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Producing Amino Acid by Hydrolysis of Fermented Mash and Slag of Food Waste with the Differrnt Proteases

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ABSTRACT. *With the development of economy and society, the output of kitchen waste has increased year by year, fermentation is an effective way to solve the problem of kitchen waste. After fermentation, there will be a lot of mash and slag. This study studied the optimum temperature and pH of FLA and PRO proteases. The optimum temperature and pH of FLA were 50℃, pH=6; the optimum temperature and pH of PRO were 50 ℃, pH=6. In the case of two enzyme substrate concentrations of 0.05%, 0.1%, 0.2% and 0.4% (g/g), the mash and slag were hydrolyzed to determine the amino acid content at 36 h and 72 h respectively. In order to further improve the hydrolysis effect, the mash and the slag are mixed in equal amounts to carry out hydrolysis. Under the action of FLA enzyme, good hydrolysis effect can be achieved. When the amount of FLA enzyme is 0.4% (g/g), 72 h, the maximum content of amino acid is 26.79 mg/ml. PRO enzyme failed to achieve the desired effect. This study is the first time to study the mash and slag residue after fermentation of kitchen waste, and try to establish a new industrial method to provide some ideas for the treatment of kitchen waste and slag.*

KEYWORDS: *Mash and slag of food waste, Proteases, Enzymatic hydrolysis*

1. Introduction

With the continuous development of economy and society, people's living standards are constantly improving, the production of kitchen waste is increasing year by year [1]. Kitchen waste not only has high water content, but also contains more organic matter. The major chemical components of kitchen waste are starch, protein, fat, cellulose, and others [2]. Therefore, if it is arbitrarily discharged, it will not only cause a large number of toxic and harmful microorganisms to breed, but also have a serious impact on the environment. However, traditional incineration methods have low economic benefits and potential environmental hazards, Bio-

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treatment is an ideal method to dispose organic waste or organic pollution [3], Commonly used biological treatment methods include anaerobic fermentation to produce methane, volatile fatty acids [4]; aerobic compost to produce fertilizer and microbial strains have been used to produce substances such as ethanol [5], butanol and lactic acid [6]. Although it effectively increases the value of kitchen waste, microbes cannot use up all the organic matter in kitchen waste. All of the fermentation residues are produced as industrial microbial fermentation proceeds [7]. This residue includes both liquid and semi-solid, which we call mash and slag. During the process of alcohol fermentation of kitchen waste, the microorganisms basically use up the sugars such as starch, and the fermentation process is completed by oil separation, alcohol distillation, centrifugation, etc, and the fermentation mash and slag are obtained [8].

Since the protein substances that microorganisms can utilize during the fermentation process are limited, the remaining protein substances in the kitchen waste remain in the fermentation mash and slag. A large amount of slag and mash are discarded at will, which not only causes a large number of bacteria to breed, but also causes serious environmental pollution. At the same time, the rate and amount of fermentation mash and slag will increase. Finding an effective way is necessary to use mash and slag [9].

Amino acids are monomers that make up proteins, they are widely found in nature [10]. They are important components in the body and in maintaining human health. They play an important role in food, medicine and agriculture. At present, the global demand for amino acids is growing rapidly. The slag and mash are rich in protein resources, and the protein resources are converted into amino acids. It can provide a large amount of raw materials for food, medical and agriculture, which not only solves the pollution problems of mash and slag, but also The secondary use of waste resources will truly turn waste into waste [11].

Novozymes Flavourzyme (FLA) and Novozymes Protamex (PRO) are two commonly used proteases [12] that have a good hydrolysis effect on proteins and are widely used in food, medical and agricultural fields [13]. Because mash and slag are rich in protein resources, this study, the proteins in mash and slag were hydrolyzed by Novozymes Flavourzyme and Novozymes Protamex proteases respectively, and the hydrolysis conditions were optimized to produce amino acids for secondary use of kitchen waste.

2. Methods

2.1 Enzyme, material

Novozymes Flavourzyme (FLA) and Novozymes Protamex (PRO) were purchased from Novozymes; This study, the mash and slag of food waste was obtained from Guangdong Lishikang Low Carbon Technology Co. Ltd.

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2.2 Determination of physical and chemical properties of FLA and PRO

In this study, the optimal temperature and optimum pH of FLA and PRO enzymes were determined by using kitchen waste mash as a hydrolysis substrate for proteases. Dispense the kitchen waste mash into 50 g per bottle, adjust the pH to 7 with Na_2HPO_4, and add two proteases FLA and PRO in the amount of 0.1% (g/g), respectively, at 30℃, 40℃, 45℃, 50℃, 60℃ temperature conditions, 200 rpm, after hydrolysis for 36 h, the supernatant was taken and the amino acid content was determined by ninhydrin colorimetry. Dispense the kitchen waste mash into 50 g per bottle, Adjusting pH 3 4 5 6 7 with Na_2HPO_4, respectively. Adding FLA and PRO proteases.

2.3 Different concentrations of FLA and PRO respectively hydrolyze the kitchen mash and slag

Dispense the kitchen waste mash into 50 g per bottle, adjust the pH to 6 with Na_2HPO_4, and add two proteases to FLA and PRO in an amount of 0.05 %, 0.1 %, 0.2 %, 0.4 %, respectively. FLA is hydrolyzed at 50 ℃,200 rpm for 72 h, PRO is hydrolyzed at 45 ℃,200 rpm for 72 h, the supernatant was taken and the amino acid content was determined by ninhydrin colorimetry. Dispense the kitchen waste slag into 50 g per bottle, adjust the pH to 6 with Na_2HPO_4, add two proteases to FLA and PRO in an amount of 0.05 %, 0.1 %, 0.2 %, 0.4 %, respectively, detection of amino acids in the supernatant after hydrolysis is completed. Dispense the kitchen waste 25 g slag and 25 g mash into per bottle, adjust the pH to 6 with Na_2HPO_4, add two proteases to FLA and PRO in an amount of 0.05 %, 0.1 %, 0.2 %, 0.4 %, respectively, detection of amino acids in the supernatant after hydrolysis is completed.

2.4 Calculations and statistical analysis

All experiments were conducted in duplicate. The data are presented as mean values ± standard deviation (SD). Statistical analysis was carried out by the IBM SPSS statistics 22 using oneway ANOVA and Duncan's multiple range tests. Results were considered statistically significant at 95% confidence interval ($p < 0.05$).

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3. Result

3.1 Effects of temperature on enzyme activity

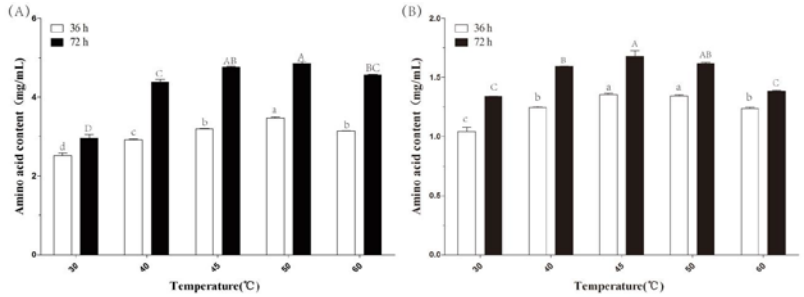

Figure. 1 Effects of temperature on enzyme activity (A) represents the amino acid content of FLA at 36 h and 72 h (B) represents the amino acid content of PRO at 36 h and 72 h. Values with different letters within the figures indicate significant differences ($p < 0.05$). $n = 2$.

Using the kitchen waste mash as a substrate, the two enzymes were hydrolyzed at different temperatures using FLA and PRO to determine the amino acid content. The amino acid content of FLA reached the highest at 36 h under 50 ℃. With the prolongation of hydrolysis time, 72 h was significantly higher than 36 h under various temperature conditions. At 72 h, the amino acid content was highest at 50 ℃, and the difference was not much different from 45 ℃. There was no significant difference in PRO at 36 h, 45 ℃and 50 ℃, but with the increase of hydrolysis time, the amino acid content reached the highest at 72 h and 45 ℃.

3.2 Effects of pH on enzyme activity

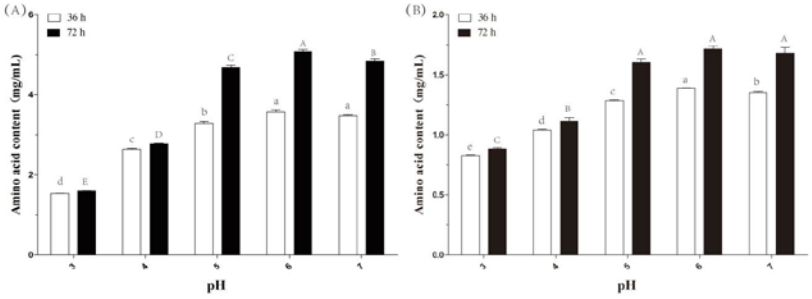

Figure. 2 Effects of pH on enzyme activity (A) represents the amino acid content of FLA at 36 h and 72 h (B) represents the amino acid content of PRO at 36 h and 72 h. Values with different letters within the figures indicate significant differences ($p < 0.05$). $n = 2$.

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Using the kitchen waste mash as a substrate, set different pH gradients, hydrolyze the mash by using FLA and PRO enzymes. The amino acid content of FLA reached the highest under the condition of pH=6 7 at 36 h. With the increase of hydrolysis time, the amino acid content reached the maximum under the condition of pH=6, indicating that pH=6 is the optimum pH of FLA enzyme. The FLA and PRO proteases have lower amino acid content at pH=3, probably because the low pH affects the stability of the protease, which in turn affects the activity of the protease. The amino acid content of PRO reached the maximum at 36 h and pH=6, but at 72 h, pH=5 6 7, there was no significant difference in amino acid content.

3.3 Hydrolysis of kitchen waste mash by FLA and PRO

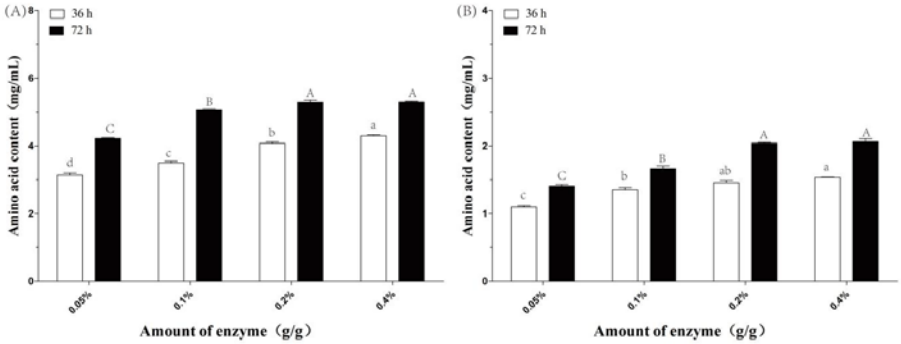

Figure. 3 (A) FLA hydrolysis of kitchen waste mash (B) PRO hydrolysis of kitchen waste mash. Values with different letters within the figures indicate significant differences ($p < 0.05$). n = 2.

Using the kitchen waste sputum as a substrate, the fermentation mash was hydrolyzed by different proteases of FLA and PRO, respectively (Fig.3). Under the action of FLA, with the increase of protease concentration, the content of amino acid increased gradually. At 36 h, under the condition of FLA content of 0.4% (g/g), the amino acid content was the highest, reaching 4.3 mg/ml, which was significantly higher than the other three Group of amino acids ($p < 0.05$). However, with the prolongation of hydrolysis time, there was no significant difference in amino acid content between the two groups at 72 h, 0.2% and 0.4% FLA. Under the hydrolysis of PRO protease, although the amino acid content is increasing with the increase of enzyme amount and hydrolysis time, the overall amino acid content is low, and the highest amino acid content is 2.04 mg/ml. The two groups of enzymes of FLA and PRO hydrolyze the kitchen waste mash, and the amino acid content is low. The possible reason is that the protein content in the whole mash is low, the water content is high, and the protease molecules are diluted to make the substrate and the enzyme. The mutual contact between molecules is reduced. Although the amino acid

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content is increased with the increase of certain enzyme amount, the improvement effect is not satisfactory.

3.4 Hydrolysis of kitchen waste slag by FLA and PRO

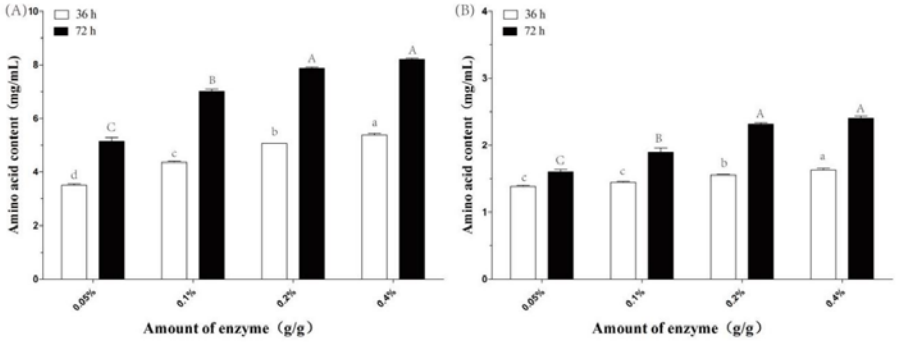

Figure. 4 (A) FLA hydrolysis of kitchen waste slag (B) PRO hydrolysis of kitchen waste slag. Values with different letters within the figures indicate significant differences ($p < 0.05$). $n = 2$.

Compared with the kitchen waste mash, the kitchen waste contains more protein, all of which are hydrolyzed by FLA and PRO, and the amino acid content is determined (Fig.4). Under the action of FLA protease, the amino acid content is gradually increasing with the increase of hydrolysis time. At 36 h, the enzyme content was 0.4% (g/g), the amino acid content reached the highest at 5.07 mg/ml. At 72 h, there was no significant difference in amino acid content between 0.2% and 0.4% (g/g) enzymes, and the amino acid content reached 7.87 mg/ml and 8.2 mg/ml, respectively. Under the action of PRO protease, the amino acid produced by the hydrolysis of kitchen waste slag is higher than that of the hydrolyzed kitchen waste mash, but the overall level is still at a lower level. With the increase of the content of protease, the hydrolysis time is prolonged, and the amino acid content is also obviously improved, the highest amino acid concentration reached 2.4 mg/ml. Although the protein content in the kitchen waste slag is significantly higher than that of the kitchen waste mash, the hydrolysis of the slag is carried out by two proteases, and the amino acid content is not greatly improved. Although the protein content in the slag is high, due to the small amount of water, the whole is in a viscous state, which affects the effective diffusion of the enzyme molecules. It may also be because a large number of protein molecules agglomerate, so that proteases can not act on these proteins, resulting in poor hydrolysis.

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3.5 Hydrolysis of kitchen waste mash and slag mixture by FLA and PRO

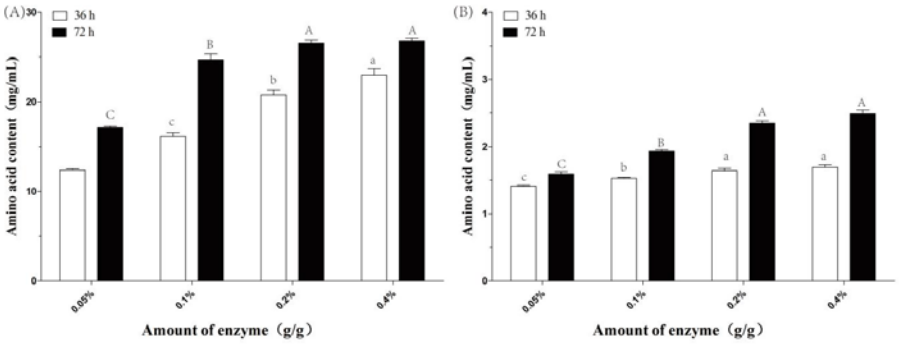

Figure. 5 (A) FLA hydrolysis of kitchen waste mash and slag (B) PRO hydrolysis of kitchen waste mash and slag. Values with different letters within the figures indicate significant differences ($p < 0.05$). $n = 2$.

In order to compensate for the low protein content in the kitchen waste mash and the high viscosity of the slag, the mash and slag are mixed in equal amounts and then hydrolysis with two proteases (Fig.4). Compared with the hydrolyzed slag and mash, the two are mixed to make a substrate, and the amino acid content is obviously improved. Under the action of FLA at 36 h, with the increase of FLA content, the amino acid content increased rapidly, and the maximum amino acid content reached 23.01 mg/ml. At 36 h, there was a significant difference in the amino acid content of 0.2% (g/g) protease content and 0.4% (g/g) enzyme hydrolysis, however, at 72 h, the amino acid content of the two was 26.53 mg/ml and 26.89 mg/ml, respectively, without much difference. In order to achieve the goal of reducing costs, the amount of enzyme added to FLA during hydrolysis is controlled at 0.2% (g/g). The amino acid content of the enzyme hydrolyzed by the PRO enzyme was also improved overall, but the amino acid content was at a lower level. At 72 h, 0.4% of the enzyme amount, the amino acid content was only 2.49 mg/ml. Therefore, compared with FLA protease, PRO protease has a low amino acid content, which can not meet the intended purpose, and FLA can achieve better results when sputum and slag is mixed as a substrate, when the enzyme content is 0.2% (g/g), it can reach a higher amino acid content.

Studies have found that FLA protease molecules may have inhibitory effects in the process of interaction with substrates [14], so in order to further enhance the hydrolysis effect, it may be from the perspective of solving the inhibition between FLA protease molecules [15]. Heat treatment can make the agglomeration of protein molecules more favorable for the binding of enzyme molecules [16]. It can also optimize the amount of enzyme and the concentration [17] and viscosity [18] of the substrate, and use external ultrasound to improve the hydrolysis of protein by protease [19].

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4. Conclusion

With the development of economy and society, the output of kitchen waste has increased year by year, fermentation is an effective way to solve the problem of kitchen waste. After fermentation, there will be a lot of mash and slag, this study mainly uses the kitchen waste fermentation mash and slag as the research object. Because it contains more protein, it degrades the protein into amino acids and supplies raw materials for food and medical treatment. First explore the optimum temperature and pH of both FLA and PRO enzymes.Then use the different proteases of FLA and PRO to hydrolyze the kitchen waste sputum and slag, in order to achieve the maximum amino acid yield, and provide a new method for the utilization of kitchen waste sputum and slag.

Acknowledgements

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