yang dikurangkan lemak mempunyai kecerahan (L*) yang lebih besar daripada sampel kawalan. Indeks keputihan semua mayonis yang dikurangkan lemak adalah dalam julat 68%. Selain itu, apabila lemak digantikan oleh NCC, a* meningkat dengan ketara, manakala b* menurun selepas penyimpanan. Semua sampel menunjukkan emulsi yang stabil walaupun selepas 1 bulan penyimpanan. Saiz zarah emulsi minyak untuk semua mayonis adalah dalam julat teori, antara 200 hingga 500 nm. Sifat reologi mayonis

yang dihasilkan menunjukkan tingkah laku penipisan ricih. Daripada semua keputusan yang diperoleh, NCC yang diektrak berpotensi untuk digunakan sebagai pengganti lemak dalam mayonis kerana ia telah menunjukkan kestabilan terbaik dalam analisis emulsi dan sifat reologi.

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I certify that an Examination Committee has met on 22 July 2022 to conduct the final examination of Aida Safina Binti Aridi on her degree thesis entitled " Isolation of Nanocrystalline Cellulose from *Leucaena leucocephala* Pods and Its Application as a Fat Replacer in Production of Low-Fat Mayonnaise" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15th March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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LIST OF ABREVIATIONS

α	Alpha
β	Beta
℃	Degree celsius
%	Percentage
MCC	Microcrystalline cellulose
NCC	Nanocrystalline cellulose
NFC	Nanofibrillated cellulose
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
FRAP	Fluorescence recovery after photobleaching
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl radical
FTIR	Fourier transform infrared spectroscopy
XRD	X-ray diffraction
TEM	Transmission electron microscopes
FESEM	Field Emission Scanning Electron Microscope

CHAPTER 1

INTRODUCTION

1.1 *Introduction*

In recent decades, enormous efforts have been made to improve new materials and replace broadly used petroleum-based products using renewable biomass feedstock. Biocompatible composites and biodegradable plastics produced from bio-renewable resources could replace petrochemical-based polymers, reducing global dependence on non-renewable sources. These biomass feedstocks are of great interest due to the possibility of nontoxicity, renewability, biodegradability, and sustainability (Amini et al. 2017; Ilyas et al. 2019).

Malaysia is one of the richest countries in biodiversity and has a high potential for biomass crop diversification. Some underutilized biomass species are yet to be discovered, which can be planted to provide a sustainable energy source and biomaterials. *Leucaena leucocephala* (*L. leucocephala*) is being considered one of the potential forest plantation plants by the Malaysian Timber Industrial Board (MITB). It is locally known as “petai belalang” and has amazed thousands of people for its high-density wood yields (Wan Mohd Nazri et al. 2011), fast growth (Wan Mohd Nazri et al. 2009) and strong adaptability (Rasat et al. 2016).

The Malaysia Agricultural Research Development Institute (MARDI) has used this multipurpose tree for shade and wind protection in various crops, especially during early growth and food for some animals. The trees are typically felled, burnt, or left to decay when the plantation crops mature (Adnan 2012). It has motivated researchers to

turn these bio-fibres into valuable sustainability products. One solution to the problem of *L. leucocephala* being left to rot is converting them into value-added products such as nanomaterials. This nanomaterial can be isolated from cellulosic plants by chemical, mechanical or enzymatic processes.

The study reported that the use of *L. leucocephala* mature pod as raw materials to produce nanocrystalline cellulose is limited. The motivation is to convert this biomass into cellulose, particularly nanocrystalline cellulose, owing to its cellulosic nature and as carbohydrates, reserve drive to this study to be conducted. Husin et al. (2017) have reported using other parts of the *L. leucocephala* tree, particularly the seeds, to isolate the cellulose from this biomass waste. However, studies on the isolation of nanocrystalline cellulose from different parts of the plant, especially the mature pods, have not yet been reported. Therefore, this study is done to provide data on the first nanocrystalline cellulose isolated from the mature pods of *L. leucocephala*.

For the value-added utilization of nanocrystalline cellulose from *L. leucocephala*, the nanocrystalline cellulose obtained has been used as a fat-replacer in the development of low-fat mayonnaise. The selected isolated and characterized nanocrystalline cellulose were later incorporated into the mayonnaise formulation, and its physical and chemical stability was investigated for one month.

1.2 *Problem Statement*

Owing to the annual availability of the *L. leucocephala* biomass, particularly the mature pods and the continuous supply of the waste as resources, the utilization of the mature pods into a value-added product is gaining much attention from researchers.

The goals are to use the waste resources contributing to environmental pollution to produce valuable products. Therefore, the isolation of nanocrystalline cellulose (NCC) from *L leucocephala* mature pods has been proposed to widen the utilization of the *L. leucocephala* tree.

Many research has been focused on investigating the parameters involved during acid hydrolysis. Still, little study has explored how pre-treatment, especially bleaching, can affect the NCC obtained. The bleaching step is critical to cellulose quality. Therefore, in this study, four different bleaching agents (sodium chlorite, sodium hypochlorite, potassium permanganate, and oxalic acid) have been used to investigate the suitable bleaching agents in the purification of cellulose fibres. Then, the best percentage of sodium hypochlorite (3%, 5% and 7%) used during the pre-treatment is also evaluated to obtain the best nanocrystalline cellulose. Sulfuric acid hydrolysis is used to obtain nanocrystalline cellulose as it will produce NCC particles with anionic sulfur groups on the surface, leading to an electrostatically stabilized NCC aqueous suspension. The NCC obtained will be comparable for their properties in terms of yield, thermal stability and crystallinity.

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LIST OF PUBLICATIONS

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