JTE HCMUTE
ISSN: 2615-9740

JOURNAL OF TECHNICAL EDUCATION SCIENCE
Ho Chi Minh City University of Technology and Education
Website: https://jte.hcmute.edu.vn/index.php/jte/index
Email: jte@hcmute.edu.vn

Study on the Production of Cellulase by Using *Aspergillus Oryzae* and its Application on the Green Coffee Treatment

Duy Q. Nguyen[1*], Thinh D. Lam[1], Hoang V. Nguyen[1], Nhi Y. Dinh[2], Phu H. Le[2]

[1]*Department of Food Technology, Faculty of Chemical and Food Technology, Ho Chi Minh City University of Technology and Education, Vietnam.*

[2]*Department of Food Technology, School of Biotechnology, International University – Vietnam National University in Ho Chi Minh City, Vietnam.*

[*]Corresponding author: Duy Q. Nguyen. *Email: duynq@hcmute.edu.vn*

ARTICLE INFO

Received: 15/03/2022
Revised: 18/05/2022
Accepted: 08/12/2022
Published: 28/12/2022

KEYWORDS

Aspergillus oryzae;
Cellulase;
Coffee treatment;
Enzyme activity;
Total soluble solids.

ABSTRACT

The objective of this study was to investigate the production of microbial cellulase by using *Aspergillus oryzae* and its application in the treatment of coffee. The cellulase producing method used for microbe medium was in submerged fermentation with malt extract medium. The method used for determining the cellulase activity was spectrophotometry at the wavelength 540 nm with 3,5-DNS reagent to measure the production of reducing sugar (the product of enzymatic reaction). Then, cellulase was applied on green Robusta coffee bean to enhance the extraction of total soluble solids. The result showed that at 6% (v/w) cellulase and 50°C of enzyme treatment, lasting for 24 hours, the extraction of soluble solids was highest at 4.47±0.06°Brix. The efficiency of extraction improved 21.79% compared to the control. This result could be applied in the production of coffee to improve the quality of coffee products.

Doi: https://doi.org/10.54644/jte.73.2022.1173

1. Introduction

The production of microbial enzymes has various advantages, including huge production, high enzyme activity and low cost [1], [2]. There are many researches which studied the production of enzymes by bacteria, yeast, and/or molds. The paper [3] produced cellulase from *Candida tropicalis*, *Bacillus subtilis*, *Aspergillus niger* and applied them into the treatment of coffee to demucilage and increase the extraction of soluble solids content of coffee [3]. The paper [4] studied the production of cellulase by using *Aspergillus oryzae* (*A. oryzae*) in molasses. *Aspergillus oryzae* is belong to *Aspergillus* family and is considered as GRAS (generally recognized as safe) which has been applied for years in the making of "tuong ban" (or Vietnamese fermented soybean paste) in Vietnam or miso in Japan. They can produce cellulase enzyme at appropriate conditions and substrates [5].

Cellulase is one kind of enzymes which catalyze the hydrolysis of cellulose into its monomers – glucoses. Going along with amylase (25%) and protease (18%), cellulase accounts for 15% of the enzyme market and also plays important roles in the industry [6]. In green coffee bean, cellulose appears in the insoluble or holocellulose fraction and takes 13-23% of the polysaccharides content in Robusta coffee [7]. The cellulose located mostly in the cell wall of the bean and enzymatic treatment hydrolyzes them to form tiny pores on the surface of cell wall and initiates the liberation of soluble solids content of coffee bean [8].

Coffea canephora or commonly named Robusta coffee is responsible for nearly 90% of the coffee planted area in Vietnam and make this country becoming one of the largest producers of green coffee in the world [9]. Robusta coffee has bitter taste and higher total soluble solids content compare to Arabica coffee. However, its price is low since Arabica has better flavor, aroma and is preferred by many

Western countries. This leads to a demand in increasing added value for Robusta coffee and the treatment of coffee by microbial enzymes is the solution for this problem [4].

There were some previous researches on the microbial enzymes from bacteria, yeast and mold… For instances, article [3] produced cellulase from *Candida tropicalis*, *Bacillus subtilis*, *Aspergillus niger* and applied them on coffee cherries to remove the mucilage layer and increase the extraction of soluble solids content from three species of coffee, including *Coffea arabica*, *Coffea robusta and Coffea chari*. In 2016, Nguyen at. al. in [4] also investigated the enzyme production from *Aspergillus oryzae* by submerged fermentation using molasses and treated on *Coffea robusta*. However, there are limited studies focus on the production of cellulase by *Aspergillus oryzae* using different cultured media and applied on coffee to improve the extraction of soluble solids content.

This research aimed to study the production of cellulase by using *A. oryzae* using malt extract as cultured media and its application on the green *Coffea robusta* treatment to enhance the extraction of total soluble solids from coffee.

2. Materials and Methods

2.1 Materials

2.1.1 Microorganism

The microorganism used in this research was *Aspergillus oryzae* and was purchased from the Institute of Microbiology and Biotechnology – Vietnam National University in Hanoi (Accession number: VTCC-F-037).

2.1.2 Green coffee bean

The green bean of *Coffea robusta* (*Coffea canephora*) was brought from Quang Tin commune, Dak R'lap district, Dak Nong province, Vietnam.

2.1.3 Chemicals

For malt extract cultured medium: Carboxymethyl cellulose (CMC), $(NH_4)_2SO_4$;

For analysis of cellulase activity: 3,5-dinitrosalicylic acid (3,5-DNS), glucose, potassium sodium tartrate ($C_4H_4O_6KNa.4H_2O$), and sodium hydroxide (NaOH).

2.2 Methods

2.2.1 Preparation of cultured medium

Cultured medium used in this study was malt extract media with the supplement of 1% $(NH_4)_2SO_4$ for nitrogen source and carboxymethyl cellulose (CMC) for substrate. The composition of medium was as following: Distilled water: 1000 mL, ground malt: 250 g, pH was 5.5 – 6.

2.2.2 Production of microbial cellulase

Microbial cellulase was produced by submerged fermentation in a 50 mL fancol with the above culture medium. It was sterilized at 121°C for 15 min, and then *A. oryzae* was cultured into the medium with the ratio of 10^7 spores/mL. The fermentation occurred for 2 days (48 hours). After fermentation, the fancol was centrifuged at 7000 rpm for 30 min using the equipment namely ROTANTA 460 Hettich Zentrifugen (Germany) and filtered by sterilized filter paper with the pore's size 0.2 µm to completely eliminate the microorganisms. Crude enzyme was collected in the supernatant after centrifugation.

2.2.3 Determination of cellulase activity

One unit of cellulase activity was defined as the amount (µmol) of glucose produced by the hydrolysis reaction of CMC 1% for 1 min. To measure, 1 mL of cellulase (sample) was reacted with 1 mL of CMC 1% for 10 min. Then, 1 mL of 3,5-DNS was added and the reaction was boiled for 5 min. The absorbance (OD) of reacted solution was measured by the spectrophotometer at wavelength $\lambda = 540$ nm. Finally, concentration of glucose was calculated based on the standard curve of glucose 0.1%. Blank sample was the sample without enzymatic reaction.

$$\text{Cellulase activity (U/mL)} = (A_T - A_B).F.\frac{1000}{180}.\frac{1}{10}.D \quad (1)$$

In which:

A_T: The absorbance of samples F: Glucose efficient

A_B: The absorbance of blank D: Dilution factor

2.2.4 Investigation of the optimal conditions for cellulase production

2.2.4.1 Effect of total soluble solids of the cultured media

The total soluble solids of the cultured media were varied from 2 to 12°Brix with the interval of 2°Brix. The other factors were unchanged. The duration of fermentation was 48 hours (2 days) at room temperature, pH was 5.5.

2.2.4.2 Effect of initial pH

The initial pH of cultured media was varied from 3.5 to 6 with the interval of 0.5. The value of total soluble solids was obtained from the previous experiment. The duration of fermentation was 48 hours (2 days) at room temperature.

2.2.4.3 Effect of duration of fermentation

The duration of fermentation was varied from 48 to 96 hours with the interval of 12 hours. The values of total soluble solids and initial pH of cultured media were obtained from the previous experiments. The fermentation occurred at room temperature.

2.2.5 Investigation of the optimal conditions for enzymatic treatment to improve the extraction of total soluble solids from coffee

The green beans of Robusta coffee were immersed into the mixture including cellulase and distilled water, using 250 mL Erlenmeyer flask as a container. It was put in the shaking incubator for treatment.

2.2.5.1 Effect of concentration of enzyme

The concentration of cellulase was varied from 2 to 10% (w/w – in distilled water) with the interval of 2%. The duration and temperature of treatment were unchanged for 18 hours, at 35°C.

2.2.5.2 Effect of duration of treatment

The duration of treatment was varied from 12 to 28 hours with the interval of 4 hours. The value for concentration of enzyme was collected from the previous experiment. Temperature of treatment was at 35°C.

2.2.5.3 Effect of temperature of treatment

The temperature of treatment was varied from 30 to 60°C with the interval of 10°C. The values for concentration of enzyme and duration of treatment were obtained from the previous experiments.

2.2.6 Determination of total soluble solids from coffee

First, 50 gram of green coffee beans was roasted at 200°C for 15 mins. Then, it was ground into powder. For the extraction of soluble solids, 10 grams of coffee powder was stirred well with 70 mL of hot distilled water. The mixture was shaken at 175 rpm for 10 mins. Finally, it was filtered by filter paper two times before measuring the total soluble solids in the coffee extract using the refractometer ATAGO Master (0.0 – 33.0°Brix), Japan.

2.2.7 Statistical analysis

All experiments were in triplicate. The data were collected and analyzed by IBM SPSS Statistics 20 Program with One-way ANOVA and Duncan Standard ($\alpha \leq 0.05$) to calculate the means, standard deviation and significant difference between samples. All the graphs were drawn by Microsoft Excel 2010.

JTE
HCMUTE
ISSN: 2615-9740

JOURNAL OF TECHNICAL EDUCATION SCIENCE
Ho Chi Minh City University of Technology and Education
Website: https://jte.hcmute.edu.vn/index.php/jte/index
Email: jte@hcmute.edu.vn

3. Results and Discussion

3.1. Production of microbial cellulase

3.1.1 Effect of total soluble solids of the cultured media

Figure 1 shows the effect of total soluble solids on the production of cellulase by using *A. oryzae*. As the result, the highest cellulase activity was 6.20 U/mL and achieved at 8°Bx. This result is in the agreement with the study of **Nguyen et al. in** [4] which also claimed that the optimal total soluble solids for cellulase production by *A. oryzae* was from 6 to 8°Bx.

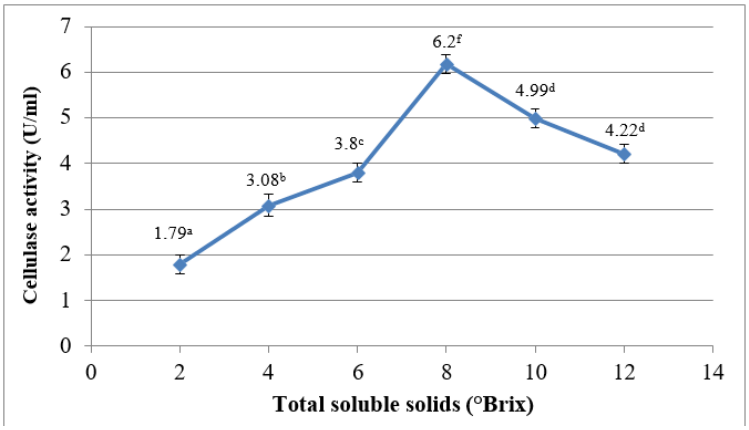

Figure 1. *Effect of total soluble solids on cellulase activity*

(The values with the same superscipt were not significantly different)

Total soluble solids in cultured medium play an important role in the growth of microorganisms. They not only supply the required nutrients, but also influence the osmotic pressure of the cells. If the concentration of total soluble solid in molasses was too high, the hypertonic environment could be occurred, the cell of microorganism would be dehydrated and shrunk. In contrast, the low concentration of total soluble solid in molasses could cause hypotonic in which the cell would absorb solution from the outer environment. Then, the cell would be swollen and busted [10]. Moreover, total soluble solids supplied the nutrients for the growth of *A. oryzae*. If the nutrients were excessive, the mold would use this source of energy instead of producing enzyme. On the other hand, if the nutrients were insufficient, the mold could not grow well [11].

3.1.2 Effect of initial pH

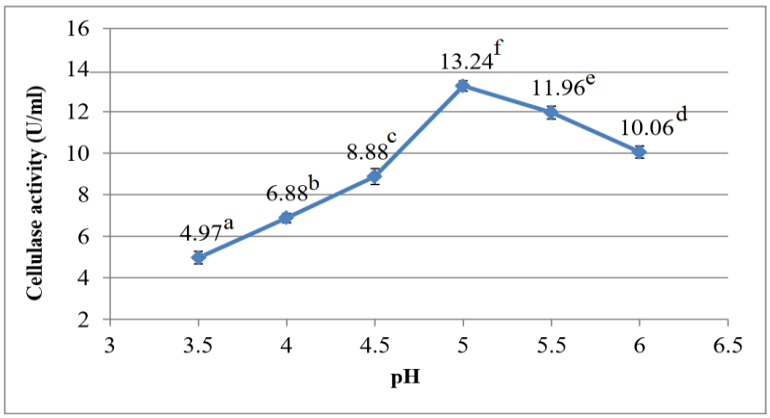

Figure 2. *Effect of initial pH on cellulase activity*

(The values with the same superscipt were not significantly different)

The result indicated that initial pH 5 was optimal for the production of cellulase by using *A. oryzae*. The enzymes from mold worked well in acidic rather than in basic medium [12]. The enzymes would

JTE
HCMUTE
ISSN: 2615-9740

JOURNAL OF TECHNICAL EDUCATION SCIENCE
Ho Chi Minh City University of Technology and Education
Website: https://jte.hcmute.edu.vn/index.php/jte/index
Email: jte@hcmute.edu.vn

lose their activity when the pH was out of the optimal range [13]. This also in the agreement with the studies [14]– [17] which claimed that the pH 5 was optimal for cellulase activity. Enzyme activity depends on the level of ionization of the amino acids in the active site, therefore pH plays an important role in maintaining the proper structure of an enzyme [18]. Changes in pH can significantly affect the degree of dissociation of the active sites of enzyme and substrate [14].

3.1.3 Effect of duration of fermentation

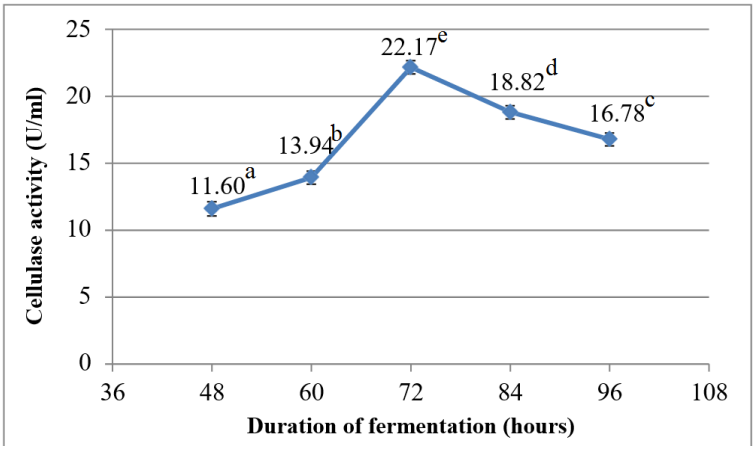

Figure 3. *Effect of duration of fermentation on cellulase activity*
(The values with the same superscipt were not significantly different)

The cellulase activity increased by the time of fermentation, reaching the peak at 22.17 U/mL for 72 h. After that, cellulase activity decreased significantly.

The results obtained from the experiment are similar to the data of [19] and [20]. Besides, this fermentation time is shorter than the results obtained by [21], which are 4 days and 5 days by [22]. While the data obtained on the fermentation time of *A. oryzae* in [23] only 60 h is optimal. The results in the present study differ from previous studies due to different organisms, isolated from different types of cellulolytic materials, and climatic conditions [24].

The curve of microbial growth describes an entire growth cycle. When filamentous fungi were added to the cultured medium, the enzyme could not be produced immediately to the maximum extent. They first used monosaccharides and minerals in the cultured medium as an immediate source of nutrients for the lag phase. This trend maintained until the beginning of the log phase. During the log phase, the fungus grew and reproduced at the maximum rate. The biosynthesis of enzyme began only when most of these immediate sources of nutrients were exhausted. The type of enzyme produced and its quantity depended on the type of substrate. During the lag phase and early log phase, the fungus tried to adapt and only a limited amount of enzyme was produced. As a result, the enzyme will be excreted at a faster rate. And this rate will reach the stationary phase. Then, in the early stages of death, enzyme activity decreases due to certain toxins present in the culture media that affect microbial growth and lead to cell death [25]. Moreover, in the early stages, microorganisms only grow and create biomass, not producing enzyme. If the duration is too long, the source of nutrients decreased, then microorganisms had to use other sources of nutrients, such as cellulose, for growth. Therefore, enzyme activity would also decrease when it catalyzes the hydrolysis reaction of cellulose into its simpler molecules to generate energy for the use of microorganisms [26].

3.2 Application of microbial cellulase on green Robusta coffee

3.2.1 Effect of the concentration of enzyme

The concentration of enzyme is one of the important factors in coffee treatment. The insufficient amount gives low quality of treatment, while the excess amount of enzyme can waste both the resources and money.

JTE
HCMUTE
ISSN: 2615-9740

JOURNAL OF TECHNICAL EDUCATION SCIENCE
Ho Chi Minh City University of Technology and Education
Website: https://jte.hcmute.edu.vn/index.php/jte/index
Email: jte@hcmute.edu.vn

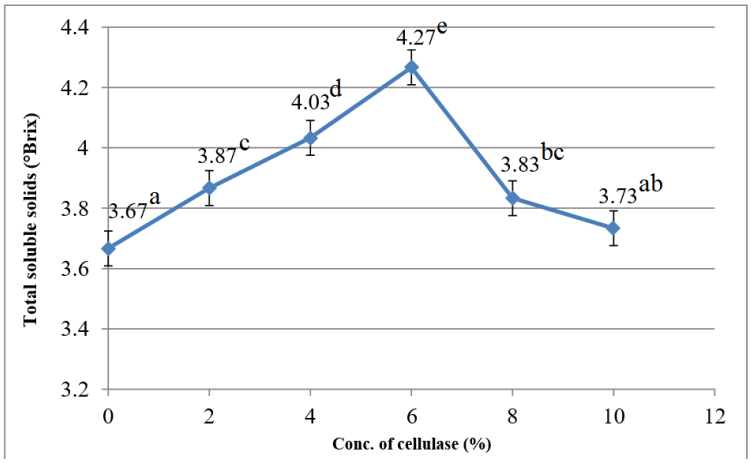

Figure 4. *Effect of concentration of cellulase on the enzymatic treatment of coffee*
(The values with the same superscipt were not significantly different)

Green Robusta coffee was treated by microbial cellulase at 2, 4, 6, 8 and 10% (volume of enzyme/ weight of green coffee sample). Control is sample without enzymatic treatment. All samples were incubated at 35°C for 18 h. As the result, the highest extraction of total soluble solids was 4.27°Bx with 6% of enzyme. The crude enzyme ratio below 6% is not enough to extract the solute, while the ratio above 6% will dissolve the solute into the medium, resulting in a decrease in the extraction of total soluble solids of green coffee beans. In addition, when increasing the amount of enzyme used, the cost for production would be also higher.

The results of Ouyang et al. in [27] mentioned that the yield of corn cob hydrolysis was increased with the increase of cellulase rate under treatment. The high content of hydrolysates in the solution indicates that carbohydrates are released into the solution, confirming that hydrolysis of cellulose has occurred. Cellulase enzyme hydrolyzes β - 1,4 glycosidic bonds in cellulose to form oligosaccharides or glucose. The results in [28] also showed that increasing the enzyme dosage beyond the optimal level did not improve enzymatic hydrolysis. In addition, the paper [29] demonstrated that when using higher enzyme concentrations, similar yields were obtained in a shorter time period.

3.2.2 Effect of the duration of treatment

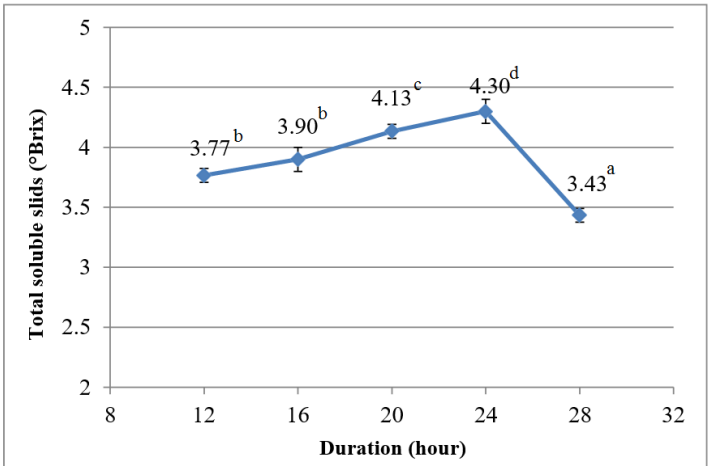

Figure 5. *Effect of duration of treatment on the enzymatic treatment of coffee*
(The values with the same superscipt were not significantly different)

Enzymes can break down the cell walls and help release biologically active substances, increasing the efficiency of the extraction process. Enzymes need time to function and work under specific conditions. If the time is too short, the enzyme will not have enough time to work. Conversely, if the processing time is too long, the cell wall of the coffee bean may undergo excessive treatment and lead

to a decrease in the extraction of soluble content since it is dissolved from the coffee bean into water. The results showed that the optimal duration of treatment was 24 h and the total soluble solids reached 4.30°Bx. This result was similar to the research [30] which pointed out that 24 h was the optimal time to reach 90% of the yield by cellulase treatment.

Furthermore, the washing step in the process also plays an important role. In this step, after undergoing enzymatic treatment, the coffee beans are washed under running water to remove the silk skin and residual enzymes. However, this step can also wash away the dissolved substances, leading to a reduction in their content in the final coffee bean product. After washing, the beans are dried at 60°C to the initial moisture content, roasted and ground into a powder before measuring the extract content with a refractometer.

3.2.3 Effect of the temperature

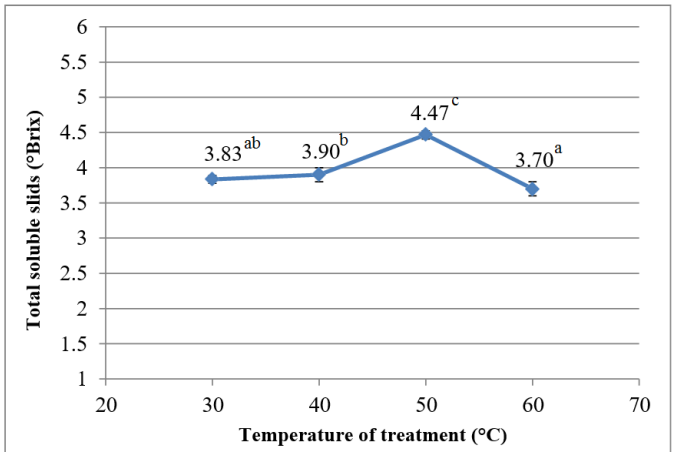

Figure 6. *Effect of temperature on the enzymatic treatment of coffee*

(The values with the same superscipt were not significantly different)

Temperature plays an important role in enzyme processing, because enzymes are protein and sensitive to heat. If the temperature of treatment is too low, the enzyme cannot be activated. On the other hand, high temperature can denature proteins and lead to the loss of enzyme activity due to the destruction of the secondary or tertiary structure of the enzyme.

The results showed that at 50°C, the highest extraction of total soluble solids was 4.47°Bx. According to the paper [31], the soluble solids reached 17% by mannanase treatment. Cellulase enzyme hydrolysis at 60°C is not optimal. However, when treating cellulase, the yield was equivalent to that of mannanase treatment [31]. The results are similar to those of study [32], who suggested that 60°C was not optimal for cellulase activity [32]. Meanwhile, 45°C is the optimum temperature for cellulase purified from *A. oryzae*, according to [33] and 55°C was optimal for cellulase activity obtained from strain *A. oryzae* according to [34]. Cellulase from other sources also gives a result of 55°C [15], [16]. Comparing the results with [17], [30], [35], also gave similar results to this research, showing that the optimal temperature for cellulase is 50°C. High optimum temperature is a characteristic of many filamentous fungi. The chemical nature of enzymes is protein. Therefore, it is different from chemical reactions, the speed of catalysis reaction of enzymes only increases when the temperature is increased within a certain limit, without affecting the structure of enzyme [36]. Temperature affects enzyme activity, leading to an impact on the extraction of total soluble solids. Besides, temperature also regulates product quality during the enzymatic treatment [37].

4. Conclusions

This research successfully produced microbial cellulase by using *A. oryzae* with the conditions of cultured medium as following: total soluble solids was 8°Bx, pH 5, duration of fermentation was 72 h. The highest cellulase activity was 22.17 U/mL. Then, cellulase was applied in coffee treatment to enhance the extraction of total soluble solids. The highest total soluble solids extracted from the treated

coffee was 4.47°Bx (21.79% higher than the control) and achieved at 6% (v/w) cellulase, 50°C and for 24 h of treatment. This result could be applied in the production of coffee to not only improve the efficiency of treatment, but also the whole quality of coffee products.

Acknowledgments

We would like to thanks the Department of Food Technology, Faculty of Chemical and Food Technology, Ho Chi Minh City University of Technology and Education (HCMUTE) for all the support in the implementation of this research.

REFERENCES

[1] D. L. Nguyen, *Technology in Microbiology: Industrial microbiology*, vol. 2. Publisher of Vietnam National University in Ho Chi Minh City, 2002.
[2] P. F. Stanbury, A. Whitaker, and S. J. Hall, *Principles of Fermentation Technology*, Butterworth-Heinemann, Elsevier, p. 2, 1995.
[3] P. H. Le, "Study on the production of microbial enzymes cellulase, pectinase and their application on coffee fermentation", Doctoral thesis, University of Science, Vietnam National University, Ho Chi Minh City, Vietnam, 2012.
[4] D. Q. Nguyen, H. N. D. Huynh, and P. H. Le, "Production of cellulase and pectinase by using Aspergillus oryzae in molasses and their application for the extraction of soluble solid content from coffee," *International Journal of Modern Engineering Research*, vol. 6, no. 11, pp. 41–45, Nov. 2016.
[5] A. Kuila and V. Sharma, *Principles and applications of fermentation technology*. Scrivener Publishing, 2018.
[6] S. Sajith, P. Priji, S. Sreedevi, and S. Benjamin, "An overview on fungal cellulases with an industrial perspective," *J Nutr Food Sci*, vol. 6, no. 1, pp. 461–465, 2016.
[7] R. J. Clarke and R. Macrae, *Volume 1: Chemistry*, Springer Netherlands, Dordrecht, 1985.
[8] D. Q. Nguyen, H. N. D. Huynh, P. H. Tran, and P. H. Le, "Optimal Conditionsof Enzymatic Treatment for Improvement of Total Soluble Solids Extraction and Antioxidant Capacity of Coffee Bean," *International Journal of Modern Engineering Research*, vol.1, no. 1, pp. 17-21, 2019.
[9] D. Giovannucci, B. Lewin, and R. Swinkels, "Socialist Republic of Vietnam: Report on the coffee production," The International Bank for Reconstruction and Development, Agriculture & Rural Development Department, Washington, US, 2004.
[10] D. P. Luong, *Fermentation technology*. Publisher of Education, Vietnam, 2010.
[11] D. Chu and D. J. Barnes, "The lag-phase during diauxic growth is a trade-off between fast adaptation and high growth rate," *Scientific Reports*, vol. 6, pp. 1–5, 2016.
[12] M. F. Begum and A. R. Alimon, "Assessment of some wild Aspergillus species for cellulase production and characterization," *African Journal of Microbiology Research*, vol. 5, no. 27, pp. 4739–4747, 2011.
[13] D. H. El-Ghonemy, T. H. Ali, A. M. El-Bondkly, M. E. S. Moharam, and F. N. Talkhan, "Improvement of *Aspergillus oryzae* NRRL 3484 by mutagenesis and optimization of culture conditions in solid-state fermentation for the hyper-production of extracellular cellulase," *Antonie Van Leeuwenhoek*, vol. 106, no. 5, pp. 853–864, 2014.
[14] J. Han *et al.*, "The development of nanobiocatalysis via the immobilization of cellulase on composite magnetic nanomaterial for enhanced loading capacity and catalytic activity," *International journal of biological macromolecules*, vol. 119, pp. 692–700, 2018.
[15] J. S. Lima, P. H. Araújo, C. Sayer, A. A. Souza, A. C. Viegas, and D. de Oliveira, "Cellulase immobilization on magnetic nanoparticles encapsulated in polymer nanospheres," *Bioprocess and biosystems engineering*, vol. 40, no. 4, pp. 511–518, 2017.
[16] W. Liang and X. Cao, "Preparation of a pH-sensitive polyacrylate amphiphilic copolymer and its application in cellulase immobilization," *Bioresource technology*, vol. 116, pp. 140–146, 2012.
[17] C. Riou, J. M. Salmon, M. J. Vallier, Z. Günata, and P. Barre, "Purification, characterization, and substrate specificity of a novel highly glucose-tolerant β- glucosidase from Aspergillus oryzae," *Applied and Environmental Microbiology*, vol. 64, no. 10, pp. 3607–3614, 1998.
[18] S. Wang, P. Su, F. Ding, and Y. Yang, "Immobilization of cellulase on polyamidoamine dendrimer-grafted silica," *Journal of Molecular Catalysis B: Enzymatic*, vol. 89, pp. 35–40, 2013.
[19] A. H. Moubasher, M. A. Ismail, R. A. Mohamed, and O. A. Al-Bedak, "Xylanase and cellulase production under extreme conditions in submerged fermentation by some fungi isolated from hypersaline, alkaline lakes of Wadi-El-Natrun, Egypt," *J Basic Appl Mycol*, vol. 7, pp. 19–32, 2016.
[20] Y. I. Yamane *et al.*, "Production of cellulose-and xylan-degrading enzymes by a koji mold, Aspergillus oryzae, and their contribution to the maceration of rice endosperm cell wall," *Journal of Bioscience and Bioengineering*, vol. 93, no. 1, pp. 9–14, 2002.
[21] S. P. Thota *et al.*, "Macro-micro fungal cultures synergy for innovative cellulase enzymes production and biomass structural analyses," *Renewable Energy*, vol. 103, pp. 766–773, 2017.
[22] D. H. El-Ghonemy, T. H. Ali, A. M. El-Bondkly, M. E. S. Moharam, and F. N. Talkhan, "Improvement of Aspergillus oryzae NRRL 3484 by mutagenesis and optimization of culture conditions in solid-state fermentation for the hyper-production of extracellular cellulase," *Antonie Van Leeuwenhoek*, vol. 106, no. 5, pp. 853–864, 2014.
[23] S. Ueno, M. Miyama, Y. Ohashi, M. Izumiya, and I. Kusaka, "Secretory enzyme production and conidiation of Aspergillus oryzae in submerged liquid culture," *Applied microbiology and biotechnology*, vol. 26, no. 3, pp. 273–276, 1987.
[24] A. J. Adeleke, S. A. Odunfa, A. Olanbiwonninu, and M. C. Owoseni, "Production of cellulase and pectinase from orange peels by fungi," *Nature and Science*, vol. 10, no. 5, pp. 107–112, 2012.
[25] N. B. Ma, D. Q. Nguyen, and P. H. Le, "Study on Culture Conditions for A Cellulase Production from *Aspergillus unguis*," *International Journal of Modern Engineering Research* , vol. 7, no.2, pp. 31-35, 2017.
[26] D. Q. Nguyen, H. D. N. Huynh, and P. H. Le, "Production of Cellulase and Pectinase by Using *Aspergillus oryzae* in Molasses and Their Application for the Extraction of Soluble Solid Content from Coffee," *International Journal of Modern Engineering Research* , vol. 6, no. 11, pp. 41–45, 2016.
[27] J. L. Ouyang, L. Zhenjiang, Y. Xin, Y. Hanjie, and Y. Qiang, "Enhanced enzymatic conversion and glucose production via two-step enzymatic hydrolysis of corncob residue from xylo oligosaccharides producer's waste," *Bioresources*, vol. 4, pp. 1586–1599, 2009.

[28] I. Chiyanzy, M. Brienzo, M. García-Aparicio, R. Agudelo, and J. Görgens, "Spent coffee ground mass solubilisation by steam explosion and enzymatic hydrolysis," *Journal of Chemical Technology & Biotechnology*, vol. 90, no. 3, pp. 449–458, 2015.
[29] P. A. Delgado, J. A. Vignoli, M. Siika-aho, and T. T. Franco, "Sediments in coffee extracts: Composition and control by enzymatic hydrolysis," *Food chemistry*, vol. 110, no. 1, pp. 168–176, 2008.
[30] R. H. Y. Chang, J. Jang, and K. C. W. Wu, "Cellulase immobilized mesoporous silica nanocatalysts for efficient cellulose-to-glucose conversion," *Green Chemistry*, vol. 13, no. 10, pp. 2844–2850, 2011.
[31] T. Jooste, M. P. García-Aparicio, M. Brienzo, W. H. VanZyl, and J. F. Görgens, "Enzymatic hydrolysis of spent coffee ground," *Applied biochemistry and biotechnology*, vol. 169, no. 8, pp. 2248–2262, 2013.
[32] A. V. Buntić *et al.*, "Screening for Factors Affecting Cellulase Adsorption from Solutions by Modified Coffee Residues," 2014.
[33] F. B. Most and A. Nurul, "Purification and Characterization of Intracellular Cellulase from *Aspergillus oryzae* ITCC-4857.01," *Mycobiology*, vol. 37, no. 2, pp. 121–127, 2009.
[34] G. A. Youssef, "Physiological studies of cellulase complex enzymes of *Aspergillus oryzae* and characterization of carboxymethyl cellulase," *African Journal of Microbiology Research*, vol. 5, no. 11, pp. 1311–1321, 2011.
[35] Y. Qin, Y. Fu, Q. Li, F. Luo, and H. He, "Purification and enzymatic properties of a difunctional glycoside hydrolase from *Aspergillus oryzae* HML366," *Indian Journal of Microbiology*, vol. 60, pp. 475–484, 2020.
[36] C. Pham and N. Phan, *Biotechnology: Enzymes and their applications*, Vietnam Education Publishing House, 2006 (in Vietnamese).
[37] L. Tran, *Technology of fermented products*, Agriculture Publisher, 2006 (in Vietnamese).

Nguyen Quang Duy is a lecturer at the Department of Food Technology, Faculty of Chemical and Food Technology, Ho Chi Minh City University of Technology and Education (HCMUTE), Vietnam. He received his Bachelor of Engineering and Master of Engineering by research from International University – Vietnam National University in Ho Chi Minh City in 2016 and 2019, respectively. His background focuses on microbial enzymes and their application on industrial plants, such as coffee, tea and cocoa. The researches aim to increase nutritional and added value to those products as well as innovate new food products in the market. He is the author and co-author of more than ten scientific papers which published in the international as well as national journals. He has experience in doing research, conducting projects and especially in technology transfer from 2014 until now.

Lam Duc Thinh – In 2022, he graduated from the Department of Food Technology, Faculty of Chemical and Food Technology, Ho Chi Minh City University of Technology and Education (HCMUTE) – Vietnam.

Nguyen Van Hoang - In 2022, he graduated from the Department of Food Technology, Faculty of Chemical and Food Technology, Ho Chi Minh City University of Technology and Education (HCMUTE) – Vietnam.

Dinh Yen Nhi – graduated from Food Technology Department at International University - Vietnam National University, Ho Chi Minh City. She is the author of two papers related to the effect of coffee processing to antioxidant properties and sensory profile; application of the digestive bioprocessing model on coffee fermentation. She is teaching assistance for Enzyme and Food Fermentation, Technology of Coffee, Tea, and Cocoa classes. She has supported the coffee flavor research.

Le Hong Phu currently works at International University - Vietnam National University Ho Chi Minh City as a role as an associate professor for Food Technology Field. He received his bachelor's degree in biology from University of Science - Vietnam National University, Ho Chi Minh City in 2000. He received his master and Ph. D degrees in Biochemistry from University of Science - Vietnam National University, Ho Chi Minh City in 2003, 2012, respectively. He was promoted to associate professor in 2017. His research areas cover applied biochemistry, applied microbiology, as well as functional food. He is the author of nearly 50 international and national articles and the leader of 4 transfer research projects. He has successfully guided more than 100 students and graduates in Food Technology and Biotechnology. The articles are focused on objects such as coffee, pepper, cocoa, and others such as ginseng and algae that are being cultivated in the central provinces and the Central Highlands in Vietnam. Other research is aimed at producing convenient functional products that can be used immediately, such as instant tea. He focuses on research on fermentation conditions for the production of fermented coffee with microbes, using a combination of enzymes to process the extraction with high efficiency, as well as peel off the skins in the production of white pepper, honey coffees.