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Functional Properties of Pineapple Plant Stem for Enhanced Glucose Recovery in Amino Acids Production

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Abstract: Pineapples generate large amounts of agricultural wastes during their production. To reduce environmental impacts due to poor handling of these wastes, the underutilised pineapple plant stem (PPS), which has a high starch content, can be explored for its sugar recovery. To achieve this, gelatinisation is a key process in increasing enzymes' susceptibility. Therefore, this study aimed to enhance glucose recovery from PPS by studying the effects of gelatinisation temperature and time on its functional properties. Afterwards, the fermentable sugar obtained was used for amino acids production by *Bacillus subtilis* ATCC 6051. PPS has a high gelatinisation temperature (T_o = 111 °C; T_p = 116 °C; T_c = 161 °C) and enthalpy (ΔH = 263.58 J/g). Both temperature and time showed significant effects on its functional properties, affecting enzymatic hydrolysis. Gelatinisation temperature of 100 °C at 15 min resulted in maximum glucose recovery of 56.81 g/L (0.81 g/g hydrolysis yield) with a 3.53-fold increment over the control. Subsequently, utilisation of PPS hydrolysate in the fermentation by *B. subtilis* ATCC 6051 resulted in 23.53 mg/mL amino acids being produced with productivity of 0.49 g/L/h. This opens up new opportunities for the applications of PPS as well as *B. subtilis* ATCC 6051 in the amino acids industry.

Keywords: pineapple plant stem; gelatinisation; hydrolysis; fermentation; amino acids; *Bacillus subtilis*

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1. Introduction

Although corn and cassava are widely used as sources of starch, especially in Southeast Asian countries, issues such as the rising cost of these sources, overexploitation, and food security have raised the need to utilise agricultural residues as a sustainable alternative starch [1,2]. The abundance and availability of PPS make it a cost-effective and great source of substrate for the fermentation process by exploiting the recovered fermentable sugars, targeting mainly glucose. According to the Food and Agriculture Organization (FAO) of the United Nations [3], the production of pineapples has been increasing and reached 28 million tonnes, with one million hectares of harvested area, in 2019. The production shares by region showed that Asia has the highest production of pineapple at 42.1%, followed by the Americas (37.0%), Africa (20.5%), and Oceania (0.4%). Among these, Costa Rica, Philippines, and Brazil have become the top three countries for pineapple production. The huge production of pineapple has resulted in a massive amount of by-products generated from the industry, which may lead to various waste management and environmental issues due to their susceptibility to microbial spoilage [4]. Therefore, there is a need to make use of these by-products in value-added products.

PPS is composed of 11.56% protein, 9.91% carbohydrate, and 8.05% fibre on a dry basis [5]. The high carbohydrate content in the PPS makes it a great source for glucose production, which can be used in the bioconversion of value-added products to help reduce the environmental issues pertaining to the poor handling of the agricultural biomass. PPS

has high starch content ranging from (dry basis) 86.7% to 97.77% [1,6]. This starch can be effectively extracted using wet milling and filtration [7]. Nakthong et al. [6] reported a 30% (*w*/*w*) high-purity starch was produced from pineapple stem. However, the utilisation of the PPS presents certain limitations in its native form as it is resistant to hydrolysis. In this case, the solubility of the PPS presents as an important characteristic during enzymatic hydrolysis. There will be a decrease in crystallinity upon heating, which in turn affects the solubility, swelling, and loss of birefringence after gelatinisation [8]. After hydrolysis, amylose is degraded and exhibits incremental solubility when swelling of granules occurs [9]. Other than its source and swelling power, the degree of intermolecular associative forces and the presence of other components may also contribute to the difference in its solubility [10,11].

Physical modification of PPS such as via the gelatinisation process can improve its properties to suit various industrial applications. This can be achieved by dispersing the PPS in water above its gelatinisation temperature [12]. During gelatinisation, the granules swell before breaking and then release their amylose content [13]. The gelatinised PPS can then be converted into glucose, which is commonly used in the fermentation industry. This can be done through enzymatic hydrolysis processes, by employing enzymes such as glucoamylase and pullulanase [14]. The fermentable sugars obtained as a result of the enzymatic hydrolysis can be applied in fermentation processes in a wide range of industries to produce value-added products, such as amino acid.

Glucose recovery from biomass often involves lignocellulosic biomass, and only few from starch-based feedstocks. Lignocellulosic biomass requires pretreatment before enzymatic hydrolysis can take place. In contrast, the utilisation of starchy biomass results in greater glucose recovery without the need of pretreatment, which in turn becomes less time-consuming and more cost-efficient. Unlike other lignocellulosic biomass that produces a complex mixture of sugars after enzymatic hydrolysis, the sugar recovered from PPS mainly consists of glucose. This acts as an added value as glucose is the most favourable fermentable sugar in amino acids production. Compared to other starch-based feedstocks, such as corn, wheat, cassava, and rice, the utilisation of PPS as the substrate does not interfere with food security as it is commonly being disposed of as waste. In term of glucose recovery, PPS also results in high hydrolysis yield when used as the substrate [5].

Bioconversion of the agricultural by-product can provide valuable insight into its potential utilisation in various industries, such as amino acids production. Amino acids have been receiving great attention due to the increasing, multi-billion USD market demand in various industries, such as the food and feed industries [15,16]. Compared to other bio-based products which have applications as specialty chemicals or food ingredients, amino acids are one of the major pillars in the field of industrial biotechnology due to their wide range of contributions to GDP and the bio-economy. With the animal feed industry among their top applications, amino acids can also be applied in food, pharmaceutical, and cosmetic industries, as dietary supplements, as well as a precursor for other bio-based products and chemicals [17]. Current approaches in the production of amino acids include protein hydrolysate extraction, chemical synthesis, enzymatic synthesis, and fermentation [18]. Among these, fermentation is the most favourable for the industrial scale utilising low-cost substrates. Genetically modified *Corynebacterium glutamicum* is being widely used in the fermentation of amino acid [19]. In addition, the production of amino acids has also been reported using *Escherichia coli* and *Pediococcus acidilactici* in other studies [20,21]. However, the discovery of alternative amino acid producers utilising agricultural biomass would be useful in providing a new approach in the industry.

To the best of our knowledge, despite its wide range of abilities to produce various enzymes, there have been few studies conducted on use of *Bacillus subtilis* for production of amino acids, such as lysine and methionine. Being generally recognised as safe (GRAS), *B. subtilis*, a Gram-positive bacterium, has received much attention from industry [22]. It is commonly applied as the microbial host for various productions, such as of succinate, ethanol, D-lactate, and 2′-fucosyllactose [23,24]. Furthermore, *B. subtilis* is also

widely used for enzymes production; namely of alpha-amylase, pullulanase, cellulase, and protease [22,25,26]. However, there is a lack of study on its application for amino acids production from agricultural biomass.

In the present study, the gelatinisation properties of PPS and the effects of temperature and time on its functional properties were studied to enhance its glucose recovery. We also evaluated the potential of *B. subtilis* ATCC 6051 as an amino acids producer utilising agricultural biomass. Although the physicochemical properties of starch isolated from pineapple stem have been well documented in recent studies, limited studies have been done to investigate the functional properties of the PPS by employing all its compositions for enhanced glucose recovery. Most studies on PPS have mainly focused on the isolation of pure starch and its physicochemical properties. Apart from lower yield, the isolation and extraction of pure starch from PPS is time-consuming and labour-intensive. In view of this, the determination of the functional properties of the PPS, such as water solubility, water absorption, and swelling power, as well as gelatinisation properties, is crucial to further utilise all its components for higher glucose production. In brief, the amino acid-producing ability of *B. subtilis* ATCC 6051 using the enhanced glucose recovered from PPS is discussed in this study.

2. Materials and Methods

2.1. Sample Collection and Preparation

The PPS was collected from Ladang Nanas Dengkil, Selangor, Malaysia. The sample was prepared based on our previous study [5]. The dried PPS was then pulverised using a grinder before sieving with a sifter. The fine-ground sample was then stored at room temperature.

2.2. Characterisation of Pineapple Plant Stem

The PPS was subjected to starch and initial sugar analysis. The soluble sugar was obtained from the liquid fraction of PPS solution prior to enzymatic hydrolysis and analysed using high-performance liquid chromatography (HPL) to determine the initial sugar content. Total starch content was determined using the dinitrosalicylic acid (DNS) method as described by Jeong et al. [27] with slight modification. A 7% (w/v) of pineapple plant stems in distilled water was incubated in a water bath at 90 °C for 15 min, followed by centrifugation at 4000 rpm for 10 min. The supernatant was analysed for the initial soluble sugars whereas the pellet was air-dried for several minutes, followed by enzymatic hydrolysis using 5.56 U/mL Dextrozyme. The supernatant resulted from the hydrolysis, also referred to as pineapple plant stem hydrolysate, was then analysed for reduced sugars concentration using the DNS method and the absorbance was measured at 575 nm. The total starch content was then calculated as shown in Equation (1) [12]:

$$\text{Starch content (\%)} = \text{Total sugar (\%)} \times 0.9 \quad (1)$$

A correction was made for the enzyme blank of Dextrozyme DX 1.5 X and initial soluble sugars in the calculation of total sugar. A correction factor of 0.9 was made for the conversion of starch to the measured glucose value.

2.3. Gelatinisation and Functional Analysis of Pineapple Plant Stem

2.3.1. Gelatinisation Properties Using Differential Scanning Calorimetry

Gelatinisation properties of PPS were analysed using a differential scanning calorimeter (STARe System, Mettler-Toledo, Greifensee, Switzerland) as described by Zeng et al. [28]. A 40 µL aluminium pan was used throughout the analysis and 1.0 g of PPS on a dried basis was dispersed in 2.0 g deionised water and left to equilibrate for 1 h at room temperature. Approximately 30 mg of the starch suspension was placed on the aluminium pan and hermetically sealed. The heating process was carried out at a rate of 10 °C per min over a temperature ranging from 25 °C to 300 °C. The aluminium pan was used as the reference

standard. The result obtained was analysed for its gelatinisation onset temperature (T_o), peak temperature (T_p), and conclusion temperature (T_c), as well as the enthalpy change (ΔH) of PPS. The start temperature (T_s) and end temperature (T_e) were also determined.

2.3.2. Water Solubility, Swelling Power, and Water Absorption of Pineapple Plant Stem

Water solubility, swelling power, and water absorption of PPS were determined as described by Atukuri et al. [29], Kaur et al. [30], and Chisenga et al. [31] with slight modifications. One gram of PPS suspended in 10 mL distilled water was subjected to heating in the water bath at various temperatures (60 °C, 70 °C, 80 °C, 90 °C, 95 °C, and 100 °C) and times (5, 10, 15, 20, 25, 30 min) using the one-factor-at-a-time (OFAT) approach. The suspension was swirled continuously during the process and left to cool at room temperature until it reached ambient temperature. It was then subjected to centrifugation at 4000 rpm for 30 min. The supernatant was collected and oven-dried at 85 °C for 24 h in a pre-weighed aluminium dish for determination of solubility. The wet sediment in the centrifuge tube was weighed for the determination of swelling power in PPS. After weighing, the sediment was allowed to dry at 85 °C for 24 h and the dried sediment was weighed for water absorption. Water solubility, swelling power, and water absorption of PPS were calculated as shown in Equations (2)–(4), respectively.

$$\text{Water solubility } (\%) = \frac{\text{Dry weight of solubilised sample (g)}}{\text{Dry weight of sample (g)}} \times 100\% \quad (2)$$

$$\text{Swelling power (g/g)} = \frac{\text{Wet weight of swollen sediments (g)}}{\text{Dry weight of sample (g)} \times (100 - \%\text{ solubility})} \quad (3)$$

$$\text{Water absorption index} = \frac{\text{Wet weight of sediments (g)}}{\text{Dry weight of sample (g)}} \quad (4)$$

2.4. Enzymatic Hydrolysis of Pineapple Plant Stem

The PPS was subjected to enzymatic hydrolysis using Dextrozyme DX 1.5 X (Novozymes, Copenhagen, Denmark) as described by reviews [14,32]. Seven percent (w/v) of PPS was suspended in 0.1 M acetate-buffered solution at pH 4.2. Gelatinisation was carried out by boiling in a water bath at 100 °C for 15 min. The beaker containing the PPS suspensions was covered with aluminium foil to reduce solvent loss in heating. It was then allowed to cool down at room temperature until it reached 60 °C. Then, 5.56 U/mL Dextrozyme DX 1.5 X with glucoamylase activity of 156.36 U/mL was added to the gelatinised PPS. The solution was incubated in a water bath at 60 °C for 60 min with continuous stirring. It was then allowed to cool down to room temperature and subjected to centrifugation at 3000 rpm for 10 min. The PPS hydrolysate was analysed for glucose and total reduced sugars concentration. The hydrolysis yield of PPS was calculated based on Equation (5).

$$\text{Hydrolysis yield } (\%) = \frac{\text{Amount of reduced sugar produced (g/L)} \times 0.9 \times 100\%}{(\text{Starch } (\%) \times \text{Amount of substrate used (g/L)})} \quad (5)$$

A value of 0.9 was used as the correction error for the determination of the amount of starch hydrolysed, since starch is a form of polysaccharide and water is involved in the hydrolysis of polysaccharides and 1 mol of reduced sugar released requires 1 mol of water.

2.5. Amino Acids Production by B. subtilis ATCC 6051

2.5.1. Culture Preparation

B. subtilis (Ehrenberg) Cohn (ATCC 6051) purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA) was used in the production of amino acids from PPS hydrolysate. Ready-made nutrient medium was used for the routine growth of the culture, which consisted of peptone (0.5% w/v), and meat extract (0.3% w/v) with pH of

the medium adjusted to pH 7.0. Subculturing was done using 1% (*v*/*v*) of the culture, incubated at 30 °C for 24 h at 150 rpm.

2.5.2. Fermentation for Amino Acids Production

A volume of 10% (*v*/*v*) active 24 h seed culture was transferred into production medium in a 250 mL shake flask consisting of nutrient medium supplemented with 20 g/L glucose from PPS hydrolysate. Incubation was conducted at 30 °C for 72 h at 150 rpm. Sampling was done at 4 h intervals in triplicate, followed by centrifugation at 10,000 rpm for 5 min using a microcentrifuge (Sorvall Legend Micro 17, Thermo Fisher Scientific, Dreieich, Germany) to obtain the cell-free supernatant for reducing sugars and amino acids analysis.

2.6. Analytical Analysis

Determination of glucoamylase activity of Dextrozyme DX 1.5 X was done as described by Leaes et al. [33] The reducing sugars concentration was determined using the dinitrosalicyclic acid (DNS) method as described by Miller [34]. Simple sugars from PPS hydrolysate were determined using high-performance liquid chromatography (HPLC) as described by Linggang et al. [35]. The quantification was done using a Rezex RPM-Monosaccharide Pb^{+2} column with a refractive index detector. Deionised water was used as the mobile phase with a flow rate of 0.6 mL/min and the analysis was carried out at 80 °C. Cell concentration was determined using an Optizen Pop UV/VIS Spectrophotometer (Mecasys Co., Ltd., Daejon, Republic of Korea) at 600 nm, based on Toe et al. [21]. Amino acids profile of the cell-free supernatant was analysed at an accredited laboratory (UNIPEQ, UKM-MTDC, Bangi, Malaysia). After acid hydrolysis, samples were analysed for amino acids content via the ACCQ Tag Waters Method using an HPLC equipped with fluorescence detector. The statistical analysis of the experimental data was conducted by t-test, analysis of variance (ANOVA), and the means were compared by Tukey's post hoc test at a significance level of 0.05 ($p < 0.05$). All statistical and correlation analyses were conducted using the statistical software SPSS Version 28.

3. Results and Discussion

3.1. Carbohydrate Content of Pineapple Plant Stem

Quantification of the carbohydrate in the PPS revealed that it consisted of a significant amount of soluble and insoluble fractions of the carbohydrate. Sugars are the soluble carbohydrates whereas starch is the insoluble fraction of the carbohydrate. The initial sugars found in the PPS before enzymatic hydrolysis were found to be monosaccharide, with 1.17 (±0.17) g/L glucose, and disaccharides, such as 0.97 (±0.75) g/L maltose and 3.64 (±0.82) g/L sucrose. These sugars are known as the water-soluble fraction of the non-starch polysaccharides, which can be readily used as a carbon source in the fermentation process. PPS was also found to be composed mainly of starch at 78.96 (±3.60)%, which could be a great source of substrate for glucose recovery. However, the starch as the non-structural carbohydrate in the PPS needs to undergo gelatinisation and enzymatic hydrolysis for the production of glucose.

3.2. Enzymatic Hydrolysis of Pineapple Plant Stem

Gelatinisation process is dependent on the plant source and type of starch; thus, it often commences at various temperatures. Gelatinisation temperatures that fall within the range of 58–72 °C have been reported for various types of native starches, such as potato, corn, wheat, tapioca, and waxy maize [36]. Among these, the temperature of 60 °C has the highest occurrence. Therefore, the gelatinisation temperature of PPS was set at 60 °C as the control prior to enzymatic hydrolysis. This resulted in 16.61 (±0.72) g/L glucose recovery, which was equivalent to 28.32% hydrolysis yield. The sugar yield was comparable to that of total reducing sugars (28.7%) and total sugars (29.0%) obtained at 5% (*v*/*v*) sulfuric acid at 3 h of hydrolysis time for direct hydrolysis of spent coffee grounds [37]. However, compared to a study conducted by Awg-Adeni et al. [14] with 52.72% hydrolysis yield, the

glucose recovered obtained from the control in this study was at a lower value. The low glucose recovery from PPS at 60 °C could be due to the biomass, which had yet to reach its onset gelatinisation temperature. Other than that, low water solubility and swelling power at the gelatinisation temperature used for the control could also be the reasons for the low hydrolysis yield obtained. Therefore, studies on the gelatinisation properties as well as the functional properties of PPS are needed to enhance the production of fermentable sugars, specifically in glucose recovery. In enzymatic hydrolysis of PPS, gelatinisation is deemed to be a vital step as the higher degree of gelatinisation will ease the enzymatic process [38]. The hydrothermal processing will increase the availability of disordered α-glucan chains, which makes the gelatinised sample more susceptible to hydrolysis [39].

3.3. Enhancement of Glucose Recovery from Pineapple Plant Stem

Gelatinisation properties of PPS were studied to understand its behaviour and optimum temperature for the process to take place. This allowed the determination of the gelatinisation temperature of PPS before it was subjected to enzymatic hydrolysis. As an added value to enhance the recovery of the fermentable sugar from the biomass, study on the effects of temperature and time on the functional properties of PPS also allows a better understanding of its behaviour. These functional properties include water solubility, swelling power, and water absorption of PPS.

3.3.1. Gelatinisation Properties of Pineapple Plant Stem

Gelatinisation is a two-stage endothermic reaction that takes place in an aqueous environment [40]. The PPS suspension was set at 7% (*w*/*v*) as reported by Awg-Adeni et al. [14] due to the high-fermentable-sugars conversion based on that respective study. Although high biomass loading may lead to more glucose being recovered, it can lead to a high inhibitors concentration and limit mass transfer [41]. The thermogram of gelatinisation temperature and enthalpies of PPS after determination using differential scanning calorimetry is depicted in Figure 1 and the values of thermal properties are reported in Table 1. The results show that the PPS has a high gelatinisation temperature and enthalpy.

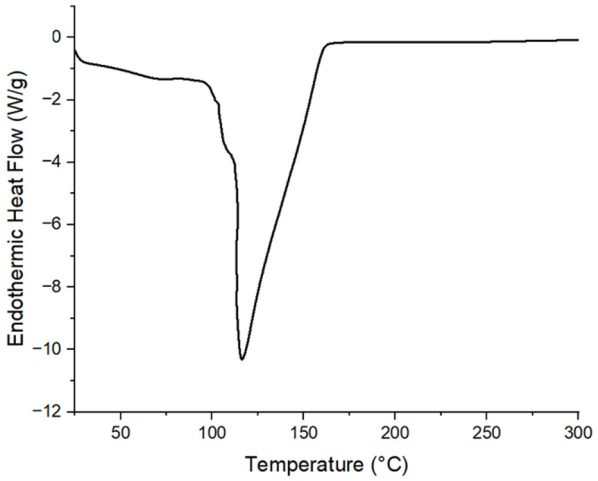


Figure 1. Differential scanning calorimetry thermogram of pineapple plant stem.

The onset gelatinisation temperature of the PPS was 111 °C. At 95 °C, the PPS reached its start gelatinisation temperature, in which the first crystallite started to gelatinise, followed by the T_o as the gelatinisation process continued. As explained by Chisenga et al. [31], high T_o results from the high content of protein, lipid, and fibre. A high lipids content will lower the susceptibility of granules to gelatinisation as it may prevent the water from diffusing into the granules, thus retarding the gelatinisation. The protein concentration also increases the temperature by competing with granules for water molecules and results in the inhibition of swelling [42].

Table 1. Thermal properties of pineapple plant stem.

Parameter	Value
Start Temperature, T_s (°C)	95
Onset Temperature, T_o (°C)	111
Peak Temperature, T_p (°C)	116
Conclusion Temperature, T_c (°C)	161
End Temperature, T_e (°C)	167
Gelatinisation Enthalpy, ΔH (J/g)	263.58

The peak gelatinisation temperature of the PPS was 116 °C. A higher peak temperature in PPS indicated that more thermal energy was required to initiate the gelatinisation process. Similar to T_o, T_p also showed a weak positive correlation with amylose, protein, and lipid content of the granules; however, it showed a weak negative correlation with swelling powers [31].

The conclusion gelatinisation temperature of PPS was 161 °C. Upon reaching the temperature of 167 °C, it reached its end gelatinisation temperature, in which the most perfect crystallites of the PPS gelatinised. At this temperature, the PPS had reached the endpoint of its complete gelatinisation. Differences in amylose, protein, and lipid contents, as well as amylopectin chain length, could be the reasons affecting the T_c [31].

The enthalpy of gelatinisation of PPS was 263.58 J/g. During the endothermic process of gelatinisation, energy is required to break the hydrogen bond, which is known as enthalpy of gelatinisation [43]. The higher enthalpy of gelatinisation in the PPS, which reflects the loss of double-helical and crystalline order, indicated that more energy would be needed to melt the granules. The enthalpy of gelatinisation in the PPS may also be attributed to the presence of other components, such as protein, lipid, and fibre content. This was further demonstrated by Sirivongpaisal [44], wherein the enthalpy of gelatinisation in the flour of Bambara groundnut was higher than that in the starch obtained from the groundnut.

3.3.2. Effects of Temperature and Time on Water Solubility of Pineapple Plant Stem

In swelling volume determination, solubility is the percentage amount of starch or granules that is leached out into the supernatant [30,45]. The granules are generally insoluble in cold water; therefore, heat is needed for the granules to absorb water and swell in the presence of excess water [40]. For a deeper understanding of the water solubility of the PPS, the process was carried out at various temperatures and times. The water solubility of the PPS increased from 15.88% to 37.01% at a temperature ranging from 60 °C to 100 °C after 15 min of gelatinization, as depicted in Figure 2. However, there was a trivial decrease of water solubility with increase in time from 15 to 30 min. During gelatinisation, the structure becomes tighter, more rigid, and ordered due to the effects of the heating on the hydrogen bonding, correlating to the internal rearrangement and reassociation of the granules [46,47]. The water solubility of the PPS was contributed to by not only starch but also proteins, fibre, and other compositions. After heating for a long period, a new network is formed when the protein molecules aggregate, thus reducing the water solubility of the protein component after extrusion. Other than that, the amount of insoluble dietary fibre also decreased [48]. This in turn affected the overall water solubility of the PPS. There was a strong positive correlation between temperature and water solubility of the PPS ($r = 0.894$, $p < 0.001$). This suggests that the water solubility of the PPS was temperature-dependent and it increased as the temperature increased.

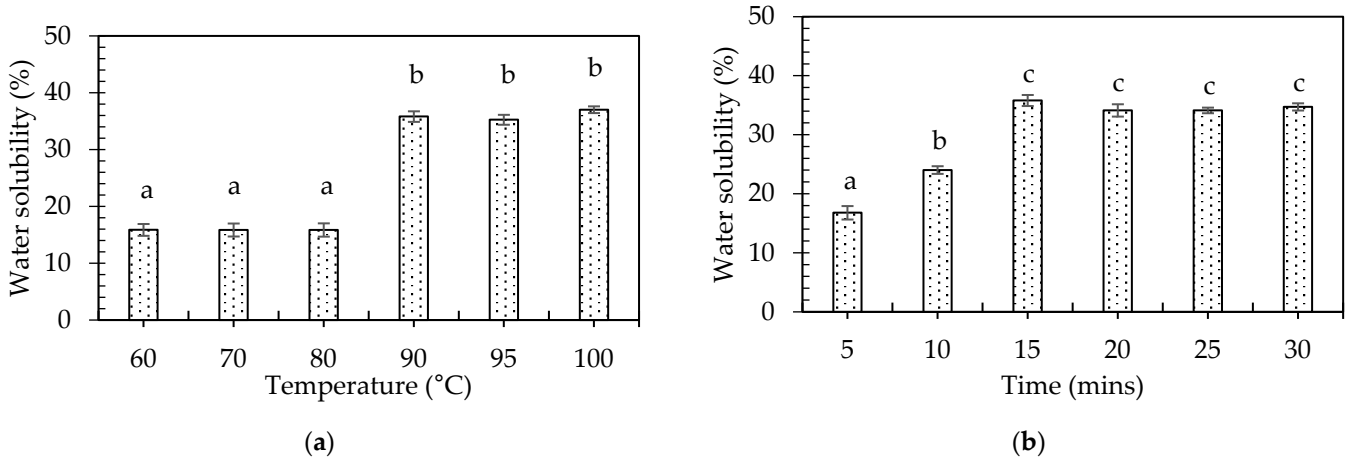


Figure 2. Water solubility of pineapple plants stems under different conditions: (**a**) effect of temperature; (**b**) effect of time. Error bars indicate the mean value ± standard deviation of triplicate of the sample. Values with different letters are statistically different at $p \leq 0.05$.

When tested at different times ranging from 5 to 30 min at 90 °C, the water solubility of the PPS increased significantly from 16.79% at 5 min and reached its peak at 35.81% at 15 min, and the trend decreased beyond 15 min, insignificantly. There was also a strong positive correlation between time and water solubility of the PPS (r = 0.894, $p < 0.001$). This suggests that the water solubility of the PPS increased as the time taken for the dispersion process increased. The water solubility of the PPS showed a two-stage process, whereby the values were insignificant when heated at 60 to 80 °C, followed by a significant increase when the heating temperature changed from 80 to 90 °C, where gelatinisation may have occurred. At high temperature, the PPS may have lost its crystallinity and molecular order due to the distraction of granules and the release of amylose from the starch content, which led to an increase in the water solubility.

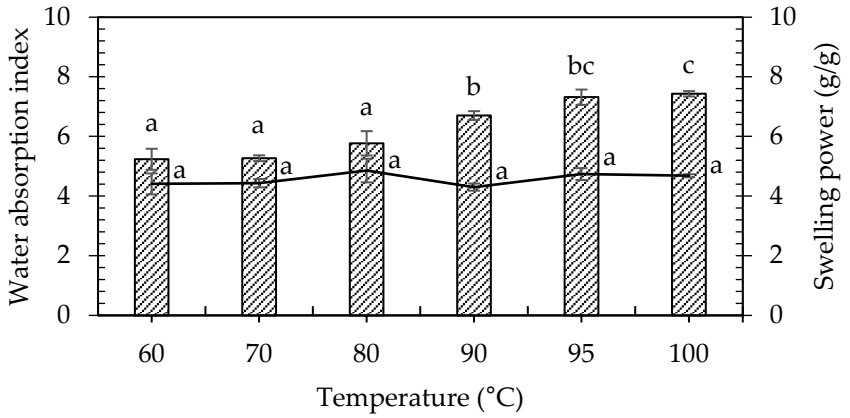
3.3.3. Effects of Temperature and Time on Swelling Power of Pineapple Plant Stem

Swelling power is usually characterised as an important structural characteristic during the processing and ascertaining of its suitability in food applications [31]. During heating, the crystallinity will decrease due to alteration in the crystalline structure, which will lead to the absorption of more water and result in changes in the solubility, granule swelling and loss of birefringence. The penetration of water will swell the granules before they are finally ruptured [8].

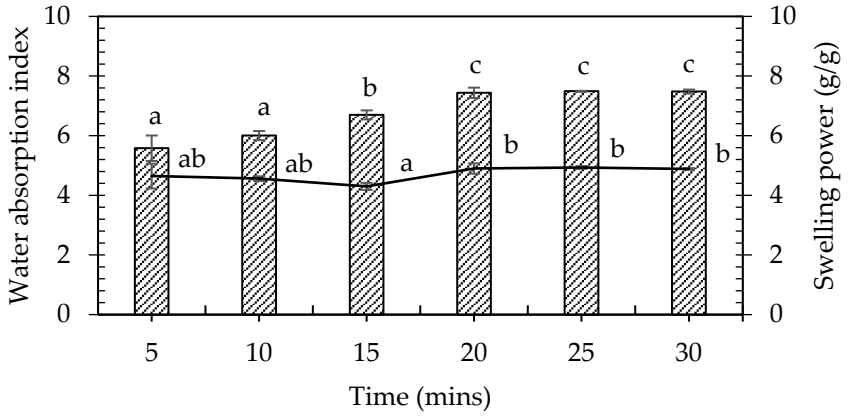
There was a very strong positive correlation between water solubility and swelling power of the PPS (r = 0.953, $p < 0.05$). When starch granules are heated and become swollen, the molecules become smaller and more soluble, making them more easily dispersed in the water, thus increasing the water solubility [6]. The swelling power of the PPS differed in a range from 60 to 100 °C after 15 min of gelatinization, as shown in Figure 3, showing a continuous increase with the increase in temperature.

At 60 to 80 °C, the temperature was below the start and onset gelatinisation temperature, leading to the lower value of swelling power in the PPS. There was a very strong positive correlation between temperature and swelling power of PPS (r = 0.961, $p < 0.05$). This is in line with the study conducted by Chen et al. [49], in which the swelling power of corn starch increased significantly as temperature increased. When the PPS was heated for different durations at 90 °C, the swelling power increased over time from 5 to 30 min. There was a significant increment from 10 to 20 min, where it reached its peak swelling power. The correlation between time and swelling power of PPS also showed a high positive value (r = 0.942, $p < 0.05$), indicating that as time increased, the swelling power also increased. A similar trend was observed by Kumoro et al. [47], whereby the swelling power of gadung (*Dioscorea hispida* Dennst) starch increased as time increased to a certain extent when the

temperature was set at 110 °C. This is also in agreement with another study conducted by Ji et al. [50], in which the swelling factor of maize starch increased as the heating time increased. As the heating time increased, the expansion of the granules was induced and granules swelled to a larger size, thus increasing their swelling factor.



(**a**)



(**b**)

Figure 3. Water absorption and swelling power of pineapple plants stems at different parameters: (**a**) effect of temperature; (**b**) effect of time. Error bars indicate the mean value ± standard deviation of triplicate of the sample. Values with different letters are statistically different at $p \leq 0.05$.

Swelling power often correlates with amylose and amylopectin content. There is a weak negative correlation between swelling power and amylose content [10,31]. Other than the amylose content, the protein and lipid contents, as well as trace elements such as phosphorus, sodium, and potassium, are also factors contributing to the variation in swelling power [31,51]. The negative correlation of swelling power and protein content is due to the ability of the protein compounds to restrict the swelling of the granules; they may cause an increase of hydrophobicity and eventually reduce the uptake of water, resulting in a decrease in swelling power [31,42].

3.3.4. Effects of Temperature and Time on Water Absorption of Pineapple Plant Stem

The water absorption index is the ability of the gelatinised granules to absorb water, which can be affected by the availability of hydrophilic groups and their capacity to

form gels [52]. It measures the amount of water absorbed by granules in the presence of excess water after the swelling of granules and is often related to their gelatinisation properties [13,53]. The water absorption index of the PPS was determined at various temperatures, ranging from 60 to 100 °C for 15 min, with a peak value of 80 °C, as depicted in Figure 3. However, the changes in the water absorption index were insignificant at all the temperatures used in the determination. Unlike water solubility and swelling power, there was only a weak positive correlation between the temperature and water absorption index (r = 0.395, $p < 0.05$). When compared to swelling power, it showed a weak positive correlation with the water absorption index (r = 0.321, $p < 0.05$). The effects of heating time on the water absorption index of PPS were studied at 5 to 30 min of gelatinisation. A significant increase in the water absorption index was reported after 15 min of heating before it reached its peak value at 25 min of heating. However, the correlation between time and water absorption index showed a relatively high positive interaction (r = 0.622, $p < 0.05$).

The water absorption index gives information on the number of solids that are dissolved in the water, acting as an indication of starch degradation [54]. When heated in water, disruption of inter- and intra-molecular bonds will disturb the compact structure of the PPS. The hydroxyl groups in the PPS will be converted into alkoxides in an aqueous environment. This will in turn lead to a disruption of the hydrogen bond between the glucose monomers. Swelling will eventually occur when a hydrogen bond is formed between water molecules and the amylose or amylopectin in the PPS. This will then lead to extensive swelling as the water molecules penetrate the internal structure of the granules [55]. The polysaccharide chains are then uncoiled in their swollen state and this transition is irreversible [12]. The water absorption capacity may be affected by the presence of other components, such as protein and carbohydrates [44]. The presence of protein may affect the hydrophobicity of the PPS, hence affecting its water absorption ability. Furthermore, variations of granular structure and the loose association of amylose and amylopectin molecules in the PPS could also affect its water-binding capacity [51].

3.3.5. Glucose Recovery from Pineapple Plant Stem at Optimised Gelatinisation Condition

Enzymatic hydrolysis of PPS led to a significant increment in terms of glucose recovery when gelatinisation was conducted at the optimum temperature and time, based on the results obtained from its functional properties and gelatinisation temperature. Considering that higher water solubility and swelling power may enhance the susceptibility of PPS towards enzymatic hydrolysis, the reaction time of 15 min was selected for the gelatinisation prior to hydrolysis for enhanced glucose recovery. This led to an enhanced enzymatic hydrolysis yield when the temperature was increased from 60 °C to 90 °C, in which the glucose recovered resulted in a 2.76-fold increase in hydrolysis yield at 78.18%, with 49.91 (±2.87) g/L glucose being produced. When the temperature was increased to 90 °C, the significant increase in its solubility and swelling power resulted in higher glucose recovery as the PPS was reaching its start and onset gelatinisation temperature. Although the water solubility and swelling power of the PPS did not increase significantly from 90 °C to 100 °C, the enzymatic hydrolysis still resulted in a 1.28-fold increase in hydrolysis yield, in which 56.81 (±2.15) g/L glucose was recovered at 100 °C with 0.81 g/g hydrolysis yield. This could be due to the gelatinisation properties of the PPS: it was reaching its peak gelatinisation temperature at 100 °C. In short, the hydrolysis yield showed a total 3.53-fold increment when the gelatinisation temperature was increased from 60 °C (control) to 100 °C before enzymatic hydrolysis, thus making PPS a potential biomass for glucose production and recovery.

3.4. Amino Acids Production by B. subtilis ATCC 6051 from Pineapple Plant Stem Hydrolysate

Growth profiling of *B. subtilis* ATCC 6051 using glucose (20 g/L) as the carbon source was carried out, as illustrated in Figure 4, to understand the growth of the strain and its glucose utilisation throughout the fermentation process, showing a total of 12.05 g/L

glucose being consumed. When PPS hydrolysate was used, the consumption was slightly higher than that of commercial glucose, as shown in Figure 5, in which 15.17 g/L carbon source was being consumed.

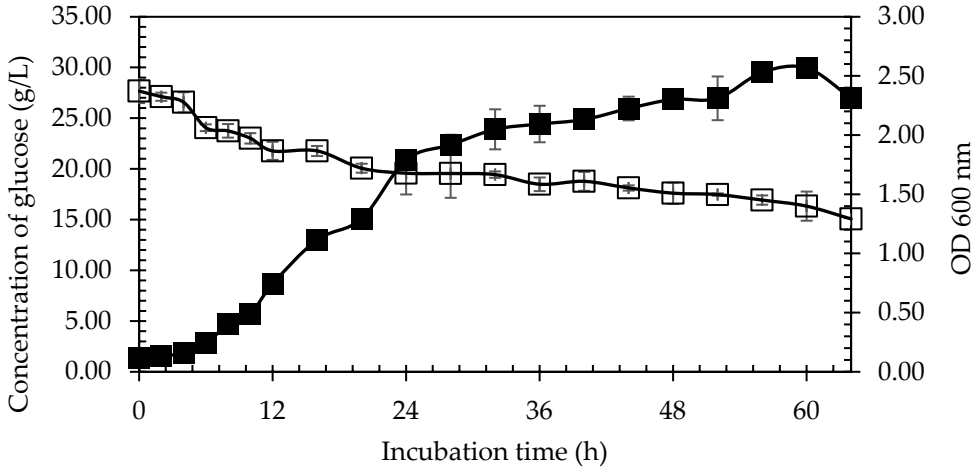


Figure 4. Growth profiling of *B. subtilis* ATCC 6051 using glucose as carbon source. Symbols represent: □: glucose; ■: OD_{600}. Error bars indicate the mean value ± standard deviation of triplicates of the sample.

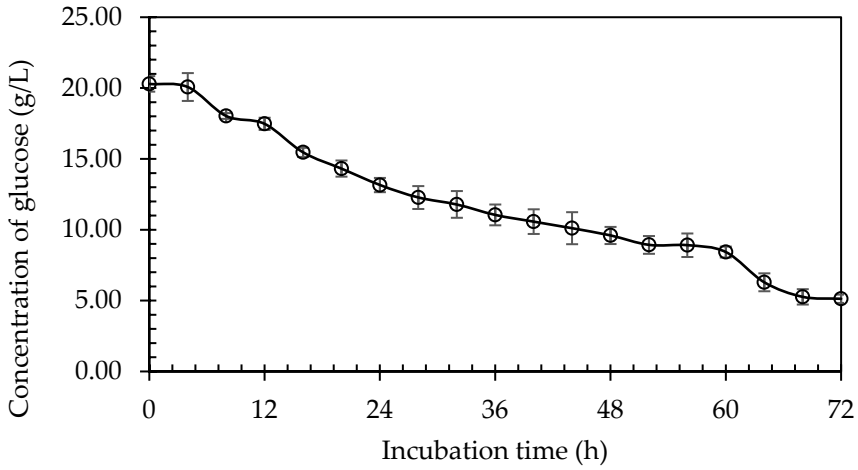


Figure 5. Glucose consumption of *B. subtilis* ATCC 6051 using pineapple plant stem hydrolysate as carbon source. Symbol represents: ○: glucose. Error bars indicate the mean value ± standard deviation of triplicates of the sample.

As a result, a maximum of 23.53 mg/mL total amino acids were being produced extracellularly during the fermentation. This indicates that glucose recovered from the PPS was suitable for the growth of the microbe in amino acids production. The increment of total amino acid produced throughout the fermentation (as presented in Table 2) suggested that *B. subtilis* ATCC 6051 has the ability to produce amino acids using PPS hydrolysate through separate enzymatic hydrolysis and fermentation.

It is noteworthy that the strain was able to produce various essential amino acids. A maximum of 21.43 mg/mL essential amino acids were produced, including threonine, valine, methionine, lysine, isoleucine, and leucine. As for non-essential amino acids, a lesser amount of production was detected as compared to the essential ones, with a total of 2.89 mg/mL being produced. Among the 17 amino acids detected, lysine (20.81 mg/mL) and alanine (1.36 mg/mL) were the most notable productions, followed by glutamic acid (0.52 mg/mL) and aspartic acid (0.34 mg/mL). The production of lysine obtained in this study was higher than that reported in a study using glucose by KiBeom et al. [56], in

which 0.22 mg/mL and 0.12 mg/mL lysine were produced from *Pediococcus acidilactici* and *Lactobacillus salivarius*, respectively. The lysine produced was also higher compared to that in other studies using jackfruit seed hydrolysate (8.00 mg/mL) [57] and corn stover hydrolysate (14.70 mg/mL) [58], where *C. glutamicum* was used in both studies. In the food industry, glutamic acid has been in great demand, followed by aspartic acid. Glutamic acid is commonly used as a flavour enhancer in the form of monosodium glutamate (MSG), whereas aspartic acid is the starting material for peptide sweeteners, namely L-aspartyl L-phenylalanyl methyl ester (aspartame) [59]. In the animal feed industry, the so-called feed amino acids, including lysine, methionine, and threonine, make up the largest share of the total amino acid market at 56% and they are expected to keep fueling the market growth in future [60].

Table 2. Maximum increment in amino acids production from pineapple plant stem hydrolysate through separate enzymatic hydrolysis and fermentation using *B. subtilis* ATCC 6051. Superscripts with different letter are significantly different from each other within the same column at $p \leq 0.05$.

Amino Acid	Maximum Increment		
	Amount (mg/mL)	Time (h)	Productivity (mg/L/h)
Essential Amino Acid			
Histidine	0.00 ^{a}	72	0.00 ^{a}
Threonine	0.25 ^{h}	24	10.44 ^{j}
Valine	0.16 ^{f}	72	2.23 ^{e}
Methionine	0.03 ^{ab}	72	0.42 ^{b}
Lysine	20.81 ^{m}	48	433.57 ^{n}
Isoleucine	0.11 ^{e}	72	1.53 ^{c}
Leucine	0.07 ^{cd}	24	2.92 ^{g}
Phenylalanine	0.00 ^{a}	48	0.00 ^{a}
Non-Essential Amino Acid			
Hydroxyproline	0.00 ^{a}	72	0.00 ^{a}
Aspartic Acid	0.34 ^{j}	48	7.10 ^{h}
Serine	0.29 ^{i}	24	12.11 ^{l}
Glutamic Acid	0.52 ^{k}	48	10.86 ^{k}
Glycine	0.06 ^{bc}	24	2.51 ^{f}
Arginine	0.00 ^{a}	24	0.00 ^{a}
Alanine	1.36 ^{l}	48	28.39 ^{m}
Proline	0.21 ^{g}	24	8.77 ^{i}
Tyrosine	0.10 ^{de}	48	2.09 ^{d}
Total Amino Acid	**23.53**	**48**	**490.15**

In contrast to the amino acids produced, our study showed a reduction in the arginine profile, indicating that the strain cannot produce arginine from PPS hydrolysate. This is in line with studies carried out by Lim et al. [61] and Lee et al. [62], which showed a drastic reduction of arginine was found in several strains of *Pediococcus* sp. and *Lactobacillus* sp. for amino acids production. Hydroxyproline, histidine, and phenylalanine also showed a reduction during the fermentation, indicating that they were being consumed for cell growth by *B. subtilis* ATCC 6051.

All in all, the production of various amino acids indicates that PPS can be an alternative carbon source in the bioconversion of amino acids. The production of amino acids also throws light on the application of *B. subtilis* ATCC 6051 in the amino acid industry. Apart from being rich in starch, PPS also consists of other components, such as cellulose, hemicellulose, and protein, which could be potential sources for amino acids production. However, due to the different structures and mechanisms of hydrolysis, different enzymes are required to break down different components of the PPS. Therefore, this study can serve as a baseline for the production of amino acids from PPS using *B. subtilis* ATCC 6051 by enhancing the glucose recovery. Future study can be conducted on direct utilisation

of starch and other components of PPS for the production of amino acids as well as its optimisation process.

4. Conclusions

The present work showed that PPS has a high gelatinisation temperature (T_o = 111 °C; T_p = 116 °C; T_c = 161 °C) and gelatinisation enthalpy (ΔH = 263.58 J/g). The optimum temperature and time for gelatinisation of PPS were at 100 °C and 15 min, respectively. This led to a maximum glucose recovery of 56.81 g/L of fermentable sugar. The glucose recovered resulted in 23.53 mg/mL amino acids being produced after fermentation. Thus, the current work has successfully demonstrated the ability of *B. subtilis* ATCC 6051 in the production of amino acids by utilising the glucose recovered from PPS as the carbon source. In short, the present findings throw light on the potential valorisation of PPS for its application in the amino acid industry and open up a new opportunity for the application of *B. subtilis* ATCC 6051 as an amino acid producer from agricultural wastes. The optimisation of the production of the amino acid from PPS using *B. subtilis* ATCC 6051 can be conducted as future studies of this work.

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