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Kinetic Studies of Enzymatic Hydrolysis Pre-Treatment of Waste Bread Under Preliminary Conditions

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Abstract: This study investigate the process conditions of enzyme concentration (2.0, 4.0, 6.0% w/v), temperature (30, 40, 50 °C), and reaction time (0, 60, 90, 120, 180, 240 min) of enzymatic hydrolysis for sugar recovery from waste bread. The kinetics of the enzyme (α-amylase) was studied to analyse the chemical reaction and the activity of the α-amylase towards the starch present in the waste bread. Enzymatic hydrolysis with different substrate concentration (5, 10, 15, 20 g) was conducted to analyse the kinetics of the enzyme with increasing amount of substrate concentration. The highest reducing sugar yield from the waste bread was 119.73 mg/mL at 15g of substrate concentration, 6.0% w/v enzyme concentration, temperature 50°C and 180 mins reaction time. From this value, the percentage recovery of the reducing sugar was 71.84%. The Michaelis-Menten equation was applied to study the kinetics of enzymatic hydrolysis of waste bread. The Vmax value was 3443.234 µmol/L/min and the Km value was 6.0g.

Keywords: *Enzyme, waste bread, kinetics, sugar, substrate*

1.0 INTRODUCTION

Bread is one of the most wasteful types of food. Out of the 100 million tonnes of bread produced each year, hundreds of tonnes are thought to be thrown away every day. This is due to the fact that bread is prone to staleness and spoilage, resulting in significant waste in both households and stores. According to the food waste action organisation WRAP, surplus bread is one of the largest waste problems facing food merchants. Surplus bakery products, including freshly baked lines, are estimated to account for about one-third of the UK's total retail food waste, or 67500 tonnes. Due to the development of poor products or processing variables, edible bread is also wasted throughout the manufacturing process. Consumer preferences, such as the high desire for crustless sandwich breads, also contribute to bread waste. While a tiny portion of bread trash is used for animal feed, the most of it ends up in landfills, emitting greenhouse gases in addition to the carbon footprint involved with its manufacturing. Now, scientists have devised a method for reusing waste bread as a platform for growing bacteria, yeasts, and other microbes that may be used to make fermented foods [1].

Waste bread also mostly used for animal feed production, sourdough production, and a possible source to yield extruded products. Another common application for leftover bread is hydrolysis and fermentation, as bread is an excellent substrate for enzymes and microorganisms. This granular matrix, which is primarily composed of gelatinized starch, will be further altered after cooling as the starch retrogrades. Furthermore, gelatinized starch can be attacked by amylases, either naturally occurring or introduced, to produce sugars [2]. Waste bread contains saccharides and other nutrients that can be fermented. Microorganisms could turn it into profitable goods like biofuels, valuable chemicals, or monomers for bioplastics manufacture. Food waste contains

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polysaccharides that must be hydrolysed prior to fermentation. Before fermentation, the substrate must be pretreated and enzymatic hydrolysis is a common pretreatment procedure. Finding the best hydrolysis conditions is important for getting a better yield of fermentable saccharides. By optimising enzymatic hydrolysis conditions of waste bread, 99 percent of theoretical maximum glucose yield, a key fermentable sugar, is attained. The viscosity both drop significantly with the addition of -amylase enzyme. The viscosity of the slurries remains very low and does not vary significantly [3].

In this study, bread waste was used as a substrate to yield glucose. The starch hydrolysis of waste bread was carried out by using enzyme α-amylase with different conditions of concentration, temperature, and reaction time. The process of hydrolysis was determined by the concentration of reducing sugar yield. The kinetics of the chemical reaction was further studied by using Michaelis-Menten equation. Hydrolysis process involves the breakdown of polymer of the bread waste into the simplest sugar that is assisted by the enzymatic reaction. The use of waste bread as a substrate with presence of enzyme α-amylase will produce high sugar concentration as bread as rich in carbohydrate and α-amylase is a biocatalyst that increases the chemical reaction.

2.0 MATERIALS AND METHODS

2.1 Raw material preparation

The waste breads (after shelf life) were collected from local mart. Mold had not infected the raw material. The waste breads were cut into small pieces and dried at room temperature for two days, and grinded with a laboratory blender into fine powder. The ground powder was then sieved by using 500µm siever, autoclaved, and stored for further use. The moisture, ash and the starch content of the waste bread were analysed and calculated.

2.2 Characterisation of waste bread

The chemical characterisation of waste bread was performed to determine the value of moisture, ash, and starch content. To determine moisture content, the mass of empty petri was weighed and recorded. 3 g of waste bread was placed in the petri dish and dried in the drying oven at 105°C [4]. After 3 hours, the petri dish was removed from the drying oven, and the mass of the petri dish was determined and recorded. The percentage of the moisture content was calculated by using the equation (Eq. 2.1).

$$\%Moisture = \frac{Initial\ Mass(g) - Dry\ Sample\ (g)}{Initial\ Mass\ (g)} \times 100 \quad \text{(Eq. 2.1)}$$

To determine ash content, the mass of empty crucible was weighed and recorded. 3 g of waste bread was placed in the crucible and calcinated at 550°C for 4 hours. The crucible was left to cool for a while. The mass of the crucible was determined and recorded. The percentage of the ash content was calculated by using the equation (Eq. 2.2)

$$\%\ Ash = \frac{Weiht\ of\ residue\ (g)}{Initial\ weight\ of\ sample\ (g)} \times 100 \quad \text{(Eq. 2.2)}$$

To determine starch content, 1 g of waste bread was added into 15mL falcon tube with 10mL of cold water and shake. The tube was centrifuge at 5500 rpm for 10 min. The supernatant was pipette out and left only the pellet. The pellet was washed again with 10mL cold water and centrifuged at 5500 rpm for 10 min. The supernatant was discarded. This step was repeated twice. The pellet was transferred into a beaker and 30mL of distilled water was added. The solution was heated to 100°C and stirred. The solid particles were removed and the sample was tested for the absorbance value.

2.3 Enzymatic hydrolysis of waste bread

2.3.1 Gelatinization and liquefaction process

By using 250 mL Erlenmeyer flask, 15% (w/v) of homogenized waste bread was prepared in water suspension. For gelatinization, the suspension was stirred and heated at 70°C for 15 min. To adjust to pH 6, citrate buffer was added to the solution along with 1% (w/v) NaOH and 1% (w/v) H_2SO_4. The gelatinized waste bread was allowed to cool to 30°C before the liquefaction process began. 2% (w/v) of enzyme α-amylase was added into the flask and liquefaction process was carried out for 120 min. The process was done in preliminary conditions of enzymatic hydrolysis in which the temperature were (30°C, 40°C, 50°C), enzyme concentrations were (2.0, 4.0, 6.0% w/v), and reaction time were (0, 60, 90, 120, 180, 240 min).The pH was constant at pH 6. Different substrate concentration was performed at (5, 10, 15, 20 g) to determine the enzymatic activity of the hydrolysis process of the different concentration of starch.

3.0 RESULTS AND DISCUSSION

3.1 Characterisation of waste bread

The proximate analysis of waste bread revealed that the moisture content of the bread was 10.65±0.08%. This shows that the bread has lower value of water content. Moisture is stated as a percentage of the amount of water present in a system or material. For food, usually moisture content is important in setting optimum conditions for preservation, storability, packaging, and shipment. Water is presence in the bread and over time, mould will grow in the bread with such condition. However, the value is low enough for the bread to be kept in its packaging materials for human consumption. After its shelf-life and to keep the waste bread, it has to be dried to last longer [5].

The ash content is an approximation of the mineral and other inorganic matter content in waste bread from the wheat. The proximate analysis of waste bread revealed that the ash content of the bread is 6.74±4.70%. The ash in the bread derived from the flour, which was milled from the wheat, leaving only an endosperm. Minerals are distributed irregularly in the kernel. The aleurone layer and pericarp comprise roughly 68% of the total minerals, the starch endosperm 20%, and the embryo 12%. Higher ash flour is typically less refined and contains more particles of fine bran and endosperm close to the bran. Wheat has a greater level of endosperm ash by nature due to genetic variables and soil conditions [6].

The proximate analysis of waste bread revealed that the starch content in 1g of waste bread was 2.7728±0.091mg. The waste bread used in the experiment was 15g. Therefore, the composition of starch in 15g of waste bread was 41.592mg.

3.2 Enzymatic hydrolysis of waste bread

3.2.1 Gelatinization and liquefaction process

According to study from [7], during gelatinization, the starch contained in the waste bread was heated at high temperature. The starch started to swell and burst out. The structure of the starch will be opened to the hydrolysis process Next process is liquefaction, where enzyme α-amylase was added into the gelatinized solution and speeds up the reaction. The polymerisation of the starch will break and produce reducing sugar. Alpha-amylase was commonly used to improve the hydrolysis process [8].

3.2.2 Reducing sugar analysis

Sugar profile evaluation (Table 3) was performed to examine the hydrolysate from waste bread enzymatic hydrolysis using UV Spectrophotometer. The reducing sugars yields were observed from three different conditions of enzymatic hydrolysis. The experiment was done under preliminary conditions which started from different temperature conditions followed by enzyme concentrations and reaction time. The hydrolysis was conducted at different temperatures (30, 40 and 50°C) without prolong treatment of enzyme α-amylase with concentration (2, 4, 6g). The reaction time of the liquefaction was (0, 60, 90, 120, 180, 240 min). From Table 3, the conversion of sugar from 15g of bread was 71.84% towards processing conditions.

The results of waste bread starch hydrolysis are depicted in Figures 3. As temperature increase, the concentration of reducing sugar also increases. The highest reducing sugar detected were at 50°C which was 72.65 mg/ml. By using temperature 50°C, enzymatic hydrolysis with different enzyme concentrations were analysed and it was detected that 6% (w/v) of enzyme concentration had the highest reducing sugar concentration, which was 95.15 mg/ml. As enzyme concentration increase, the concentration of reducing sugar also increases. By using temperature 50°C and 6% (w/v) enzyme concentration, reaction time of the enzymatic hydrolysis were observed. It was detected that the reducing sugar increase as time increase, however the sugar started to decrease at 240 min. This may be due to the decrease in the amount of the waste bread to converse the starch to glucose and there was less substrate accessible for the enzyme to degrade [9]. From the preliminary condition of enzymatic hydrolysis at liquefaction stage, it was established that the optimum temperature for enzyme α-amylase was at liquefying temperature of 50°C, enzyme concentration of 6% w/v and reaction time of 180 minutes.

Table 3: Concentration of reducing sugar of hydrolysate from enzymatic hydrolysis of waste bread with various process conditions

Process conditions		Reducing sugar concentration (mg/mL)
Temperature (°C) at 15% w/v substrate concentration, enzyme concentration 2% w/v, pH 6 for 120 mins	30	61.31
	40	62.34
	50	72.65
Enzyme concentration (w/v) at 15%	2%	72.65

w/v substrate concentration, temperature at 50°C, pH 6 for 120 mins	4%	80.93
	6%	95.15
Reaction time (mins) at 15% w/v substrate concentration, 6% w/v enzyme concentration, temperature at 50°C, and pH 6	0	8.30
	60	66.74
	90	82.48
	120	95.15
	180	119.73
	240	109.73
Substrate concentration (w/v) at 6% (w/v) enzyme concentration, temperature 50°C, pH 6, and reaction time 180 mins	5%	49.78
	10%	94.78
	15%	96.22
	20%	111.66

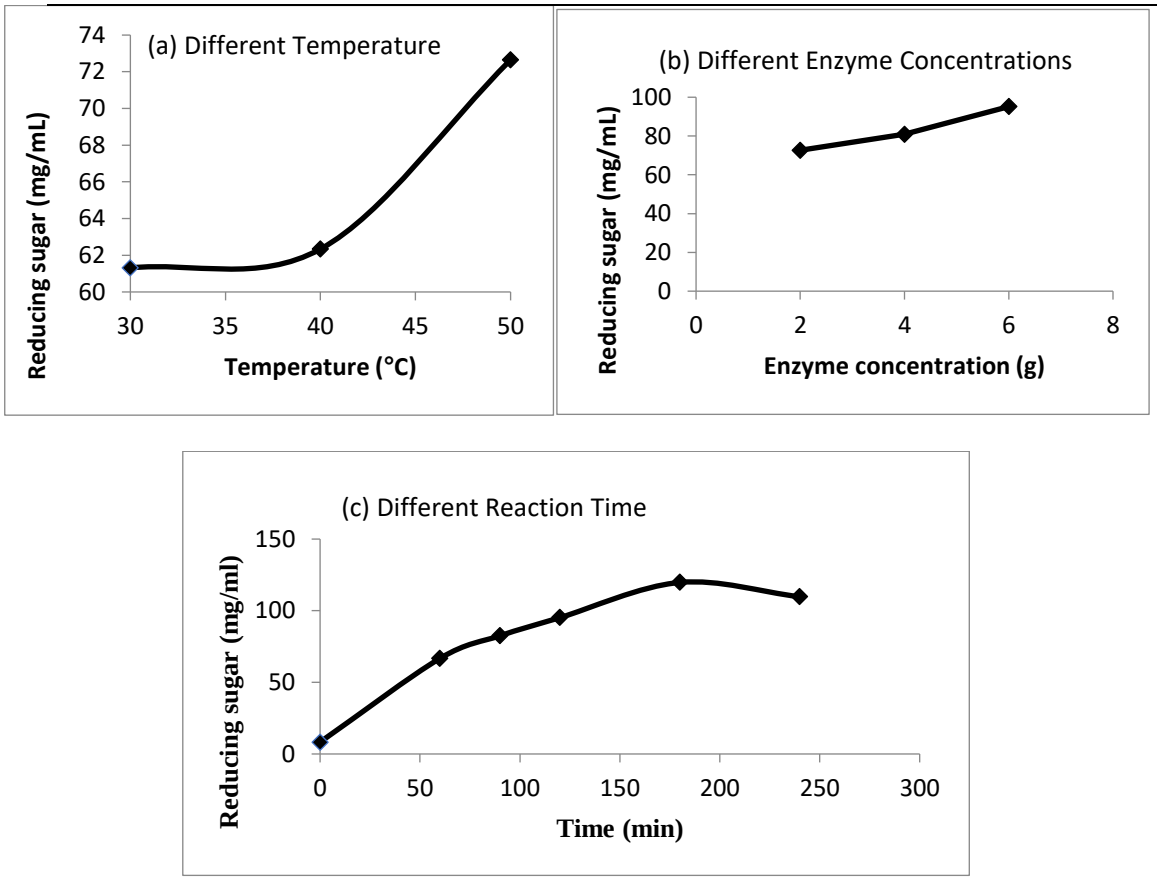

Figure 3: Concentration of reducing sugar from the process of enzymatic hydrolysis under different conditions

(a) Different Temperature (b) Different Enzyme Concentrations (c) Different Reaction Time

3.2.3 Kinetics of waste bread hydrolysis

Kinetics of hydrolysis process was studied by using Michaelis Menten equation where the formula consists of a hyperbolic relationship between enzyme activity (v) and substrate concentration ([S]) [10]. The formula of the Michaelis Menten was as follow:

$$v = \frac{v_{\max}\ [s]}{K_m + [s]} \qquad \text{[eq.3]}$$

Figure 3.1 shows the michaelis-menten plot of waste bread hydrolysis by α-amylase with different substrate concentration. Based on the plotted graph, the value of V_{max} was 34.432 µmol/L/min. V_{max} shows the maximum value of the product formed. The affinity of enzyme binding to a substrate is determined with the value of K_m. K_m is also equal to [S]. The higher value of K_m achieved, the affinity-binding to the substrate is lower. Based on the graph plotted, the value of Km is halved of the V_{max} which was 17.216 µmol/L/min. K_m is equal to [S], which was 6.0g.

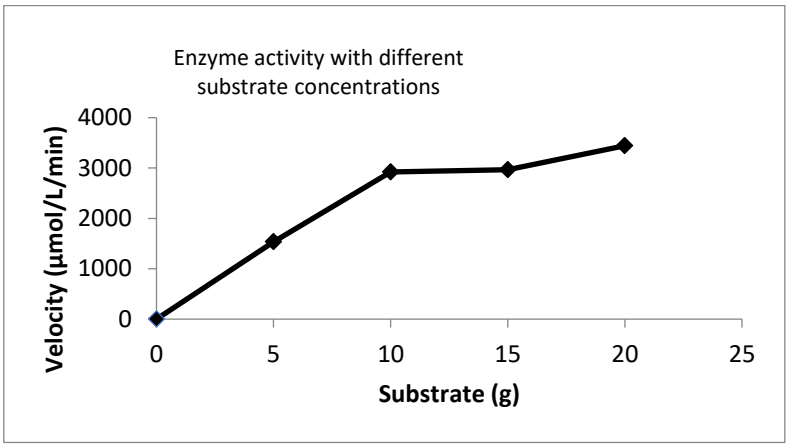

Figure 3.1: Michaelis-Menten plot of kinetics of enzymatic hydrolysis with different concentration of waste bread

4.0 CONCLUSION

The concentration of reducing sugar from enzymatic hydrolysis under different conditions was successfully determined. The kinetics of the chemical reaction of the enzyme α-amylase and different substrate concentration was also evaluated. Hydrolysis process needs a heating for the polymerisation structure of the starch to open up. This process started with gelatinisation, followed by liquefaction process where enzyme α-amylase was added into the solution under certain conditions. The highest reducing sugar recorded was under conditions (15% (w/v) substrate concentration, 6% (w/v) enzyme concentration, temperature 50°C, 180 min reaction time). The kinetics of the hydrolysis process was determined by using Michaelis-Menten equation. The V_{max} value was 3443.234 µmol/L/min and the K_m value was 6.0 g. Waste bread can serve as a carbon source to produce sugar. Instead of wasting it, the waste bread can be used to produce sugar, which has a variety of industrial applications.

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CONFLICT OF INTEREST STATEMENT
The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare absence of conflicting interests with the funders.

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