Effect of Sodium Chloride and Ethanol on Autolysis of Spent Baker's Yeast Generated from Sago Bioethanol

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Bachelor of Science with Honours
(Resource Biotechnology)
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A thesis submitted in partial fulfilment of the Requirement of The Degree Bachelor of

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Programme of Resource Biotechnology
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2022

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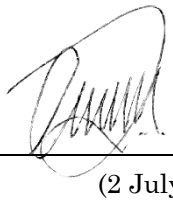
Student's signature  (2 July 2022)

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ABSTRACT

Spent Baker's yeast are generated as a by-product from brewery and bioethanol industries. The excessive production of this waste may lead to an increment in environmental pollution. Since spent yeast contains several useful components, it will be wise to convert it into valuable products such as via autolysis process. Sodium chloride and ethanol are known as great plasmolysing agents to enhance any cell lytic process. The combination use of ethanol and sodium chloride in the autolysis of spent Baker's yeast, especially those generated from sago bioethanol has received little attention in the literature. Hence, this research is conducted to investigate the effect of sodium chloride and ethanol on autolysis of spent Baker's yeast generated from sago bioethanol. The spent Baker's yeast was autolysed with using three different settings which are 5% sodium chloride, 5% sodium chloride with 5% ethanol, and 5% ethanol. The amount of protein and carbohydrate released in the autolysates were determined using lowry assay and phenol-sulphuric acid method, respectively. The results showed that the autolysis treated with 5% sodium chloride shows the highest production of protein and carbohydrate released which were 8.3-fold and 4.7-fold higher compared to the control experiment. In general, this work gives useful insight into valorisation of spent Baker's yeast generated from sago bioethanol.

Keywords: Autolysis, Spent Baker's yeast, plamolysers, sodium chloride, ethanol

ABSTRAK

Yis Baker merupakan hasil sisa bahan buangan daripada aktiviti penghasilan produk dalam industri bir dan bioetanol. Namun, penghasilan sisa buangan yang berlebihan ini boleh menyebabkan peningkatan terhadap pencemaran alam sekitar. Maka, bahan sisa buangan ini digunakan untuk penghasilan produk yang bernilai melalui proses-proses tertentu seperti autolisis, memandangkan sisa yis Baker itu sendiri mengandungi beberapa komponen yang berguna. Sementara itu, natrium klorida dan etanol dikenali sebagai agen plasmolisis bagimeningkatkan proses-proses sel lisis. Namun, penggunaan kedua-dua kompaun ini dalam autolisis Yis Baker, terutamanya yang terhasil daripada pembuatan sago bioetanol masih mendapat kurang tumpuan dalam literatur penyelidikan sains. Oleh itu, kajian ini dijalankan bagi mempelajari kesan penggunaan etanol dan natrium klorida terhadap autolisis, serta untuk menentukan strategi yang terbaik dalam menggunakan kedua-dua agen plasmolisis ini dalam autolisis sisa yis Baker yang terhasil daripada pembuatan bioetanol sago. Dalam kajian ini, tiga kondisi berbeza telah diaplikasikan dalam autolisis iaitu dengan menggunakan 5% natrium klorida, 5% etanol, dan 5% natrium klorida dengan 5% etanol. Protein dan karbohidrat yang terhasil daripada autolisis tersebut ditentukan menggunakan kaedah "lowry assay" dan "phenol-sulphuric acid". Keputusan kajian menunjukkan bahawa autolisis dengan menggunakan 5% natrium klorida menghasilkan kepekatan yang tertinggi bagi kedua-dua protein dan karbohidrat, dimana kedua-dua komponen ini menunjukkan perbezaan sebanyak "8.3-fold" dan "4.7-fold" lebih tinggi berbanding eksperimen kawalan. Kesimpulannya, kajian ini memberi gambaran yang berguna untuk mengaplikasikan nilai sisa yis Baker yang terhasil daripada pembuatan sago bioetanol.

Kata kunci: *Autolisis, sisa yis Baker, agen plasmolysis, natrium klorida, etanol*

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LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
w/v	Weight per volume
mL	Millilitre
°C	Celsius
rpm	Rotations per minute
nm	Nanometre
µl	Microliter
C_x	Amount of autolysate (g/100 g yeast dry wt.)
C_C	Amount of carbohydrate (g/100 g yeast dry wt.)
C_P	Amount of protein (g/100 g yeast dry wt.)
C_X	Amount of yeast (g dry wt./l)
k	Constant for yeast digestion rate (h-1)
α_A	Coefficient of autolysate yield
β_C	Coefficient of carbohydrate yield
β_P	Coefficient of protein yield
0	Original point
∞	Last point or equilibrium point
h	Hour

CHAPTER 1

INTRODUCTION

1.1 Introduction

Autolysis was first introduced by Ernst Leopold Salkowski in 1875 which described as a process of "self-digestion" due to some sort of subsequent changes of enzymatic reactions (Alexandre, 2011). Autolysis happens naturally as cells reach the end of their stationary stage of growth. However, it occurs at a very slow rate and takes a long time to complete. In today's industry, autolysis is carried out to obtain the yeast products, known as autolysates. Regardless of the natural autolytic phenomenon in organisms, scientists are presently investigating several methods to enhance the autolysis process utilising either physical, chemical, or biological inductors (Babayan and Bezrukov, 1985).

Sodium chloride is one of the chemical inducers that may be utilised to improve autolysis. It generally works by incorporating osmotic pressure and water retention activity which causes the cells to lyse by driving water out of the cells. This mechanism is often regarded as plasmolysis and some researchers classify sodium chloride as plasmolyser (Takalloo et al., 2020). Another plasmolyser that has previously been demonstrated to promote autolysis is ethanol. It works particularly by inducing the cell membrane to disorganize before initiating the hydrolysis of yeast nucleic acids (Trevelyan, 1977).

Nowadays, yeast autolysates are obtained due to their promising nutritional value for food production, with their bioactive compounds such as monooligosaccharides, β-glucans, minerals, and Vitamin B (Podpora & Swiderski, 2015). They are usually produced from *Saccharomyces cerevisiae*, which is also known as Baker's yeast. It is widely used in the baking industry due to its capability in generating a significant amount of carbon dioxide, which may be utilised to rise and expand dough in bread-making.

Spent yeast, on the other hand, is a waste product obtained from certain fermentation processes. According to Rakowska et al. (2017), spent yeast has been regarded as an inconvenient waste, due to its abundance in the baking and brewing industries. As a result, it is considered relatively inexpensive and thus people have been widely employed its uses in producing livestock feed.

Nevertheless, the massive amount of spent Baker's yeast produced as a waste product from industrial fermentation might have a significant impact on today's pollution. If this problem is not addressed properly, it is possible that levels of pollution may continue to rise as industrial processing expands over time. As a result, it is proposed that the value of yeast autolysates be intentionally employed for food industrial processes while reducing the environmental damage that this waste product may cause. Apart from that, there are limited studies focusing on the autolysis of spent Baker's yeast generated from sago bioethanol fermentation as well as the influence of sodium chloride and ethanol on the autolysis of spent *S. cerevisiae*.

1.2 Aim and objectives

This research aims to investigate the feasibility of autolysis of spent Baker's yeast generated from sago bioethanol using two objectives which are:

i. To study the effect of sodium chloride and ethanol on autolysis of spent Baker's yeast generated from sago bioethanol.

ii. To determine the best strategy of using sodium chloride and ethanol that will yield the best autolysis of spent Baker's yeast in terms of the protein and carbohydrate released.

CHAPTER 2

LITERATURE REVIEW

2.1 Yeast

Yeast can be classified as a unicellular eukaryotic organism that requires warmth, moisture, and nutrients to thrive. They also reproduce primarily by budding. According to Oca et al. (2016), although yeasts are a part of microorganisms, it has a completely different property from bacteria, allowing them to be resistant to sulfamides, antibiotics, and any other anti-bacterial agents. This property is specifically unique to yeast and not liable to be transformed or transmitted to other microorganisms.

Predominately, there are a variety of species of yeast that can be characterised via cell morphology and physiology which involves sugar fermentation tests, immunology through immunofluorescence technique, and molecular biology involving a few techniques such as karyotyping, AFLP, DNA reassociation, DNA base composition, and hybridization, as well as ribosomal DNA phylogeny. According to Walker (2009), these techniques have become more popular among taxonomists in discovering the new species of yeasts.

2.1.1 *Saccharomyces cerevisiae*

One of the most popular yeast species is *Saccharomyces cerevisiae*, which has the ability to undergo fermentation to convert sugars into carbon dioxide and ethanol. This allows it to be an essential microbe in the fermentation industry. Under the presence of oxygen, they undergo aerobic fermentation and anaerobic fermentation when there is no oxygen available.

This process will typically produce two molecules of adenosine triphosphate while generating carbon dioxide and ethanol as the by-products. Figure 2.1.1 illustrates the cellular morphology of *S. cerevisiae*.

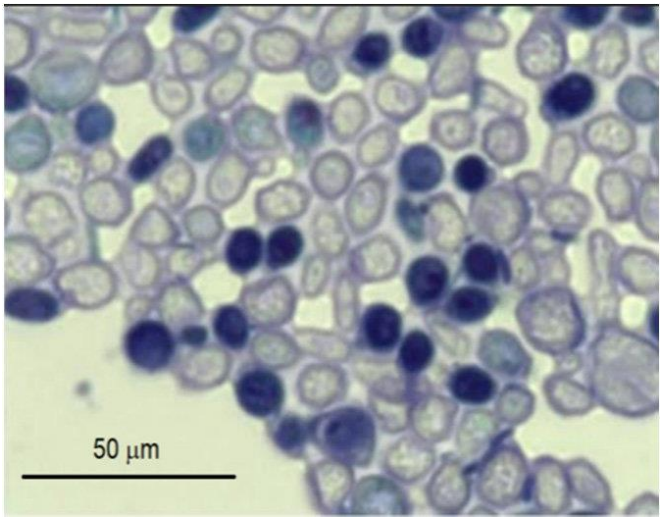

Figure 2.1.1: The yeast *Saccharomyces cerevisiae* cells in an identical with field stained (Bruzaite et al., 2020)**.**

Saccharomyces cerevisiae alone is divided into two main categories based on its mechanisms and uses. The first category is brewing's yeast that possesses more strains that produce more ethanol than carbon dioxide, making it suitable to be used in the beer production industry. On the other hand, Baker's yeast possesses more strains that produce more carbon dioxide than ethanol. This simply allows it to be utilised in the baking industry as the carbon dioxide produced can cause the dough to rise and expand. This is supported by a study conducted by Tanguler and Erten (2008), which stated that due to its ability in producing carbon dioxide to increase the volume of the dough, Baker's yeast has been used extensively in bread and baking goods production since the earlier generation.

2.1.2 Spent Baker's yeast

Industrially, spent yeast is considered relatively inexpensive, as it is known to be a waste product from certain fermentation processes, including sago bioethanol fermentation. This has sparked the interest of numerous scientists and manufacturers since it provides a possibility to utilise this advantage in the production of more industrial products. According to Waszkiewicz-Robak (2013), spent yeast was once used as a source of minerals, protein, and vitamins in animal feed production.

However, as technology advances and more research become accessible, more producers are focusing on the processing of spent yeast with bioactive properties which involve β-glucans, minerals, B vitamins, and monooligosaccharides. This has opened up more opportunities for the manufacturers to produce more functional food and yeast extracts generated from spent yeasts (Podpora & Swiderski, 2015). Rakowska et al. (2017) also reported that more published research nowadays is concentrating on the significant and rapidly increasing field of study which is yeast extracts production from spent yeast. It is often generated through autolysis, which utilises the mechanism of endogenous cellular enzymes.

2.2 Autolysis

Generally, autolysis is defined as when cells or tissues of an organism undergo self-destruction catalyzed by its own enzymes that are provided by lysosomes. According to Babayan and Bezrukov (1985), autolysis in microorganisms is divided into two types which are "exo-type" autolysis and "endo-type" autolysis. "Exo-type" autolysis mainly occurs in most bacteria in which involves the destruction of the cell wall with the help of their own

hydrolases. This process may be terminated at this stage and other cell components will not be affected. “Endo-type” autolysis, however, involves the disturbance of lipoprotein structure within the cell membranes. This type of autolysis is usually subjected to yeasts, fungi, and a few bacterial species.

Takalloo et al. (2020) also reported that yeast autolysis is a process of degradation by its endogenous enzymes which can be triggered by the activation of the intracellular enzymes of the yeast. The process begins with a cell death event by disorganising the biological membranes, which are organelle and plasma membranes.

During autolysis, the activation of hydrolysis enzymes increases, whereas the activity of the respiratory enzyme decreases. It involves the disruption of the cell wall by proteinase and glucanase, allowing the internal chemicals to be released into the surrounding media (Martínez et al., 2016). Verduyn et al. (1999), however, reported that the intracellular enzymes which are nucleases and proteases also cause the intracellular compounds, which include RNAs, DNAs, and proteins to be hydrolyzed, and eventually excrete the decomposed materials to the surrounding media. Figure 2.2 summarises the autolysis process.

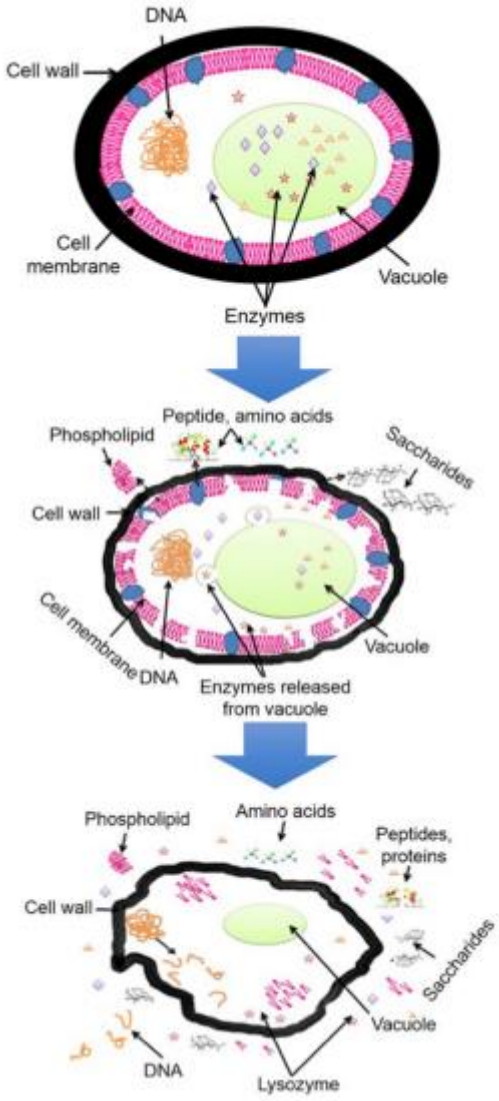

Figure 2.2: Illustration of cell autolysis process (Wang et al., 2018).

2.2.1 Significance of yeast autolysate

Yeast autolysate, which is also referred to as yeast extract is basically defined as the total content of product obtained from yeast autolysis. According to Alexandre (2011), yeast autolysate is mostly composed of the soluble cellular components found within the yeast, which includes flavour compounds, amino acids, peptides, nucleotides, proteins, vitamins,

and sugars. This has made a promising nutritional value for food production as a great source of protein, nucleotides, and vitamin B. A study conducted by Hassan (2011) also proved that yeast extracts have been essentially important in the fermentation and food industry. The compounds released during autolysis are shown in Table 2.2.2, along with their functions in sparkling wine industry.

Table 2.2.1: Applications of the released compound in sparkling wine (Alexandre, 2011).

Origin	Nature	Proved or potential impact on sparkling wine
Cell content	Nucleoside	Flavouring agent
	Nucleotide	Aromatic precursors
	Amino acid	Quality of foam
	Peptide Protein Lipids	Sweet and bitter taste
Cell wall	Glucan	Foam quality
	Mannoprotein	Increase in mouthfeel

Hatoum et al. (2012), reported that yeast extracts have been used extensively in medicine, animal nutrition, as well as in pharmaceutical industries, due to their high demand as an essential component of fermentation media. Apart from that, the process of obtaining autolysates itself provides a great opportunity for scientists to utilise yeast autolysis in studying the purification and extraction of enzymes and coenzymes.

2.2.2 Factors influencing autolysis

Despite the fact that autolysis occurs naturally in organisms, scientists currently employ inductors to induce autolysis for industrial applications. It is primarily affected by three different types of treatments which are chemical, biochemical, and physical (Alexandre, 2011). For chemical means, pH, detergents, and antibiotics play important role in regulating the process of autolysis.

Biochemical inductors, on the other hand, mainly involve the use of exogenous lytic enzymes, such as papain, helicase, protamex, favourzyme, and cellulose for the acceleration of lysis of the yeast (Bayarjargal et al., 2014). However, physical treatment predominately uses osmotic pressure, temperature, as well as alternate melting and freezing point to regulate and enhance the autolysis of *S. cerevisiae.*

A study conducted by Boonraeng et al. (2000), demonstrated the effects of these treatments on Baker's yeast autolysis using ethanol and sodium chloride for chemical inductors, papain for biochemical, and the physical treatment was performed by homogenizing and incubating the yeast samples at 54°C for 24 hours. Their final outcome illustrated that each of the treatments produced a varied concentration of yeast autolysate, depending on the maximal rate of endogenous enzymes' activity for each method.

The effects of using different treatments on autolysis of Baker's yeast in this study is illustrated in Table 2.2.1. $\alpha_{A,}$ $\beta_{P,}$ and β_{C}, stand for the coefficient of autolysate yield,

carbohydrate yield, and protein yield, respectively. This measurement was required to assess the effectiveness of yeast autolysis when it was subjected to various treatments. Boonraeng et al. (2000), also illustrated in their study that the regression coefficient (r^2) of all of the samples treated with different treatments varied in number, but for the most part, the values recorded were to be 0.90, which can be classified as a high regression coefficient value.

Table 2.2.2: The influence of different treatments on autolysis of Baker's yeast (Boonraeng et al., 2000).

Treatments	**Parameters**							
	$C_{X\infty}$ (g/l)	k (h^{-1})	α_A	(r^2)	β_P	(r^2)	β_C	(r^2)
Control	70.79	0.239	0.503	(0.87)	0.689	(0.93)	0.416	(0.94)
5% ethanol	66.47	0.306	0.625	(0.88)	0.833	(0.97)	0.257	(0.95)
5% NaCl	65.52	0.449	0.747	(0.95)	0.861	(0.98)	0.262	(0.78)
5% ethanol + 5% NaCl	61.78	0.537	0.672	(0.96)	0.894	(0.97)	0.238	(0.92)
0.1% papain	52.38	0.320	0.784	(0.96)	0.731	(0.94)	0.311	(0.80)
Homogenization (8 000 psi)	52.80	0.667	0.529	(0.78)	0.839	(0.95)	0.539	(0.88)

Subsequently, using physical treatments for autolysis frequently needs extreme requirements which make the processing very challenging. Biochemical methods involving exogenic enzymes, on the other hand, are not a cheap alternative for industrial purposes, and some manufacturers typically prefer autolysis to occur naturally, but this would have required a significant amount of time to complete the entire lytic process (Alexandre, 2011). Scientists have been studying several alternative strategies to accelerate the autolysis of yeast while limiting any negative side effects. However, the research of autolysis of yeast either by using sodium chloride or ethanol as accelerators is fairly limited, with very few publications accessible. Thereby, this study will be focusing on finding the best strategy of using the same concentration of sodium chloride and ethanol that will maximise the autolysis of spent Baker's yeast.

2.3 Sodium chloride as a plasmolysing agent

The property of sodium chloride itself is closely related to reducing water activity, and thus enhancing the yeast autolysis process. Alexandre (2011), also reported that salt is usually added to the yeast cells prior to the beginning of cell lysis. This will drive water out of the cells via osmosis and eventually initiate the process of cell breakdown. According to Takalloo et al. (2020), some researchers may refer to this process as plasmolysis, specifically when organic solvents or inorganic salts, such as sodium chloride are added to enhance the autolysis of yeast cells.

Gilpin et al. (1972), conducted a study on the effect of salt towards autolysis of microbial cells using a mutant strain of *Staphylococcus aureus.* They discovered that high concentrations of particular salts activated an autolytic enzyme activity, called N-acyl-muramyl-L-alanine amidase, which focally cleaves the cell wall of the mutant cells, causing cell wall disruption and speeding up the autolysis process. Hence, they came to the conclusion that sodium chloride has a specialised mechanism for preventing cell wall biosynthesis in microbial cells.

A subsequent study conducted by Yabu and Kaneda (1995), which focused on the growing and harvested cells of *S. aureus* IID671 strain, with growing cells cultured in growth medium while harvested cells suspended in phosphate buffer. Their finding illustrated that sodium chloride induced the autolysis process for both cell cultures. They did, however, show that the rate of the lytic process was greatly dependent on the sodium chloride content, with 0.3 to 0.4 M being the optimal value.

2.4 Ethanol as a plasmolysing agent

Ethanol, with a chemical formula of C_2H_6O, is one of the most common alcohols that has always been used in scientific studies. It is widely known for its significant properties in altering the physical structure of cell membranes. Goldstein (1986), reported that ethanol can disorder all types of model membranes, in which the disruption of these bio-membranes can be seen in the reduction of membrane order concentration upon the cell's exposure to ethanol. Particularly, cells that are induced by ethanol are more likely to undergo cell