INVESTIGATION ON THE POTENTIAL OF ORANGE PEEL WASTE IN THE PRODUCTION OF USEFUL HOMEMADE SOLUTION

AZIAN BT OTHMAN

UNIVERSITI MALAYSIA PAHANG

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ABSTRACT

Homemade solution from fermented orange peel has a high potential to be used as multipurpose cleaning agent. It was produced by undergoing fermentation process using a mixture of brown sugar, orange peel waste and water with correct proportion for at least 3 months. This proposed study was investigating the usefulness of the fermentation broth from orange peel waste as a dish cleaning detergent. In addition, it also aims to fractionate the protein mixture from different age of fermentation and identify its possible content. A proposed method for this study is the comparison of the cleaning power of the fermentation broth from different age with solvent. While, the possible content was examined by determining the distribution of molecular weight by using size exclusion chromatography (SEC), determination the molecular weight of protein in the sample by using Sodium Dodecyl Sulfate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) and by using High Performance Liquid Chromatography (HPLC) to detect possible organic acids. The results showed that 3 months fermentation broth has the highest cleaning power followed by 5 months and 1 month fermentation broths. While based on SEC result, it showed that all ages fermentation broths may contain α-amylase, protease and lipase and SDS-PAGE result proved that the existence of these three enzymes in the fermentation broths. Lastly, HPLC results showed that all ages fermentation broths contain lactic acid and succinic acid. In conclusion,3 months fermented broth from orange peel waste has the highest cleaning power and is quite comparable to the dish cleaning detergent. Other than that, all ages fermentation broths were fractionate to different kind of proteins and the possible contents were identified.

KAJIAN MENGENAI POTENSI KULIT OREN DALAM PEMBUATAN LARUTAN BUATAN SENDIRI

ABSTRAK

Larutan buatan sendiri daripada kulit oren diperam mempunyai potensi yang tinggi untuk digunakan sebagai agen pembersih serbaguna. Ia telah dihasilkan oleh menjalani proses penapaian menggunakan campuran gula perang, sisa oren kulit dan air dengan kadar yang betul untuk sekurang-kurangnya 3 bulan. Tujuan kajian untuk mengkaji kegunaan larutan penapaian dari kulit oren sebagai detergen pembersihan. Di samping itu, ia juga bertujuan untuk mengklasifkasi campuran protein dari umur yang berbeza penapaian dan mengenal pasti kandungandi dalam larutan tersebut. Satu kaedah yang dicadangkan untuk kajian ini adalah perbandingan kuasa pembersihan larutan penapaian dari usia yang berbeza dengan pelarut. Sementara itu, kandungan mungkin telah diperiksa dengan menentukan taburan berat molekul dengan menggunakan "Size Exclusion Chromatography" (SEC), penentuan berat molekul protein dalam sampel dengan menggunakan "Sodium Dodecyl Sulfate Poly-Acrylamide Gel Electrophoresis"(SDS-PAGE) dan dengan menggunakan "High Performance Liquid Chromatography"(HPLC) untuk mengesan kemungkinan kehadiran asid organik. Berdasarkan kepada hasil bagi kaedah pertama, ia menunjukkan bahawa larutan penapaian 3 bulan mempunyai pembersihan kuasa tertinggi diikuti oleh larutan penapaian 5 bulan dan1bulan.Sementara itu, berdasarkan keputusan SEC, ia menunjukkan bahawa semua peringkat umur larutan penapaian mungkin mengandungi α-amilase, protease dan lipase dan keputusan SDS-PAGE membuktikan bahawa kewujudan enzim ini tiga dalam larutan penapaian. Akhir sekali, keputusan HPLC menunjukkan bahawa semua peringkat umur larutan penapaian mengandungi asid laktik dan asid suksinik.Kesimpulannya, semua peringkat umur larutan penapaian dari sisa kulit orenmempunyai kuasa pembersihan dan boleh berguna sebagai detergen pembersihan. Selain daripada itu, semua peringkat umur larutan penapaian diklasifikasi untuk pelbagai jenis protein dan kandungan yang mungkin telah dikenal pasti.

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LIST OF SYMBOL

°C	Degree celcius
Da	Dalton
g	Gram
µg/ml	Microgram per millilitre
µm	Micrometre
mAU.s	Milli absorbance unit
ml	Millilitre
mm	Millimetre
ml/min	Millilitre per minute
mM	Millimolar
v/v	Volume per volume
w/v	Weight per volume
λ	Wavelength

LIST OF ABBREVIATIONS

CV	Column Volume
DOCC	Direct Oxidation Carbon Coulmetry
HPLC	High Performance Liquid Chromatography
MW	Molecular weight
OPC	Optical Particle Counter
OSEE	Optically Stimulated Electron Emission
SDS	Sodium Dodecyl Sulfate
SDS-PAGE	Sodium Dodecyl Sulfate Poly Acrylamide Gel Electrophoresis
SEC	Size Exclusion Chromatography
SmF	Submerged Fermentation
SSF	Solid State Fermentation
TCA	Trichloroacetic acid
UP	Ultrapure Water
UV	Ultraviolet
V	Voltage
XPS	X-Ray Photoelectron Spectroscopy

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Nowadays, people become more environmental conscious and try to recycle or reuse the waste they generated. Mostly, the typical wastes generated from the kitchen at home are fruit and vegetable wastes. Typical kitchen waste such as orange peel can be converted to a valuable product which known as “homemade enzyme” by undergoing fermentation process. Basically, this “homemade enzyme” solution is the fermentation product of fruit wastes in the mixture of brown sugar and water after a period of 3 months (Ways to Save Energy, 2008). It is the process of fermentation with the key ingredient of brown sugar that is metabolized by bacteria into alcohol which is subsequently reduced to acetic acid or vinegar. Homemade enzyme also known as garbage enzyme which was invented by Dr.Rossukon from

Thailand (SmartRanger, 2009). This “homemade enzyme” solution has been claimed that it has a cleaning power which is as effective as chemical detergent in the market.

There are several benefits of the “homemade enzyme” solution in daily life. One of the advantages is to save money by convert kitchen waste such as orange peel, pineapple peel and vegetable waste into multipurpose natural household cleaners instead of buying expensive and polluting chemical cleanser. Other than that, “homemade enzyme” solution also can act as a natural pesticide which reduces the demand for chemical pesticide and not only that; it also can be used as an organic fertilizer (Yeen, 2009).

1.2 Problem Statement

The use of the chemical substances in daily life is rapidly increased since the advancement of technology. The excessive uses of the chemical substances in daily routine such as detergent and chemical pesticide may lead to worse pollution and contribute to some dangerous health problems. Fruit waste such as orange peel has been used to produce so-called “homemade enzyme” solution which is very popular among housewives. It has been claimed to be able to act as a multipurpose cleaning agent which is effective like chemical detergent in the market.

Furthermore, it has been used to partially substitute the household cleaning agent to do the laundry, clean the dishes and floor and some even use it to partially substitute facial cleanser and shampoo. But there is no any evidence that can explain

what is exactly contained in this fermentation broth that was produced from orange peel waste. The question here is the truth of presence of the enzyme in the fermentation broth which has better cleaning power. Moreover, the concern about whether there is any harmful effect to human when they use “homemade enzyme” solution in their daily life need to be proven.

1.3 Research Objective

This study attempts:

1) To investigate the usefulness of the fermented solution from orange peel waste as a dish cleaning detergent.
2) To fractionate the protein mixture from different age fermentation of orange peel waste and identify the possible content.

1.4 Scope of Study

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

1) Compare the cleaning power of the fermentation broth from fermentation age of 1 month, 3 months and 5 months with the solvent using conventional homemade recipe.

2) To fractionate and characterize protein mixture from fermentation broth of age 1 month, 3 months and 5 months by using several methods, which are Size Exclusion Chromatography (SEC) , Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis (SDS-PAGE), and also High Performance Liquid Chromatography (HPLC).

1.5 Significance of The Proposed Study

"Homemade enzyme" solution has been claimed to be able to act as a multipurpose cleaning agent. One of the significance of this proposed study is the important to verify the cleaning power of the "homemade enzyme" solution. Other than that, this proposed study is able to determine the possible compounds that contribute to the 'magic' cleaning effect.

If proved useful, "homemade enzyme" solution that produces from the fermentation process can be used as an alternative way to replace chemical cleaner agent with a natural cleaner agent (SmartRanger, 2009). By making the "homemade enzyme" solution from fruit waste, people can reduce reliance on chemical cleaner and also compost their own waste without contribute to any pollution (Ways to Save Energy, 2008). The excessive uses of chemical substance in daily life may lead to hazardous waste and worsen pollution. Therefore, "homemade enzyme" solution not only can replace the chemical used in daily life but also can saves the environment.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

A review of literature was performed to identify studies relevant to the topic. A limited number of studies were found that discussed about the potential of orange peel waste in the production of useful homemade enzyme. Basically, the general knowledge about the potential of orange peel waste is largely based upon empirical studies to produce the specific enzyme by common fermentation in laboratory, but not by a conventional homemade fermentation. The emergent keywords may be divided into four broad areas which are composition of orange peel, state of fermentation, cleanliness measurement method and fractionation of protein.

2.2 Orange Peel

Orange peel has a potential valuable composition that can be developed into high quality products. According to Rivas et al. (2008), orange peel composed of 16.9% soluble sugar, 9.21% cellulose, 10.5% hemicelluloses and 42.5% pectin. Mamma et al. (2007) mentioned that orange peel is a raw material which can be direct utilized in daily life such as animal feed and organic fertilizer.

Orange peel has been fed to cattle, where it can drastically reduce the existence of pathogenic bacteria, such as *E. coli* and *Salmonella*, in the intestine of cattle (Lopez et al., 2010). Ultimately, cutting down the internal bacteria number in living cows could reduce into lower rates of dangerous pathogens in the meat. Other than that, orange peel can be used as an organic fertilizer by composting up to 3 months (Guerrero et al., 1995). The use of orange peel waste as an organic fertilizer seems to be low cost if compared with chemical fertilizer which can pollute the soil.

According to Grohman et al. (1996), orange peel also can be used to produce fuel ethanol. Bioethanol is an alternative fuel derived from biologically renewable resources. It is a good substitute for gasoline in spark-ignition engines. Fermentation process to produce value-added products such as fuel ethanol from orange peel while minimizing waste disposal would enhance the profitability of the citrus industry. Rivas et.al (2008) mentioned that orange peel also can be used for production of citric acid. Citric acid is a natural preservative mainly used to add an acidic flavor and more than a million tonnes are produced every year by fermentation. Orange peel has a potential as one of the waste which can convert into something that is valuable.

2.3 State of Fermentation

There are two common state of fermentation which is solid state fermentation (SSF) and submerged fermentation (SmF). Solid state fermentation is a process of fermentation involving solids without the presence of water. The substrate need enough moisture to support growth of natural microorganism. There are several important aspects that need to be considered in solid state fermentation, which are selection of suitable microorganism and substrate, optimisation of process parameters, and isolation and purification of the product (Pandey, 2002). While submerged fermentation (SmF) is a process of fermentation with the presence of water. For this study, the proposed state of fermentation is a submerged fermentation which is more suitable.

For solid state culture, the culture media are simple. Some substrate can be used directly as a solid media or being enriched it with nutrient. In this case, the products of interest are concentrated, which facilitates its purification. The quantity of waste generated is smaller than submerged culture (Asther et al., 2002). In addition, the waste generated from SSF has lower humidity content. The disadvantages of SSF are that the microorganisms are limited to those able to grow in the reduced levels of humidity. The determination of parameters such as humidity, pH, free oxygen and carbon dioxide, constitute a problem in solid state fermentation (SSF) due to the lack of monitoring devices.

Submerged fermentation is the cultivation of microorganism in liquid nutrient broth. Submerged culture fermentation has been widely used for production of enzyme because in submerged fermentation unwanted metabolites are not produced

and purification of enzymes can take place easily. This involves growing carefully selected microorganisms (bacteria and fungi) in closed vessels containing a rich broth of nutrients and a high concentration of oxygen. As the microorganisms break down the nutrients, they release the desired enzymes into the solution. Basically, the desired product of fermentation is in the liquid form; thus, easy to concentrate and purify. Since the volume of water used in this study is more than 10 % of the fermentation volume, it is, therefore, a submerged fermentation.

2.4 Cleanliness measurement method

Cleanliness measurement method is a method to measure the cleanliness of metal parts in the manufacturing industry. The purpose of the cleanliness method is to test how clean a part will be by specifying the process used to do the cleaning. This method is also used to monitor the contamination levels in the cleaning solution in order to determine when the solution needs to be replaced. So, when the suitable cleaning agent replacement is qualified, the performance of replacement solution need to be compared with the current product used in order to know the effectiveness. According to Farella et al (1991), ozone depletion increases when chlorinated fluorocarbon (CFC) and methyl chloroform are used for cleaning metal parts. Therefore, replacement of cleaning metal agent is necessary to reduce ozone depletion.

Farella et al. (1991) stated that there are two methods for cleanliness measurement, which are direct method and indirect method. The examples of direct method are Magnified Visual Inspection, Black Light, Water Break Test, Contact Angle, Gravimetric Measurement, Optically Stimulated Electron Emission (OSEE), Direct Oxidation Carbon Coulmetry (DOCC) and X-Ray Photoelectron Spectroscopy (XPS). While the examples of the indirect method are Gravimetric Analysis, Ultraviolet (UV) Spectroscopy and Optical Particle Counter (OPC).

As stated by Wilkie and Montague (1994), effective cleaning process selection requires quantifying the cleanliness. For this study, it is proposed to use Ultraviolet (UV) Spectrographic Determination because this method is commonly used in industry to analyze the flux residue on printed circuit boards and also used to analyze oil and grease residue remaining on the cleaned metal parts. The concept of this method can be applied in this study because the objective of this study is to investigate the cleaning power of the fermentation broth. Pure cooking oil was used to soil a stainless steel spoon, and then the analysis on the residue that collected after the spoon was washed with fermentation broth. This analysis determined the cleaning power effect by using UV-Vis Spectrophotometer.

2.5 Fractionation of Protein

Protein fractionation is a separation of protein mixture by using the inherent differences of each protein. Fractionation involves changing the conditions such as the temperature or the acidity of the proteins, so that soluble proteins become insoluble and large clumps which are known as precipitate. The insoluble protein can

be collected by centrifugation. There are several types of protein precipitation which are ammonium sulfate precipitation, pH adjustment and also solvent precipitation. According to Campbell (2007), ammonium sulfate precipitation is a common way to precipitate the protein in order to get protein of interest.

As stated by Oliveira et al. (1999), pH adjustment and addition of Trichloroacetic acid (TCA) are some of the methods of protein fractionation in precipitation. Both methods used in fractionation of proteins shows different result, but the differences between these two results are not too much. For this study, it is proposed to use solvent precipitation to precipitate the fermentation broth of age 1 month, 3 months and 5 months because it is expected that the protein of interest is amylase, protease and lipase. Solvent precipitation is the common way to precipitate these three standard enzymes as mentioned above.

Other than that, SEC is one of the methods that can be used to determine the distribution of molecular weight of the protein samples. Principle of SEC is the separation technique based on the molecular size of the components. Separation is achieved by the differential exclusion from the pores of the packing material, as the sample molecules pass through a bed of porous particles. SEC separate molecules in different size by elute the bigger molecular size first then following by the smaller size. The bigger size molecule will flow out following the mobile phase, while the smaller size molecules will flow into the pores, retain and separate based on the molecular size.

SDS-PAGE is the separation of macromolecules in an electric field. The technique is based upon the principle that a charged molecule will migrate in an electric field towards an electrode with opposite sign. The general electrophoresis techniques cannot be used to determine the molecular weight of biological molecules

because the mobility of a substance in the gel depends on both charge and size. To overcome this, the biological samples needs to be treated so that they acquire uniform charge, then the electrophoretic mobility depends primarily on size. For these different protein molecules with different shapes and sizes, they need to be denatured with the aid of SDS so that the proteins lost their secondary, tertiary or quaternary structure. The proteins being covered by SDS are negatively charged and when loaded onto a gel and placed in an electric field, it will migrate towards the anode and separated by a molecular sieving effect based on size. After the visualization by a staining technique, the size of a protein can be calculated by comparing its migration distance with that of a known molecular weight ladder (marker). Other than that, SDS-PAGE is also one of the methods that can be used to determine the number of protein in the samples of 1 month, 3 months and 5 months with their possible molecular weight (Davey et al., 2003). Protein separation by SDS-PAGE can be used to estimate relative molecular mass, to determine the relative abundance of major proteins in a sample, and to determine the distribution of proteins among fractions. The purity of protein samples can be assessed and the progress of a fractionation or purification procedure can be followed. Different staining methods can be used to detect rare proteins and to learn something about their biochemical properties. The SEC results sometimes cannot be conclusive about the molecular weight of the protein sample, so SDS-PAGE is used to justify the molecular weight of the protein sample and its purity.

Lastly, the determination of other possible compound such as organic acid can be done by using High Performance Liquid Chromatography (HPLC). For this equipment, the standards used are acetic acid, succinic acid, lactic acid and citric acid. It is assumed that the other possible compound in the fermentation broth with

different age of fermentation might be the organic acid that has been used as a standard in this study.