



UNIVERSITI PUTRA MALAYSIA

***PHYSICOCHEMICAL CHARACTERISTICS, MICROBIAL SAFETY AND
BIOLOGICAL ACTIVITIES OF FERMENTED BLACK SESAME
(*Sesamum indicum* L.) SEEDS DREGS***

NUR KAMARIAH BINTI ROSNI

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BIOLOGICAL ACTIVITIES OF FERMENTED BLACK SESAME (*Sesamum
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By

NUR KAMARIAH BINTI ROSNI

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

June 2020

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

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Utilization of dregs has been widely used, especially in Indonesia, such as peanut dregs into local Indonesian food known as “oncom”. Sesame seed dregs are derived from the process of making sesame oil. The seed dregs were then proceeded with spontaneous fermentation for 7 days at room temperature (23-27°C). During fermentation, physicochemical characteristics of the food product may undergo changes with microorganisms and bioactivities involvement in the process. The aims of this study were to analyse physicochemical characteristics and microbiological safety and quality of black sesame seed dregs during fermentation, to determine antimicrobial and antioxidant activity, and to identify bioactive compounds in raw black sesame seeds and fermented seed dregs extract. Physicochemical characteristics including internal temperature, pH value, total soluble solid (TSS), water activity (a_w), colour determination, proximate analysis and texture profile analysis of seed dregs during fermentation were analysed using Association of Analytical Communities (AOAC) methods for food analysis. The present of microorganisms including total plate count (TPC), *Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus*, lactic acid bacteria (LAB), coliform bacteria, mould and yeast in seed dregs during fermentation were isolated using microbial selective media. The collected samples for analysis were raw seeds and seed dregs at day 0, 1, 3, 5 and 7 of fermentation. Raw seeds and fermented seed dregs (day 7) were extracted using water and ethanol and the extracts were further determined for antimicrobial activity against foodborne pathogens using Clinical and Laboratory Standard Institute (CLSI) methods. The total phenolic content was determined using a Folin-Ciocalteu assay and antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The bioactive compounds in ethanol extract of raw seeds and fermented seed dregs were determined using Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS). The results showed that internal temperature, pH and TSS showed no significant differences

while a_w shows the significant increased from 0.53 to 0.92% during fermentation. The colour determination of final product had the highest lightness and yellowness but had the lowest redness. Moisture was significantly increased while ash, crude protein and carbohydrate were decreased and crude fat and fibre were showed no significant differences during fermentation. A gradually increased in hardness and decreased in springiness, chewiness and resilience were observed while cohesiveness showed no significant differences. Microbial counts were reduced in number of *P. aeruginosa* and *B. cereus* while increased in number of TPC, *S. aureus*, lactic acid bacteria, coliform bacteria and mould and yeast. The extraction yield using ethanol showed higher yield than water. Diameter of inhibition zones of extracts against 16 pathogenic microorganisms were in the ranged from 6.50 to 11.00 mm. Minimum inhibitory concentrations (MICs) of water extract were in the range from 3.13 to >50.00 mg/mL, mostly effective against *B. cereus* ATCC33019. Meanwhile, MICs of ethanol extract were in the ranged from 6.25 to 50.00 mg/mL, mostly effective against *P. aeruginosa* ATCC9027, *B. megaterium* ATCC14581 and *C. albicans* ATCC10231. Minimum bactericidal/fungicidal concentrations of water and ethanol were spread from 6.25 to >50.00 mg/mL and 25.00 to >50.00 mg/mL, respectively. Time-kill curve study showed *S. aureus* ATCC29737, *E. coli* ATCC43895 and *B. cereus* ATCC33019 were completely killed when exposed to ethanol extract of raw seeds and fermented seed dregs in 0.5 h incubation at 4× MIC. One hour had been taken to completely killed *P. aeruginosa* ATCC9027 and *Candida albicans* ATCC10231 after exposing to raw seeds extract while two hours had been taken after exposing to fermented seed dregs extract at 4× MIC. The fermented seed dregs extract showed higher total phenolic contents rather than raw seeds and seed dregs extracts. IC₅₀ of the fermented seed dregs extract showed lower concentration than raw seeds and seed dregs extracts. There were 19 bioactive compounds in raw seeds and 24 compounds in fermented seed dregs extract detected by GC-MS including sesamin and sesamol. Six compounds were identified to be the first time reported in sesame seeds by LC-MS which were cyclo(L-leucyl-L-prolyl), docosahexaenoic acid (DHA), enoxolone, α-eleostearic acid, 16-hydroxyhexadecanoic acid and oleanolic acid. In conclusion, there were changes on physicochemical characteristics during fermentation and fermented seed dregs had low level of microbial counts, rich of lactic acid bacteria and detected bioactive compounds were potentially good for antimicrobial and antioxidant activities.

Keywords: antimicrobial activity, antioxidant activity, bioactive compounds, fermented black sesame seed dregs, microbial safety, physicochemical characteristics

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

**CIRI-CIRI FISOKOKIMIA, KESELAMATAN MIKROB DAN AKTIVITI
BIOLOGI HAMPAS BIJI BIJAN (*Sesamum indicum* L.) HITAM YANG
DITAPAI**

Oleh

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Penggunaan hampas telah digunakan secara meluas terutamanya di Indonesia, seperti hampas kacang tanah ke dalam makanan tempatan Indonesia yang dikenali sebagai "oncom". Hampas biji bijan terhasil daripada proses pembuatan minyak bijan. Hampas biji kemudian akan melalui proses penapaian secara langsung selama 7 hari di suhu bilik (23-27°C). Semasa penapaian, ciri-ciri fisikokimia produk makanan akan mengalami perubahan dengan penglibatan mikroorganisma dan aktiviti bio dalam pemprosesan. Tujuan kajian ini adalah untuk menganalisis ciri-ciri fisikokimia serta keselamatan dan kualiti microbiologi terhadap hampas biji bijan hitam semasa penapaian, untuk mengkaji aktiviti antimikrob dan antioksidan, dan untuk mengenalpasti sebatian bioaktif dalam ekstrak biji bijan hitam mentah dan ekstrak hampas biji yang ditapai. Ciri-ciri fisikokimia termasuk suhu dalaman, nilai pH, jumlah pepejal larut (TSS), aktiviti air (a_w), pengenalpastian warna, analisis proksima dan analisis profil tekstur ke atas hampas biji semasa penapaian telah dianalisis menggunakan kaedah Komuniti Persatuan Analitikal (AOAC) untuk analisis makanan. Kehadiran mikroorganisma termasuk plat kiraan keseluruhan (TPC), *Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus*, bakteria asid laktik (LAB), bakteria koliform, kulat dan yis ke atas hampas biji yang ditapai semasa penapaian dianalisis menggunakan media selektif mikrob. Sampel yang di ambil untuk analisis adalah biji mentah dan hampas biji pada hari penapaian yang ke 0, 1, 3, 5 and 7. Biji mentah dan hampas biji yang ditapai (hari ke 7) diekstrak menggunakan air dan etanol, dan ekstrak berkenaan telah digunakan untuk mengenalpasti aktiviti mikrobial menentang patogen bawaan makanan menggunakan kaedah Institut Standard Klinikal dan Makmal (CLSI). Jumlah kandungan fenolik ditentukan menggunakan ujian Folin-Ciocalteu dan aktiviti antioksidan ditentukan menggunakan ujian memerangkap radikal 2,2-diphenyl-1-picrylhydrazyl (DPPH). Sebatian bioaktif dalam ekstrak etanol biji mentah dan hampas biji yang ditapai ditentukan oleh Gas Kromatografi-Jisim Spektrometri (GC-MS) dan Cecair Kromatografi-Jisim

Spektrometri (LC-MS). Keputusan menunjukkan suhu dalaman, pH dan TSS menunjukkan tiada perbezaan signifikan manakala a_w menunjukkan peningkatan signifikan daripada 0.53 kepada 0.92% semasa penapaian. Penentuan warna bagi produk akhir mempunyai cahaya dan kekuningan yang tinggi tetapi kemerahan yang rendah. Kandungan kelembapan meningkat secara signifikan manakala kandungan abu, protin dan karbohidrat mengalami penurunan, dan kandungan lemak dan serat menunjukkan tiada perbezaan signifikan semasa penapaian. Peningkatan secara beransur-ansur dalam ciri kekerasan dan penurunan dalam kekenyalan, kelembutan dan daya tahan telah dikenalpasti manakala ciri kohesif menunjukkan tiada perbezaan signifikan. Kiraan mikrob menunjukkan penurunan pada *P. aeruginosa* dan *B. cereus* manakala peningkatan pada TPC, *S. aureus*, bakteria asid laktik, bacteria koliform, dan kulat dan yis. Hasil ekstrak etanol lebih tinggi berbanding ekstrak air. Diameter zon penghambatan ekstrak menentang 16 jenis mikroorganisma patogenik menunjukkan julat perencatan di antara 6.50 sehingga 11.00 mm. Kepekatan Perencatan Minimum (MICs) ekstrak air bagi biji mentah dan hampas biji yang ditapai adalah di antara 3.13 sehingga >50.00 mg/mL, paling efektif menentang *B. cereus* ATCC33019. Manakala, MICs ekstrak etanol pula adalah di antara 6.25 sehingga 50.00 mg/mL, paling efektif menentang *P. aeruginosa* ATCC9027, *B. megaterium* ATCC14581 dan *C. albicans* ATCC10231. Kepekatan minimum bakterisida/fungisida untuk ekstrak air dan etanol adalah masing-masing di antara 6.25 sehingga >50.00 mg/mL dan 25.00 sehingga >50.00 mg/mL. Analisis keluk-masa pembunuhan menunjukkan *S. aureus* ATCC29737, *E. coli* ATCC43895 dan *B. cereus* dibunuh sepenuhnya apabila terdedah kepada ekstrak etanol bagi biji mentah dan hampas biji yang ditapai dalam pengeraman 0.5 h pada 4×MIC. Satu jam diambil untuk *P. aeruginosa* dan *Candida albicans* dibunuh sepenuhnya apabila terdedah kepada ekstrak biji mentah manakala dua jam diambil apabila terdedah kepada ekstrak hampas biji yang ditapai pada 4×MIC. Ekstrak hampas biji yang ditapai mempunyai kandungan jumlah fenolik lebih tinggi berbanding ekstrak biji mentah dan hampas. IC₅₀ ekstrak hampas biji yang ditapai menunjukkan kepekatan lebih rendah berbanding ekstrak biji mentah dan hampas. Terdapat 19 sebatian bioaktif dalam ekstrak biji mentah dan 24 sebatian dalam ekstrak hampas biji yang ditapai dapat dikenalpasti oleh GC-MS termasuk sasamin dan sesamol. Enam sebatian telah dikenalpasti pertama kali dilaporkan dalam biji bijan oleh LC-MS iaitu cyclo(L-leucyl-L-prolyl), docosahexaenoic acid (DHA), enoxolone, α -eleostearic acid, 16-hydroxyhexadecanoic acid dan oleanolic acid. Kesimpulannya, terdapat perubahan ciri-ciri fisikokimia semasa proses penapaian dan hampas biji yang ditapai mempunyai nilai kiraan mikrob yang rendah, kaya dengan bakteria asid laktik dan sebatian-sebatian bioaktif yang dikesan berpotensi baik untuk aktiviti antimikrob dan antioksidasi.

Kata kunci: aktiviti antimikrob, aktiviti antioksidasi, sebatian bioaktif, hampas biji bijan hitam yang ditapai, keselamatan mikrob, ciri-ciri fisikokimia.

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LIST OF ABBREVIATIONS

°C	Degree Celsius
%	Percentage
µL	Microlitre
a^*	Red/greenness
a_w	Water activity
ANOVA	Analysis of variance
ASE	Accelerated solvent extraction
AOAC	Association of Official Analytical Chemists
ATCC	American type culture collection
b^*	Yellow/blueness
BaCl ₂	Barium chloride
BHA	Beta-hydroxy acid
BHT	Butylated hydroxytoluene
BSA	<i>Bacillus cereus</i> selective agar
CA	Coliform Agar
CFU	Colony forming unit
CHX	Chlorhexidine
CLSI	Clinical and Laboratory Standards Institute
cm	Centimetre
Da	Dalton
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl

EMB	Eosin methylene blue
Eth	Ethanol extract
EU	European Union
FFAs	Free fatty acids
FSD	Fermented seeds dregs
g	gram
GAE	Gallic acid equivalent
GRAS	Generally recognized as safe
GC-MS	Gas chromatography-mass spectrometry
h	Hour
H ₂ SO ₄	Sulphuric acid
HPLC-MS	High-pressure liquid chromatography mass spectrometry
IBS	Institute of Bioscience
IC ₅₀	Half-maximal inhibitory concentration
ICMSF	International Commission on Microbiological Specifications for Foods
kHz	Kilohertz
kPA	Kilopascal
L*	Lightness
L	Litre
LAB	Lactic acid bacteria
LC-MS	Liquid chromatography-mass spectrometry
<i>m/z</i>	Mass-to-charge ratio
MAE	Microwave assisted extraction
MBC	Minimum bactericidal concentration
MF	Molecular formula

MFC	Minimum fungicidal concentration
mg	Milligram
MHA	Mueller Hinton agar
MHB	Mueller Hinton broth
MIC	Minimum inhibitory concentration
min	Minute
mL	Millilitre
mm	Millimetre
MRS	Man, Rogosa and Sharpe
MSA	Mannitol salt agar
MW	Molecular weight
N	Normality
NaOH	Sodium hydroxide
Nm	Nanometre
PBS	Phosphate buffered saline
PCA	Plate count agar
PDA	Potato dextrose agar
ppm	Parts per million
psi	Pounds per square inch
PUFA	Polyunsaturated fatty acid
RI	Retention index
rpm	Revolutions per minute
RRI	Relative retention index
RS	Raw seeds
s	Second
SI	Similarity index

spp.	Species
t _R	Retention time
TPA	Texture profile analysis
TPC	Total plate count
TSS	Total soluble solid
UPM	Universiti Putra Malaysia
w/v	Weight per volume
WE	Water extract
XLD	Xylose lysine deoxylate



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CHAPTER 1

INTRODUCTION

1.1 Background

Sesame seeds have beneficially on nutritional and medicinal that easily extract, good stability and high resistance in dry season (Sheahan, 2014). Black sesame seeds have been said more beneficial to human health; therefore, the price is higher than white sesame seeds. Wang et al. (2018) stated that the black sesame seeds can be classified as a healthy food as their identified compounds on the research were similar with specific characteristics in other studies. The use of sesame oil has become increasingly popular these days for food ingredient, pharmaceutical and cosmetic uses especially in Korea and Indonesia. Sesame oil is acknowledged rich in oleic and linoleic acids (essential unsaturated fatty acids) and have abundant of vitamin E (Hansen, 2011; Asghar et al., 2014). The extraction of sesame oil produced meaningful by-product that rich of protein which called as sesame oil meal or cake or dregs. This sesame dregs are beneficial source of protein for animal (Oplinger et al., 1990; Hansen, 2011). According to Suja et al. (2005), sesame dregs can be food grade (from dehulled sesame seeds), or used as a feed for livestock, especially ruminants and poultry (from undecorticated sesame seeds) or utilized as fertilizer regarding to the extracted methods used. In addition, Surono (2016) indicated that dregs of products such as coconut dregs, peanuts dregs and sesame seeds dregs can be used as food or functional food through fermentation process.

Interestingly, in West Java (Indonesia) the sesame seeds dregs are consumed after spontaneous fermentation namely *dage* while in Central Java (Indonesia) called *cabuk*. *Dage* and *cabuk* are defatted sesame seed-based fermented food. The difference between *dage* and *cabuk* is, *dage* is only spontaneous fermentation of sesame seeds dregs while *cabuk* is alkaline solid-state fermentation as it is produced by adding rice straw ash water with sesame seeds dregs (Sarkar and Nout, 2014). In Sierra Leone, Nigeria, fermented sesame seeds or called as *Ogiri* is one of their traditional foods that used as low-cost protein by Nigerian especially poor families to change the fish during rainy season as the price of fish is too expensive during that time (Jonsyn, 1990). Hence, the fermented sesame seeds that have longer shelf life can provide some protein during that season to replace fish.

Fermentation is one of the studies in food microbiology which is included in preservation technique. It is the process of freeing up water molecules and the complex compound will convert into simpler compounds. It will increase the area of raw materials and indirectly produced edible food products and removing anti-nutritional factors to ensure the safety of food (Steinkraus, 2018). The plant-based processing foods such as fermented food have improve in taste and flavour that indirectly surging the acceptability (Ejoh et al., 2007), however the

processing such as heating processes can give some effect on the chemistry of these foods. During the fermentation process, the microorganism might be present and can change physical characteristic and biological of the food product. The evaporation and metabolism of the microorganism cause the water activity, a_w might be varies (Tsao, 1999) while the pH value may drop along the with fermentation day. Based on previous studies, it is confirmed that fermentation process can enhance the nutritious content while increasing the food digestibility (Ray and Didier, 2014; Hasan et al., 2014; Tamang, 2016).

Fermented sesame seeds dregs usually made manually. Hence, a good hygiene practices should be emphasized during fermentation to lower or discards especially from microorganisms that dangerous for health. Previous studies had shown major microorganisms usually involved in this fermented food are bacilli family like *Bacillus subtilis* and *B. cereus* (Steinkraus, 2018). Besides, as studied by Gadaga et al. (2007) and Capozzi et al. (2017), *Bacillus cereus*, *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus* and *Aeromonas* are the most commonly encountered pathogens in African fermented foods. Steinkraus (2018) also stated that *tempe bongkrek*, which is also one of fermented dregs as it is produced from coconut dregs, has been reported to be contaminated by *Pseudomonas* species such as *Pseudomonas aeruginosa* and *P. cocovenenans* if no proper handling during process of fermentation. Fermented coconut dregs it not common fermented food, but well known as Indonesia cuisines other than tempeh and *oncom* which also food from fermentation process.

Natural antioxidants are one of the essential components found in plant have ability in scavenging free radical inside the biological system (Anjaneyulu et al., 2003). Moreover, extract of plants potentially has antimicrobial compounds against disease and food spoilage causing microorganisms. Sesame seeds have two lignans which are called sesamin and sesamol that helps to prevent rancidity (Bennett et al., 1997; Hemalatha and Rao, 2004W; Moazzami et al., 2006; Asghar et al., 2014; Wu et al., 2016; Qadir et al., 2018). The sesamol will converted into sesamol during roasting process. Sesamol have anti-oxidative effects that induce the growth arrests and apoptosis in cancer cells (Tunde-Akintunde et al., 2012). It also has phenolic and benzodioxide group that helps in antioxidant activity.

To the best of our knowledge, there are still no studies regarding to physicochemical characteristics and microbiological safety of black sesame seeds dregs during fermentation. Furthermore, there are also lacks of studies on determination of bioactivities and identification of bioactive compounds of black sesame seeds dregs and fermented seeds dregs extracts. Therefore, this research is aiming to analyse physicochemical characteristics and microbiological safety of black sesame seeds dregs during fermentation, to determine the antimicrobial and antioxidant activity and to identify the bioactive compounds using Liquid Chromatography Mass-Spectrometry (LC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS).

1.2 Problem Statements

During the fermentation process, physicochemical characteristics and biological of the food product may undergo changes (Steinkraus, 2002) with microorganisms' involvement in some of the process (Caplice and Fitzgerald, 1999). Spontaneous fermentation is an uncontrolled process, involving succession of different types of microorganism including pathogenic and non-pathogenic bacteria that results in variety of end product of fermented food. Mehta et al. (2012) stated that bioactivities on foods that involve chemical reactions during fermentation process could give effects on food properties and bioactive compounds (antioxidant and antimicrobial compounds) inside food product. Natural antioxidants are one of the essential components found in plant that have potential in scavenging of free radical inside the biological system (Anjaneyulu et al., 2003) and plant extract also potentially have antimicrobial compounds against disease and food spoilage causing microorganisms. There are a lot of studies are done on raw sesame seeds and fermented sesame seeds. However, no systematic study has been reported regarding fermented black sesame seeds dregs and its extracts. Thus, the physicochemical characteristics and microbiological safety of black sesame seeds dregs during fermentation need to be analysed and the antimicrobial and antioxidant of raw black sesame seeds, seeds dregs and fermented seeds dregs extract need to be determined. On the other hand, bioactive compounds that contributed in antimicrobial and antioxidant activity of raw black sesame seeds, seeds dregs and fermented seeds dregs extract also need to be identified.

1.3 Objectives

The objectives of this study are:

1. To analyse the physicochemical characteristics and microbiological safety of black sesame seeds dregs during fermentation.
2. To determine the antimicrobial and antioxidant activity of raw black sesame seeds and fermented seeds dregs extracts.
3. To identify the bioactive compounds in raw black sesame seeds and fermented seeds dregs extracts by Liquid Chromatography Mass-Spectrometry (LC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS).

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LIST OF PUBLICATIONS

Manucript

- Rosni, N. K., Sanny, M., Bahranor, N. S. A. and Rukayadi, Y. (2020). Physicochemical characteristics, microbiological safety and sensory acceptability of coconut dregs during fermentation using *Rhizopus oligosporus*. *Food Research*, 4(5): 1402-1411. (Published)
- Rosni, N. K., Sanny, M., Mustaffer, N. H. and Rukayadi, Y. Antimicrobial and antioxidant activities of ethanolic extract of fermented black sesame (*Sesamum indicum* L.) seed dregs. *LWT – Food Science and Technology*. (Submitted)
- Rosni, N. K., Yusof@Daud, N. A. M. and Rukayadi, Y. Effect of *Nigella sativa* L. seed extract on foodborne pathogens and microflora in raw chicken wing. *Journal of Food Safety*. (Submitted)

Oral presentation

- Rosni, N. K. Physicochemical characteristics, microbiological safety and sensory acceptability of coconut dregs during fermentation using *Rhizopus oligosporus*. Monash Science Symposium 2018. Monash University Malaysia, Subang Jaya, Selangor. 21st - 23rd November 2018.



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