OPTIMIZATION OF PROCESSING PARAMETERS ON THE YIELD OF OLEORESIN FROM *ZINGIBER ZERUMBET* AND ITS ANTIBACTERIAL ACTIVITY

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A thesis submitted in fulfillment of the
requirements for the award of the Degree
of Master of Engineering (Bioprocess)

Faculty of Chemical Engineering and Natural Resources Engineering
Universiti Teknologi Malaysia

NOVEMBER 2008

Dedicated to my beloved Husband, Mak, Abah, Ariff, Iman and Affiq

ACKNOWLEDGEMENT

I am grateful to Allah that I could finish my thesis. In preparing this thesis, I was in contact with many people, researchers, academicians, and practitioners. They have contributed towards my understanding and thoughts. In particular, I would like to convey my gratitude to my thesis supervisor Prof. Dr. Mohamad Roji bin Sarmidi for his guidance and advice throughout this project. Without his continued support and interest, this thesis would not have been the same as presented here.

I also indebted to Chemical Engineering Pilot Plant (CEPP) and Chemical Analysis Laboratory for giving me the permissions to use the facilities and to the staff, the technicians and lab assistances, special thanks for their guides and assistances.

A great appreciation for Mrs. Norhayati for her helps, advice and suggestions towards the application of statistical software and analysis of the process.

To Mrs. Mariani, Mrs. Roshanida, Mrs. Zalina, Mrs. Zarani and Ms Roslinda, thank you. Finally, to all those whom directly and indirectly had helped in making this project a success, thank you.

ABSTRACT

The effect of processing parameters on the yield of oleoresin from *Zingiber zerumbet* and its antibacterial activity was investigated. The preliminary experiments were carried out to determine the main parameters that affect the yield of oleoresin, zerumbone content and antibacterial activity on the *Escherichia coli* and *Staphylococcus aureus* of *Z. zerumbet* oleoresin. The main parameters were found to be extraction time, type of solvent used and blanching treatment. The range of those parameters that was investigated to get the highest of all yield were blanching treatments (untreated, boiled, steamed), solvent (acetone, ethanol and methanol) and extraction time (6 hours to 18 hours). A D-optimal design was employed to study the effect of different range parameter of *Z. zerumbet* oleoresin on four response variables; the yield of oleoresin, the zerumbone content, the antibacterial activity on the *E. coli* and *S. aureus.* Analysis of variance and response surface methodology were applied to identify the optimal processing parameter. The optimal processing parameters that fulfilled the requirement for yield of oleoresin, zerumbone content, antibacterial activity on the *E. coli* and *S. aureus* with high desirability were found to be 18 hours of extraction time, methanol as the solvent used and steaming as the blanching treatment. The desirability value was 84 %. Meanwhile, the optimal yield of *Z. zerumbet* oleoresin was 31.50 % (w/w), the zerumbone content was 0.56 % (w/w) and the antibacterial activities (inhibition zone) on the *E. coli* and *S. aureus* were 14.58 mm and 13.51 mm respectively. Processing optimization has resulted in an increase of overall antibacterial activity on the *E. coli* and *S. aureus* of *Z. zerumbet* oleoresin as well as the increment yield of oleoresin and zerumbone content.

ABSTRAK

Kesan parameter proses terhadap hasil oleoresin lempoyang (*Zingiber zerumbet)* dan aktiviti antibakterianya telah dikaji. Ujian awal dilakukan untuk menentukan parameter-parameter utama yang mempengaruhi hasil oleoresin, kandungan zerumbon serta aktiviti antibakteria terhadap *Escherichia coli* dan *Staphylococcus aureus* dalam oleoresin lempoyang. Parameter-parameter utama yang bersesuaian adalah rawatan penceluran, pelarut dan masa pengekstrakan. Julat parameter-parameter tersebut yang dikaji bagi mendapatkan keseluruhan nilai hasil yang tertinggi terdiri daripada rawatan penceluran (stim, rebus dan segar), pelarut (aseton, etanol dan metanol) dan masa pengekstrakan (6 jam hingga 18 jam). Satu kaedah rekabentuk *D-optimal* telah digunakan untuk mengkaji kesan perbezaan parameter proses oleoresin lempoyang terhadap empat pembolehubah respon; hasil oleoresin, kandungan zerumbon, aktiviti antibakteria terhadap *E. coli* dan *S. aureus*. Analisis varians dan kaedah tindak balas permukaan telah diaplikasikan untuk mengenalpasti parameter yang optimal. Parameter-parameter proses optimal yang dikenalpasti memenuhi kecenderungan ideal yang tinggi bagi hasil oleoresin, kandungan zerumbon dan aktiviti antibakteria terhadap *E. coli* dan *S. aureus* adalah masa pengekstrakan selama 18 jam, metanol sebagai pelarut dan stim sebagai rawatan penceluran. Keadaan ideal yang dinilai adalah sebanyak 84 %. Sementara itu, hasil kajian mendapati hasil optimal oleoresin ialah 31.50 % (w/w), kandungan zerumbon ialah 0.56 % (w/w), aktiviti antibakteria (zon perencatan) terhadap *E. coli* ialah 14.58 mm dan *S. aureus* ialah 13.51 mm. Parameter-parameter proses yang meningkatkan aktiviti antibakteria terhadap *E. coli* dan *S. aureus* dalam oleoresin lempoyang telah meningkatkan hasil oleoresin dan kandungan zerumbon.

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NOMENCLATURE

%	-	Percentage
µg/ml	-	Microgram per milliliter
µL	-	Microlitre
^0C	-	Degree Celsius
ANOVA	-	Analysis of Variance
CFU/ml	-	Colony Forming Unit/milliliter
DOE	-	Design of Experiment
E. coli	-	*Escherichia coli*
FID	-	Flame ionization detector
g	-	Gram
g/L	-	Gram per litre
GC	-	Gas Chromatography
$gmol^{-1}$	-	Gram per mole
hr	-	Hour
I	-	Intermediate
kg	-	Kilogram
mg/g	-	Milligram per gram
ml	-	Millilitre
mm	-	Millimeter
MS		Spectrophotometer and Mass Spectra
ppm	-	Part per million
psi	-	Pounds per square inch
R	-	Resistance
R^2	-	Coefficient of multiple determinations

RSM	-	Response surface methodology
S	-	Susceptible
S. aureus	-	*Staphylococcus aureus*
SFE	-	Supercritical fluid extraction
TLC	-	Thin Layer Chromatography
UV	-	Ultraviolet
VPC	-	Vapor phase chromatography
w/w	-	Weight per weight
Z. zerumbet	-	*Zingiber zerumbet*
ZER	-	Zerumbone

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CHAPTER 1

INTRODUCTION

1.1 Introduction of Research

The emergence of new infectious diseases, the resurgence of several infections that used to be under controlled and the increase in bacterial resistance have created the necessity for studies directed towards the development of new antimicrobials. Considering the failure to acquire new molecules with antimicrobial properties from microorganisms, the optimization for screening methods used for identification of antimicrobials from other natural sources is of great importance (Valgas *et al.*, 2007). Moreover, natural products are widely recognised in the pharmaceutical industry for their broad structural diversity as well as their wide range of pharmacological activities (William, 2000).

Currently, one of the natural sources that receive consumer attention is the use of phytochemical extract as antibacterial agent. *Zingiberaceae* species are among the most prolific plants in the tropical rainforests. Somchit and Nur Shukriyah, 2003 reported that plants from *Zingiberaceae* family have anti-inflammatory, antiulcer, antioxidant and antimicrobial properties. Among these species, *Z. zerumbet* is one of the most commonly used ingredient in Indo-Malaysian traditional medicines, health supplements and tonics (Ruslay *et al.*, 2007). In view of it popularity among the locals, it is important that the chemical profiles of this species be established as the marker compounds, as well as to validate the rationale for their efficacies (Ruslay *et al.*, 2007).

Z. zerumbet which is also known as Lempoyang contain active ingredient that act as anti-inflammatory, antitumor and antimicrobial (Somchit and Nur Shukriyah., 2003). According to Sanagi (1994), there are at least 10 groups of chemicals constituents present in *Z. zerumbet*. Zerumbone (ZER) was identified as the active ingredient in *Z. zerumbet*. It is a monocyclic sesquiterpene in *Z. zerumbet* which recently been found to suppress tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced Epstein-Barr virus activation in a potent manner (Murakami *et al*., 2002). Murakami *et al.*, 2002, also indicate that zerumbone is a food phytochemical that has distinct potentials for use in anti-inflammation, chemopreventation and chemotherapy strategies. In addition, *Z. zerumbet* reported to have the properties of antibacterial towards gram positive strains such as *S. aureus* and *Micrococcus luteus* (Azmi Muda *et al.*, 2001). Takashi *et al.*, 2001 also found that certain products derived from zerumbone selectively inhibited the growth of Gram-positive bacteria.

With increasing awareness on the role of antibacterials in phytochemical extract, their prevention during processing has become increasingly important. Processing is known to affect content, activity and bioavailability of bioactive compounds (Hashim, 2002). The choice of processing methods and parameters is important in minimizing the nutrient losses during processing (Ang *et al.*, 1982). As a result, a lot of developments in phytochemical processing have been directed toward the preservation of nutritional contents such as micronutrients, antioxidant and bioactive constituents (Nicoli *et al.*, 1999). Plant materials with retained antimicrobials are more functional towards improving the shelf life of the product and providing the health promotion compared to extracts whose antimicrobials have been removed or destroyed during processing (Smith and Paton, 2002).

Previous researchers have been applying the strategy of ‘reconstitution’, achieved by the addition of natural antibacterials to the product in order to minimize the eventual processing damages (Zivanovic *et al.*, 2005 and Burt, 2004). However, the strategy has succeeded in explaining the effects of the addition of natural antibacterials on the product rather than the processing effects on the natural antibacterials presents in the phytochemical extracts.

Based on these observations, the aim of the present study was to investigate the effect of processing parameters on the yield of *Z. zerumbet* oleoresin, zerumbone content and antibacterial activity towards *E. coli* and *S. aureus*.

1.2 Research Background

Oleoresins are solvent extractives of spice or herbs. They are concentrated liquid products that contain both volatile and non-volatile flavour components. The quality of oleoresins is typically evaluated based on the presence of active ingredients at the desired level (Hashim, 2002). Therefore, the choice of processing parameters is important in minimizing the compounds losses during processing (Ang *et al.*, 1982).

The main processing parameters of *Z. zerumbet* oleoresin are solvents, extraction time and blanching treatments. There are many choices of solvents that can be used in the extraction, such as n-hexane, short chain alcohol (methanol,

ethanol), ketone (acetone), hydrocarbons (methylene dichloride) and liquid carbon dioxide (Tiwari, 1995). The two most common and widely used blanching methods are steam blanchers and hot-water blanchers (Hashim, 2002), while the extraction time differs according to the phases of experiments involve (Hashim, 2002).

At present, the processing parameters of oleoresin are as follows (Hashim, 2002):

i. Blanching treatment – steam treated, boil treated and untreated
ii. Solvent extraction – methanol, ethanol, acetone and hexane
iii. Extraction time – 6 to 38 hours

1.3 Research Overview

In this project, yield of oleoresin, zerumbone content, antibacterial activity on the *E. coli* and *S. aureus* are the most important properties to be considered in the extraction of a *Z. zerumbet* oleoresin. In order to obtain the most appropriate parameter, statistical experimental designs were used. Design Expert Version 6.0.8 software was used to design and analyze the experiments (Stat-ease Inc., 2002). To establish the possible range of processing parameters, several preliminary experiments were carried out. These experiments were to determine the most suitable range of the parameters and identify the significant factors that affect the oleoresin quality. Statistical software of D-optimal Design Techniques was used to design the experiment.

The D-optimal design techniques were used to obtain the mathematical and polynomial relationship between the factors and the response variables to create a mathematical model from the data (Cornell, 1990). These factors are the significant ingredients that had been identified during the initial experiments. D-optimal design was used to determine the combination of the significant ingredients to obtain the best parameters in term of yield of oleoresin, zerumbone content, antibacterial activity on the *E. coli* and *S. aureus* of oleoresin.

There are four responses that were considered in this research; the yield of oleoresin, zerumbone content, antibacterial activity on the *E. coli* and *S. aureus*. All properties were determined and the result was used for further calculation using D-optimal Design Techniques. The optimization step was carried out in order to determine the optimal processing parameter of *Z. zerumbet* oleoresin. The result of optimization work was analyzed using ANOVA (analysis of variances) table as well as representation by graph or contour graph (Stat-ease Inc., 2002).

This method is an alternative to the conventional method of one-factor at a time production optimization which is time-consuming, expensive and inaccurate as the existence of interactions between various factors occurs. Statistical experimental designs allow simultaneous, systematic and efficient variation of all components. The use of user-friendly software packages has made this technique increasingly popular for production optimization (Indrani and Rao, 2001).

1.4 Research Objectives

The objective of this research was to study the effect of processing parameters on the yield of oleoresin from *Z. zerumbet* and its antibacterial activity.

1.5 Research Scopes

To achieve the objectives, four scopes have been identified in this research:

(i) To study the effect of processing parameters on the yield of oleoresin.

(ii) To study the effect of processing parameters on the zerumbone content.

(iii) To study the effect of processing parameters on the antibacterial activity on *E. coli* and *S. aureus*.

(iv) To optimize the yield of oleoresin, the zerumbone content and the antibacterial activity on *E. coli* and *S. aureus* using D-optimal Design Techniques.

The processing parameters studied were the blanching treatments, solvent and extraction time. Experiments were conducted according to an experimental design generated by Design Expert Version 6.0.8. Each sample obtained from each run of experiments was evaluated for the yield of oleoresin, zerumbone content and antibacterial activity on *E. coli* and *S. aureus*.

1.6 Contribution of the Study

The study of natural antibacterial nowadays becoming increasingly important in the developed countries due to the most available antibacterial was failure to treat infectious diseases. In addition, the study of *Z. zerumbet* oleoresin as antibacterial is an important contribution as it is one of the most important materials in Malaysian traditional medicine. Zerumbone is a major component of *Z. zerumbet* that has distinct potentials for use in anti-inflammation, chemoprevention and chemotherapy strategies. This study would help to understand the effect of processing parameter towards yield of oleoresin, zerumbone content and antibacterial activity on *E. coli* and *S. aureus* of *Z. zerumbet* oleoresin. The understanding of the processing effect is useful in designing a better processing technology to retain the antibacterial activity in *Z. zerumbet* extracts.

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