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Enzymatic Pretreatment of Waste Bread for Glucose Syrup Production

Zafirah Zenol Abidin[1], Siti Roshayu Hassan[2*]

[1]*Faculty of Bioengineering and Technology, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan, Malaysia*
[2]*Bioproduct and Bioprocessing Technology Research Group, Faculty of Bioengineering and Technology, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan, Malaysia.*

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*Corresponding author.
E-mail address:
roshayu.h@umk.edu.my

ABSTRACT

One of the pre-treatments developed to separate monosaccharide from starch polymerization is enzymatic hydrolysis. Leftover bread is abundant in bakeries and homes, and this waste can cause the build-up of carbon dioxide in the atmosphere. According to this study, due to their high carbohydrate content, expired bread can be used as a source of glucose or a substrate for various industrial processes, including ethanol and organic acids. The expired bread was heated for 15 minutes before the gelatinization process, followed by the liquefaction and saccharification processes. The liquefaction process uses different temperatures (30, 40, 50, 60, 70) ℃, enzyme concentrations (2, 4, 6) g, and reaction times (0, 60, 90, 120, 180, 240) min. Different temperatures (50, 60, 70, 80) ℃ and reaction times (30, 60, 90, 120) min were applied for the saccharification process. ATR-FTIR was used to identify the functional group present in the sugar, and a UV Spectrophotometer was used to analyze sugar concentrations. The highest concentration of glucose yield during the liquefaction process was found to be at 50 °C, 6 g, and 180 min of optimization condition. The highest glucose concentrations were found at 60 °C after 60 min for the saccharification process. The optimization of hydrolysis pre-treatment improves the yield of glucose syrup from the waste bread.

INTRODUCTION

Bread waste is one of the most wasted foods. Most wasted bread products are still fresh, raw, or barely processed despite being a staple food and a major source of carbohydrates. 1.3 billion tonnes of food are wasted worldwide each year. Bakery waste falls under the cereal food category, which accounts for about 30% of all food waste globally [1]. The growing amount of bread products being thrown away, where they end up in landfills or are used as animal feed, is a result of their short shelf lives and sensitivity to environmental moisture. In addition to the possibility of landfill exhaustion, the degradation of bread wastes also increased the release of carbon dioxide (CO_2) and methane (CH_4) gas, resulting in Green House Gas (GHG) emissions. Water use and systemic water, air, and soil pollution are additional environmental effects [2].

Because of its abundance, ease of isolation in a highly pure form, and ease of solubilization and enzymatic hydrolysis to glucose and/or other maltodextrin products, starch is currently the most significant biorenewable material. As a result, it is a natural product with numerous industrial applications, including fermentation-based production of ethanol, acetic acid, D-lactic acid, high fructose syrups, maltodextrins, and cyclomaltodextrins [3].

The primary building blocks of amylaceous materials are glucose molecules linked together by α-1,4 and α-1,6 glycosidic bonds. The native structure of amylaceous materials comprises granules packaged together in amorphous and crystalline regions. The amylopectin lateral chains that comprise the crystalline region give rise to a secondary structure of double helices. Hydrogen bonds comprise the double helices, whereas amylose and unpackaged amylopectin comprise the amorphous regions. One of the most crucial industrial processes is the enzymatic hydrolysis of amylaceous materials. It consists of three steps—gelatinization, liquefaction, and saccharification—and is typically done in a batch mode [4].

Enzymatic hydrolysis has benefits, including a more specific process, controllable conditions, lower purification costs, lower ash and residues, and reduced color damage [5]. The interactions of various factors, such as the source of the starch, the size of the granules, the length of the association between the starch components, the rate of amylose and amylopectin, the crystallinity, the polymorphic type (A, B, C), the amylose-lipid complex, the type of enzyme, and the hydrolysis conditions (concentration, pH, temperature), have been suggested as the cause of variations in the enzymatic susceptibilities of starches [6]. By conducting enzymatic hydrolysis pretreatment, this study aims to find suitable conditions for this process using waste bread as a raw material. The product yield will be the glucose syrup, highlighted for the amount produced from the waste bread.

EXPERIMENTAL

Raw Material Preparation

The raw material used in this experiment is waste bread (after shelf-life). All bread pieces were examined for mold, cut into smaller pieces, and dried for two days. Using a 500 µm sieve, the bread was ground and sieved into a fine powder. The powdered waste bread was autoclaved to sterilize it before being stored.

Enzymatic Hydrolysis Using α-Amylase

The 15% w/v suspension with distilled water as solvent was applied to the prepared substrate. The first process was gelatinization, where the suspension was heated at 70°C for 15 min. With the aid of sodium citrate buffer, the pH was changed to 6. The substrate that had been gelatinized was cooled to 30 °C. The gelatinized suspension was treated with 2% w/v α-amylase enzyme for 120 min to liquefy the process. The procedures were carried out three times. Different temperatures (30, 40, 50, 60, 70) °C, enzyme concentrations (2, 4, 6) g, and reaction times (0, 60, 90, 120, 180, 240) min were employed during the procedures.

Enzymatic Hydrolysis Using Amyloglucosidase

After the liquefaction process, the amyloglucosidase enzyme was added to carry out the saccharification process. Different temperatures (50, 60, 70, 80) °C and reaction times (30, 60, 90, 120) min were used. The samples were taken out at regular intervals to measure the sugar concentration.

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Analytical Methods

Determination of Sugar Concentration

This study used the dinitrosalicylic acid (DNS) Reagent with modification to ascertain the presence and concentration of sugar. DNS, sodium hydroxide, and sodium potassium tartrate made DNS Reagent. A sample volume of 1 mL was taken and diluted to a 100-fold concentration. In 2 mL of the diluted sample, DNS Reagent and Sodium Potassium Tartrate solution were added, and the mixture was heated for 5 minutes to increase the reaction rate for color change in the mixture. After the color changed, a UV-visible spectrophotometer was used directly to measure the wavelength of the solution at 575 nm. The sugar concentration generated by the enzymatic hydrolysis was determined using a standard glucose calibration.

Attenuated Total Reflection (ATR)-FTIR Analysis

The ATR-FTIR analysis was employed to identify the functional group of the sugar present in the sample. Data was provided based on the spectrum, and the peaks were observed after a small sample was analyzed.

RESULTS AND DISCUSSION

Enzymatic Hydrolysis Using α-Amylase

As the temperature rises, the enzyme performs better and more effectively. Heating the substrate is necessary for hydrolysis because it increases the number and size of pores and pinholes on the surface of the granules, allowing the enzyme to adhere and penetrate more easily [7]. According to Fig. 1, the sugar concentration was 61.31±7.11 mg/ml at 30 °C. As the temperature rose to 40 °C and 50 °C, the concentration of sugar began to rise to 62.34±1.91 mg/ml and 72.65±11.51 mg/ml, respectively. The sugar yield began to fall at 60 °C and 70 °C, indicating that the α-amylase enzyme performed poorly at high temperatures.

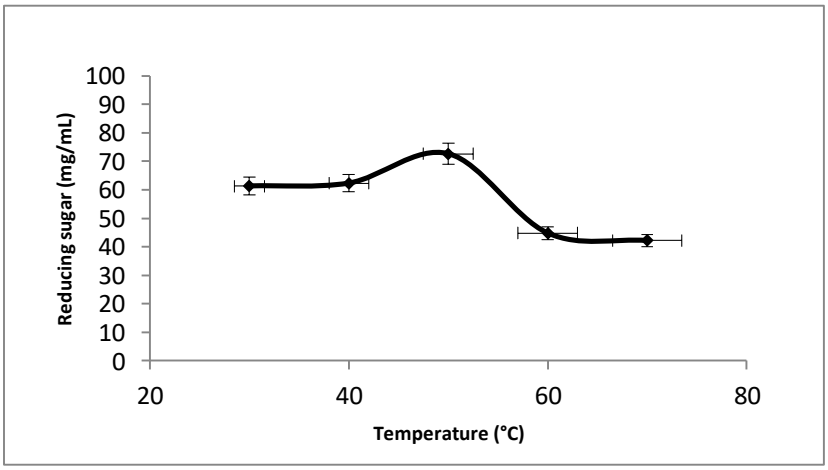

Figure 1. Hydrolysis of expired bread using α-amylase at different temperatures

The liquefaction process was then carried out with a range of enzyme concentrations after it was discovered that 50 °C produced the highest sugar yield. Fig. 2 demonstrates how sugar increases as the

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enzyme concentration rises. Higher enzyme concentrations make more enzymes available per unit of substrate. It increases mass transfer between the enzyme and the waste bread because the enzyme will be transported to the starch granule surface of the waste bread, and the increase in total reducing sugar was anticipated [8].

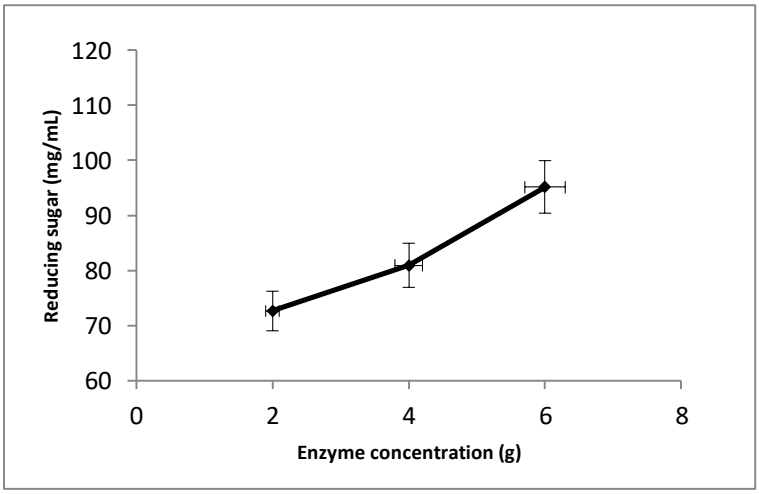

Figure 2. Hydrolysis of expired bread using α-amylase at different enzyme concentrations

The reaction time was also assessed after determining the temperature and enzyme concentration for the liquefaction process. Fig. 3 demonstrates how the sugar concentration rises over time and begins to fall at a certain point. During the initial phase of enzyme hydrolysis reactions, the yield of reducing sugar increases steadily and quickly. The yield of reducing sugar rises steadily from 0 to 180 min of reaction time; however, once the reaction time reaches 240 min, it starts to fall. The hydrolysis efficiency may decrease due to a substrate shortage, an enzyme inhibitor's presence, or the enzyme's deactivation [9]. Since the maximum sugar yield was 119.73±10.30 mg/mL at 180 min, this was the ideal reaction time for the liquefaction of the expired bread.

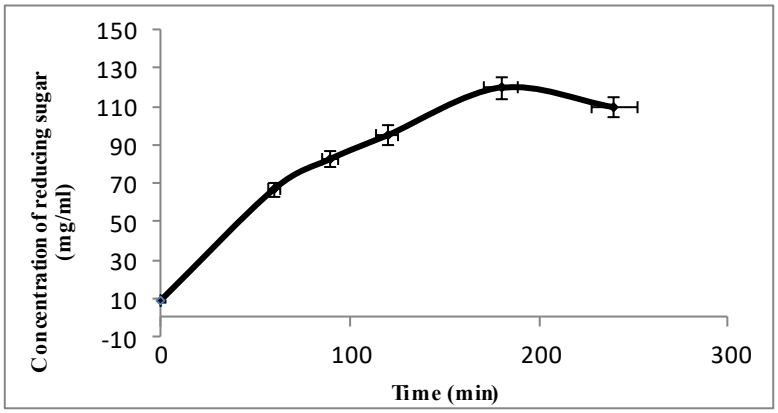

Figure 3. Hydrolysis of expired bread using α-amylase at different reaction times

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Enzymatic Hydrolysis Using Amyloglucosidase

Amyloglucosidase enzyme was used to continue the saccharification process. The process was performed at various temperatures, and the amount of sugar that was produced was periodically checked. The study was conducted with an amyl glucosidase enzyme concentration of 30–60 units/mg and a pH of 4.2. Fig. 4 shows the glucose syrup created because of this process.

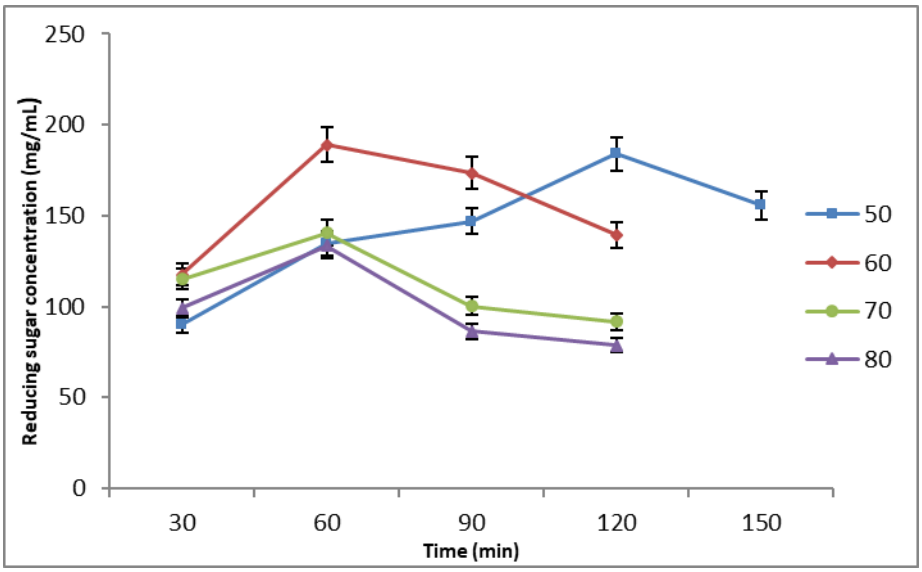

Figure 4. Hydrolysis of expired bread using amyloglucosidase enzyme at 50 ℃, 60 ℃, 70 ℃, and 80 °C for saccharification process

The glucose syrup made from the stale bread had patterns that changed depending on the temperature range. Fig. 4 shows that the enzyme amyloglucosidase had a significant impact on the production of glucose. For all temperature conditions and up to 60 min, there was an increase in the amount of glucose yield from the expired bread. At 90 min, the glucose syrup yield decreased for 60, 70, and 80 °C. The increasing temperature made the amyloglucosidase enzyme functions optimally. However, higher temperature causes the enzyme to be denatured and affects the enzyme's activity [10].

For temperature 50 ℃, the reducing sugar concentration kept increasing until 120 min and dropped after reaching 150 min. The hydrolysis process for temperature 50 ℃ had a longer reaction of amyloglucosidase enzyme than the other temperatures. This is caused by excessive amounts of the enzyme amyloglucosidase, which only has a few targets, and saccharides hydrolyzation [2]. At 60 °C for 60 min, the sugar concentration that could be produced was 188.90±15.65 mg/mL. This finding demonstrated that the amyloglucosidase enzyme can hydrolyze the expired breads effectively at 60 °C. The hydrolysis took place shorter than under other circumstances, but more sugar was produced.

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ATR-FTIR Analysis

ATR-FTIR analysis was used to determine the chemical composition of the hydrolysates made from the enzymatic hydrolysis of expired bread. The distinct peaks indicate the presence of the functional group. Carbonyl and hydroxyl functional groups can be found in sugar. Fig. 5(a) displays the ATR-FTIR spectra of a sample from the liquefaction process (6 g, 50°C, 180 min), while Fig. 5(b) displays the spectra from the saccharification process. The reducing sugar exhibits absorption bands at 1030.65 cm^{-1} due to the C-O-H and C-O-C bonds. The absorption band for the saccharification sample was located at 1036.14 cm^{-1}, as shown in the bands in Fig. 5(b).

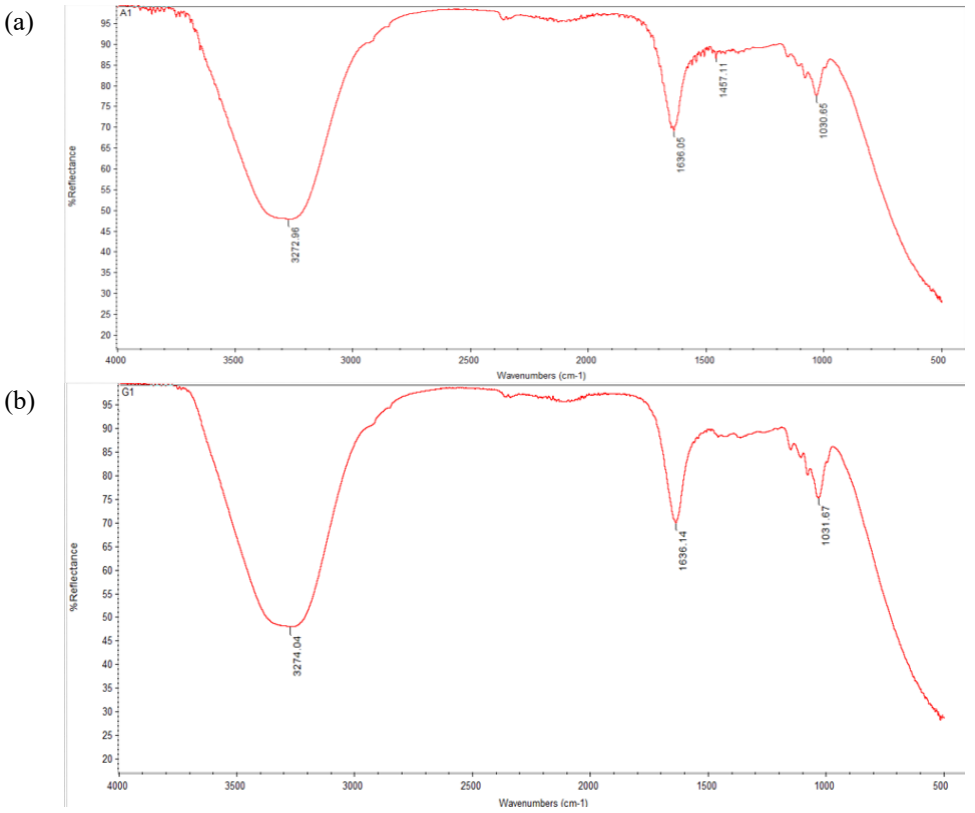

Figure 5. The FTIR spectra of (a) liquefied sample, (b) saccharified sample of hydrolyzed bread

This shows that compared to the sample during the liquefaction process, the peak in this area was formidable (intensified in terms of its width and height). This demonstrates that the glucose syrup sample used in the saccharification process produced more total sugar than the glucose syrup sample used in the liquefaction process when the amyl glucosidase enzyme was present [2]. Protein-based sweeteners contain distinct absorption bands between 1636.05 cm^{-1} for the liquefaction process and 1636.14 cm^{-1} for the saccharification process, corresponding to the protein amide absorption bands [11]. According to [12], the presence of the -OH groups was attributed to broad stretching at 3272.96 cm^{-1} for the liquefaction sample and 3274.04 cm^{-1} for the saccharification sample, respectively.

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CONCLUSION

As a result of the enzymatic hydrolysis of expired bread by α-amylase, the highest sugar yield was 119.73±10.30 mg/mL at 50 °C, 6 g of α-amylase concentration for 180 min of reaction time. The saccharification process was carried out under these ideal conditions, and the results showed that the highest sugar yield was 188.90±15.65 mg/mL at 60 °C in 60 min. The hydrolysis pre-treatment of waste bread could be advantageous in all aspects of managing waste. Only by implementing these measures can many products be produced for economic growth.

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CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted without any self-benefits or commercial or financial conflicts and declare the absence of conflicting interests with the funders.

AUTHORS' CONTRIBUTIONS

Zafirah Zenol Abidin conducted the research, designed the idea, and wrote the article. Siti Roshayu Hassan supervised the research progress, provided the theoretical framework, and reviewed and approved the article submission.

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