ISOLATION OF NANOCRYSTALLINE CELLULOSE FROM *Leucaena leucocephala* (Lam.) de Wit PODS AND ITS APPLICATION AS A FAT REPLACER IN PRODUCTION OF LOW-FAT MAYONNAISE

By

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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

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July 2022

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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 and seems that 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 that is 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 seen in liquid crystalline systems was observed in sonicated samples. Besides that, from Power law, 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% of 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 fat was replaced by NCC, a* increased significantly, whereas b* decreased after storage. All samples showed stable emulsion even after 1 month of storage.

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, mayonnaise with NCC as a fat replacer shows the best stability.

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

PENGASINGAN NANOKRISTAL DARIPADA POD *Leucaena leucocephala* (Lam.) de Wit DAN PENGGUNAANNYA SEBAGAI PENGGANTI LEMAK DALAM PENGHASILAN MAYONIS RENDAH LEMAK

Oleh

AIDA SAFINA BINTI ARIDI

Julai 2022

Pengerusi : Profesor Yus Aniza binti Yusof, PhD
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Kajian ini menyiasat sifat selulosa nanohabluran (NCC) yang diasingkan daripada buah matang *L. leucocephala*. Empat agen peluntur yang berbeza telah digunakan semasa proses pelunturan, diikuti oleh hidrolisis asid sulfurik. Sifat fizikal dan kimia, indeks kehabluran, kapasiti mengikat air, dan kelakuan terma NCC yang diperolehi ditentukan dengan menggunakan mikroskop elektron penghantaran (TEM), inframerah transformasi Fourier (FTIR), pembelauan sinar-X (XRD) dan analisis termogravimetrik (TGA), masing-masing. Keputusan menunjukkan gentian individu zarah bentuk rod dengan diameter purata bersaiz nao (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 Tmax 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, daripada undang-undang Kuasa, boleh disimpulkan bahawa penggantungan NCC berkelakuan sebagai bahan penipisan ricih. Kerja lanjut telah dijalankan untuk menghasilkan mayonis rendah lemak dengan NCC terpencil 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. Pengurangan minyak dalam mayonis memang mempengaruhi warna kerana semua mayonis yang dikurangkan lemak

mempunyai kecerahan (L*) yang lebih besar daripada sampel kawalan. 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, mayonis dengan NCC sebagai pengganti lemak menunjukkan kestabilan terbaik

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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 ABBREVIATIONS

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 because it not only acts as an unwanted colour remover but is also capable of degrading non-cellulose compounds. 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 sulphur 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.

1.3 Research Aims and Objectives

This research aims to isolate NCC from *L. leucocephala* mature pods and to investigate the suitability of this NCC to be used as a fat-replacer as an alternative to a commercial fat replacer available in the market. To achieve the aim, several objectives were set, as listed below:

i. To investigate how different bleaching agents will affect the isolation of cellulose and nanocrystalline cellulose (NCC) and how different percentage of sodium hypochlorite affect the properties of NCC obtained.

ii. To evaluate the physical and rheological properties of isolated NCC.

iii. To evaluate the isolated NCC as a fat-replacer in the production of low-fat mayonnaise

1.4 Research Hypothesis

To address the objectives, several hypotheses were stated as follows:

i. Due to the difference in chemical composition between *L. leucocephala* mature pods and wood pulp, it is reasonable to assume that the optimal bleaching conditions for the pods are different from those used for wood pulps. Thus, it is critical to investigate how bleaching agents and conditions affect yield quality.

i. Pre-treatment (delignification) process using the right bleaching agent produced a higher yield and purified cellulose. Besides that, if the right combination for acid hydrolysis of *L. leucocephala* mature pods with sulfuric acid, NCC produced should be purified, has a high aspect ratio and crystallinity index.

ii. When the purified NCC is obtained using the correct percentage of sodium hypochlorite during pre-treatment, it can be applied as a fat-replacer in low-fat mayonnaise. This is because a purified NCC can adsorb to air–water or oil-water interfaces and stabilize foams or emulsions, self-assemble in aqueous solutions to form gel networks and act as fillers or fat replacers.

1.5 Scope and Significance of Research

The novelty of the research lies in the contribution of the research data findings dealing with *L. leucocephala* and nanocrystalline cellulose. This plant is mainly burnt or left to decay, contributing to waste management problems. Therefore, researchers tend to find a solution for converting biomass into something valuable and contributing to the growth of Malaysia's economy. *L. leucocephala* is widely used in Malaysia as livestock forage, reforestation material, furniture, and construction timber. Recently, a study on cellulose-based isolation, particularly in nanocellulose fibres, gained much attention. To the best of our knowledge, isolating nanocrystalline cellulose from *L. leucocephala* mature pods has not been reported yet. Thus, this is a pioneer work to provide the data on nanocrystalline cellulose isolated from this plant, and we can claim that our research is the first to study the isolation and characterization of nanocrystalline cellulose from *L. leucocephala* mature pods.

Understanding different factors that affect the response of mayonnaise properties is vital in producing good quality NCC using sulfuric acid hydrolysis. This study uses three different percentages of sodium hypochlorite during pre-treatment. The best quality of NCC is used as a fat-replacer to replace oil in low-

fat mayonnaise. Such knowledge is also valuable for producing stable low-fat mayonnaise and provides an application for the isolated NCC.

1.6 Thesis organization

This thesis consists of seven chapters. The first chapter is an introduction to the background study, problem statement, objectives, novelty and the hypothesis of the study. Chapter 2 contains a review of published literature relating to this subject. The literature review guided the reader to understand the study's design, methods, analysis and expected trends.

Chapter 3 covered the information on the materials and chemicals that were used during the study. It also describes the experimental methods, from the preparation of samples to producing nanocrystalline cellulose. It also entails various analyses to characterise the samples, including FTIR, FESEM, TEM, XRD, TGA, and other chemical composition analyses.

In chapter 4, the properties of nanocrystalline cellulose isolated from *L. leucocephala* mature pods are discussed in detail. The physical and rheological properties of NCC suspension are discussed in chapter 5. In chapter 6, an attempt was carried out to use isolated NCC as a fat-replacer in the development of low-fat mayonnaise. The physical stability of the mayonnaise produced is evaluated for one month.

Finally, chapter 7 summarizes the conclusion of all findings from this study. Also, recommendations and suggested future works were mentioned in this chapter.

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