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Enhanced fermentable sugar production from low grade and damaged longan fruits using cellulase with algal enzymes for bioethanol production

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Abstract

Longan fruits' economic values have been increasing in recent years, for example, nutrients, medicine, cosmetic, and pharmaceutical products, etc. Especially, Southeast Asia countries are the largest longan fruit, producer. The considerable amount of low grade and damaged longan fruits are one of the interesting resources for producing bioethanol. In this study, hydrothermal pretreatment and hydrolysis through added cellulase and algal enzymes were conducted with dried low grade and damaged longan fruits. The total and reducing sugar were achieved 230.70 ± 2.01 g/L and 91.11 ± 1.11 g/L, after the pretreatment process finished, respectively. Subsequently, a rise in the total and reducing sugar in the hydrolysis case was 368.42 ± 13.16 g/L and 297.78 ± 2.94 g/L, respectively. Consequently, longan fruits are valuable edible products, and leftover or low grade/damaged longans are promising bioresources for bioethanol production.

Keywords algal enzymes bioethanol production, cellulose, damaged longan fruits, low grade, pretreatment

Introduction

Over the years, humankind's demand for the use of energy has been increasing. Therefore, the amount of fossil fuel, such as coal, petroleum, and natural gas, becomes limited. Moreover, the phenomenon of environmental pollution happens, for instance, climate change, acid rain, global warming, greenhouse effect, etc. [1-3]. This is the reason that the countries in the world are interested in using renewable energy, for example, biogas, biobutanol, biodiesel, and biohydrogen, etc. One of the potential biofuels that has received much attention is bioethanol [4]. In the world, bioethanol production of the US and Brazil are the highest, in addition to several agricultural-based countries currently focusing on bioethanol production [5].

Bioethanol is a high octane fuel due to the amount of lead that has been replaced, so its ability to combustion is higher with the compression ratio within shorter burning times. Also, bioethanol is a renewable liquid fuel that is utilized transportation, 85% bioethanol and 15% gasoline (E85) is popular [6-7]. Moreover, bioethanol is lower noxious than because of the reduction of greenhouse gas emissions (e.g., carbon monoxide (CO) and hydrocarbons (HCs).

Therefore, bioethanol is blended to gasoline to oxygenate the fuel mixture using for the internal combustion engines [8]. Ethanol can be blended with gasoline in any ratio, thus obtaining fuels with different properties. Bioethanol is made from biomass (e.g., energy crops, industrial waste, agricultural waste, woody waste and forest biomass, waste from green areas (parks and gardens), and municipal solid waste) [9]. One of the top agricultural producing countries is Thailand. Hence, agricultural wastes which produced entirely from agricultural activities related to cultivation consist of 17,083,594 tons per year (rice husk, corncob, bagasse, and cassava rhizome) [10]. So the researchers are frequently keeping an eye on this biomass due to its abundance, low cost, but it can produce the amount of significant bioethanol [11]. The low grade/damaged longan fruits are no exclusion.

Longan (*Dimocarpus longan* Lour, syn. *Euphoria longan* Lam.) is commercial fruits in Thailand with an export value of $109 million in 2006 (Office of Agricultural Economics, Thailand, 2007) [12]. Flowers of the longan tree bloom at the end of wintertime. The duration of longan fruit harvesting is over 4-6 weeks in mid-summer [13]. The Peel of this fruit has brown, thin, and its brittle when it ripe. The taste of pulp longan is sweet with translucent of the flesh color. The seed is ring shape, dark black color, and white point at the base [14]. The carbohydrates of longan are in the form of fructose, glucose, and sucrose. After extraction, the main compositions of longan juice are 2.77 % glucose, 3.91 % fructose, and 14.21 % sucrose. The pH of longan juice reaches a low acidic level due to the number of organic acids compose, such as gluconic acid, malic acid, and citric acid [15].

The microbial fermentation procedure for the production of organic chemicals is greener than the chemically synthesized ones with no release of toxic fumes or chemicals in the environment [16]. Fermentation and production of ethanol from sugar (pure sugar or final product of hydrolysis) are done. For one mole of simple sugar ($C_6H_{12}O_6$) in the presence of yeast or bacteria, two moles of ethanol (C_2H_5OH) and two moles of carbon dioxide (CO_2) are produced [17]. The fermentation process takes three days and is performed at a temperature between 25 and 30 °C [4].

In recent studies, bioethanol production by utilizing the yeast brings high productivity the research of Oberoi et al. [18], the ethanol concentration from Kinnow mandarin (*Citrus reticulata*) waste at 12 h was 42 g L^{-1}, using simultaneous saccharification and fermentation with *S. cerevisiae*. Another research, production of bioethanol using pseudo banana stem by *S. cerevisiae* NCIM 3570 gave maximum ethanol (17.1 g/L) [19]. Bioethanol production with 4.1 to 7.1% was produced from the fermented banana fruit waste, which includes skin and pulp of rotten fruit become convenient for the production of bioethanol as an alternative fuel due to decrease the cost of the original steps [20]. Therefore, in this study that dried low grade and damaged longan fruits were utilized to produce bioethanol using cellulase with algal enzymes.

Methodology

Sample Collection and Material Preparation

Low grade and damaged longans fruits were collected from the Pratupa Agricultural Cooperative company located at Pratu Pa Subdistrict, Mueang Lamphun District, Lamphun 51000 (at coordinate 18°37'44.5"N 98°59'48.5"E). Collected samples were transferred to the laboratory at Faculty of Science, Maejo University, Chiang Mai, Thailand, at which the leaves, branches, dust, soil, and other impurities of low grade/damaged longans fruits were removed, was chosen to experiment. However, this material was dried at the Energy Research Center before experimenting. The chipping disk machine (multi-purpose shredder model MJU-EB8) was used to shred dried low grade/damaged longans fruits until reaching a smaller size.

Pretreatment and hydrolysis

Thermal pretreatment (boiling) was applied with 100g of dried waste longan fruits with a ratio of 1:10 w/v. The sample was boiled at 30 minutes; afterward, this mixture was undergone an autoclaving apparatus at 121°C, 15 psi, 15 mins. After pretreatment, the samples were inoculated with 1% commercial cellulase and 10% algal enzymes for the hydrolysis process. The pH of the combined solution was adjusted at 5.0 and 7.0, respectively. Then the solution was kept in a solar dryer to perform the hydrolysis process. Pretreatment and hydrolysis were carried out step by step as Figure 1.

Figure 1. Pretreatment and hydrolysis process: (A) Boiling, (B) Using autoclave, (C) Adding cellulase enzyme, (D) Adding algal enzyme, (E) Using a solar dryer

Fermentation

The hydrolysate solution was fermented with 2% (v/v) of yeast (*Saccharomyces cerevisiae* TISTR 5020) in 1000 mL fermentor. The pH of the fermented mixture was adjusted 5.6 and kept at room temperature. A small amount of sample was withdrawn every 12 hours to check sugar analysis and alcohol determination.

Sugar analysis and alcohol determination

Total sugar estimation, 0.5 mL of the sample was combined 0.5 mL of 5% phenol (w/v) and 2.5 mL of 98% H_2SO_4 by using a vortex. The solution of total sugar estimation was kept in cold water 10 minutes and use a UV-Spectrophotometer detector DV-8000 (Drawell, Osaka, Japan) at 490 nm [21]. Fermentable sugar (i.e., reducing sugar) estimation, the mixture was created by mixing 0.5 ml of the sample and 0.5 ml of 3, 5-dinitrosalicylic acid (DNS) through a vortex. After that, the solution was boiled in water bath 15 minutes at 90°C. 4 mL of distilled water was added in this solution. A UV-Spectrophotometer detector DV-8000 (Drawell, Osaka, Japan) at 540 nm was used to read the absorbance [22]. For alcohol determination, 50 mL of the sample was removed and centrifuged before using Ebulliometer (Dujardin-Salleron, Alcohol Burner, France). Then this sample was poured into in the condenser of Ebulliomter and boiled until the steady temperature. A comparison of the resulting temperature recorded and the boiling point of the distilled water was used the Ebulliomter disc.

Statistical analysis

Three replicates were conducted in the present study to report the values. The data were shown as mean ± SE from triplicate. The program SPSS 20.0 (SPSS Inc., Chicago, IL, USA) was used to analyze and statistic data. A significant difference was examined at the level of $p < 0.05$.

Results and Discussion

Influence of physical pretreatment and enzymatic hydrolysis

Peel of longan is one of the parts of this material that plays a role as lignocellulosic biomass, which includes cellulose, hemicellulose, and lignin. Therefore, pretreatment is a necessary step to enhance the accessibility of enzymes to cellulose. This is evaluated through the results of the crystalline structure was decomposed, the lignin was removed, and the amount of total and reducing sugar yields were released [23].

In this study, boiling at 30 min and an autoclave, which was set at 121°C, 15 psi, 15 mins were conducted sequentially. However, according to Gabhane et al. [24], different heating devices were combined with the severe pretreatment of conditions such as high temperature and duration, which get more

Table 1. Sugar concentration of dried longan fruits after physical pretreatment and hydrolysis

	Total sugar (g/L)	Reducing sugar (g/L)	Degree of polymerization
Pretreatment	230.70	91.11	2.53
Hydrolysis	368.42	297.78	1.24

than 30 min. It is those of reasons not only to lead to the more significant degradation of hemicellulosic sugars but also effect to reducing sugar yield due to the conversion of reducing sugar into other compounds such as Hydroxymethylfurfural (HMF) etc.

Hence, 30 min of hydrothermal pretreatment and steam explosion at 121°C, 15 psi, 15 mins are those suitable conditions to reach the high sugar yield. It was clearly shown in Table 1 with the amount of both TS and RS 230.70 ± 2.01 g/L and 91.11 ± 1.11 g/L of sugars, respectively. In addition, it is also expressed through the degree of polymerization; this number after hydrolysis is lower than after pretreatment (Table 1), according to Manmai et al. [25], the degree of polymerization index calculates the number of monosaccharides in a polymer molecule. The higher degree of polymerization of the structure needs handles to produce the simple sugars which are suitable for the fermentation process. Therefore, cellulose with algal enzymes was added within the hydrolysis process; the result of total and reducing sugar reach 368.42 ± 13.16 g/L and 297.78 ± 2.94 g/L.

Bioethanol production

Longan fruit is one of the most significant parts of the recent food industry in the world. The feasibility of bioethanol production from waste longan and low-grade fruits by enzymatic hydrolysis (1% commercial cellulase and 10% algal enzymes) and fermentation were investigated in this study. There are several fruits that several fruits have been suggested for bioethanol fermentation, including rambutan fruit biomass (skin and juice) with different pH and temperature conditions that were researched using similar yeast in this study [26]; the bioethanol highest yields were 7.5% and 9.17%, respectively. Moreover, Shubhra et al. [27] stated that different rotten fruits, such as sapota, papaya, apple, banana, and grapes, were utilized for bioethanol production; the fruits of the grape was reached the highest bioethanol concentration (8.04%) after 4 days using *S. cerevisiae*.

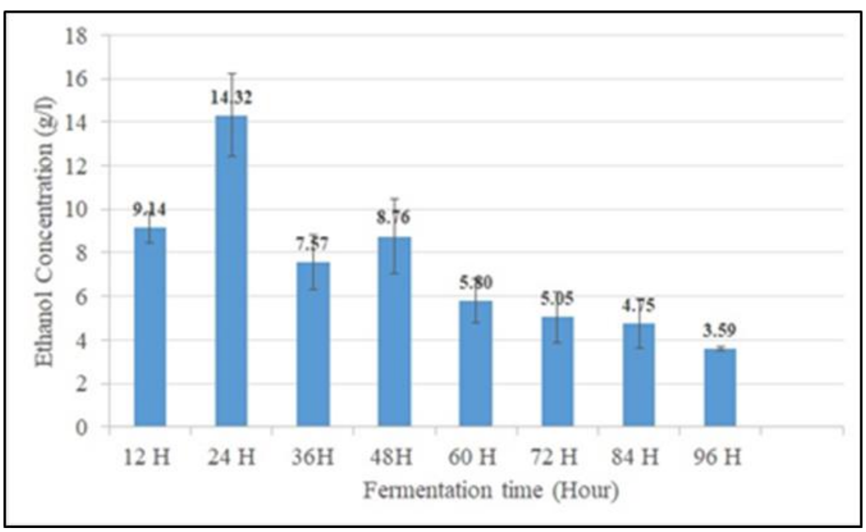

Figure 2. Bioethanol production from dried longan fruits from 96h fermentation

The product of hydrolysis was used to produce bioethanol through anaerobic respiration with 2% v/v *S. cerevisiae* (dry yeast), which was added in the fermenter for 96 h at room temperature. The ethanol concentration was checked every after 12 hours. The result of ethanol concentration was shown in Figure 2 that the highest bioethanol production reaches 14.32 ± 1.89 g/L at 24 hours; the total and reducing sugars were reduced from 103.25 ± 4.94 g/L and 63.52 ± 8.79 g/L to 92.98 ± 5.15 g/L and 55.19 ± 4.66 g/L; this is explained that *S. cerevisiae* consumed fermentable sugars to transform into alcohol, followed by 12 hours with 9.14 ± 0.71 g/L. The results show that the low grades and waste longan fruits can be a suitable feedstock for biochemical conversion into bioethanol for further use as potentially useful products such as fuel, chemical feedstock, or catalyst support.

Conclusion

Low grade and damaged longan fruits, which is agricultural waste, can be used to produce bioethanol. The hydrothermal and steam explosion was applied in the pretreatment process with significant success to enhance the accessibility of enzyme and the high sugar concentration achieved. Hence, the productivity of bioethanol production from low grade and damaged longan fruits was higher significantly.

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References

[1] K. Khunchit, S. Nitayavardhana, R. Ramaraj, V. K. Ponnusamy and Y. Unpaprom (2020). Liquid hot water extraction as a chemical-free pretreatment approach for biobutanol production from Cassia fistula pods. Fuel, 279: 118393. doi: 10.1016/j.fuel.2020.118393

[2] B. Saengsawang, P. Bhuyar, N. Manmai, V. K. Ponnusamy, R. Ramaraj, and Y. Unpaprom **(2020)**. The optimization of oil extraction from macroalgae, Rhizoclonium sp. by chemical methods for efficient conversion into biodiesel. Fuel, 274: 117841. doi: 10.1016/j.fuel.2020.117841

[3] Y. Unpaprom, T. Pimpimol, K. Whangchai and R. Ramaraj **(2020)**. Sustainability assessment of water hyacinth with swine dung for biogas production, methane enhancement, and biofertilizer. Biomass Convers. Biorefin., doi: 10.1007/s13399-020-00850-7

[4] P. T. Vu, Y. Unpaprom and R. Ramaraj **(2017)**. Evaluation of bioethanol production from rice field weed biomass. Emergent. Life Sci. Res., **3:** 42-49.

[5] M. X. He, Q. Hu, Q. Zhu, K. Pan and Q. Li **(2015)**. The feasibility of using constructed wetlands plants to produce bioethanol. Environ. Prog. Sustain. Energy, **34:** 276-281.

[6] P. Khammee, R. Ramaraj, N. Whangchai, P. Bhuyar and Y. Unpaprom **(2020)**. The immobilization of yeast for fermentation of macroalgae *Rhizoclonium* sp. for efficient conversion into bioethanol. Biomass Convers. Biorefin., doi: 10.1007/s13399-020-00786-y

[7] P. T. Vu, Y. Unpaprom, N. Whangchai and R. Ramaraj **(2017)**. The feasibility of bioethanol production from wetland plant Cyperus difformis. The 1st Maejo - Engineo International Conference on Renewable Energy (MEICRE 2017), The Empress Hotel, Chiang Mai, Thailand, 1-12.

[8] P. T. Vu, Y. Unpaprom and R. Ramaraj **(2018)**. Impact and significance of alkaline-oxidant pretreatment on the enzymatic digestibility of *Sphenoclea zeylanica* for bioethanol production. Bioresour. Technol., **247:** 125-130.

[9] K. Robak and M. Balcerek **(2018)**. Review of second generation bioethanol production from residual biomass. Food Technol. Biotechnol., **56:** 174-187.

[10] P. Thanarak **(2012)**. Supply chain management of agricultural waste for biomass utilization and CO_2 emission reduction in the lower northern region of Thailand. Energy Procedia, **14:** 843-848.

[11] N. Manmai, Y. Unpaprom, V. K. Ponnusamy and R. Ramaraj **(2020)**. Bioethanol production from the comparison between optimization of sorghum stalk and sugarcane leaf for sugar production by chemical pretreatment and enzymatic degradation. Fuel, **278:** 118262. doi: 10.1016/j.fuel.2020.118262

[12] S. Janjai, N. Lamlert, B. Mahayothee, B.K. Bala, M. Precoppe and J. Muller **(2011)**. Thin layer drying of peeled longan (*Dimocarpus longan* Lour.). J. Food Sci. Technol., **17:** 279-288.

[13] Y. Jiang, Z. Zhang, D. C. Joyce and S. Ketsa **(2002)**. Postharvest biology and handling of longan fruit (*Dimocarpus longan* Lour.). Postharvest Biol. Technol., **26:** 241-252.
[14] S. Lapsongphol, B. Mahayothee, S. Phupaichitkun, H. Leis, M. Haewsungcharoen, S. Janjai and J. Mueller (2007). Effect of drying temperature on changes in volatile compounds of longan (*Dimocarpus longan* Lour.) fruit. In Book of abstracts of the conference on International Agricultural Research for Development, Tropentag, Witzenhausen, Germany.
[15] S. Surin, P. Thakeow, P. Seesuriyachan, S. Angeli and Y. Phimolsiripol **(2014)**. Effect of extraction and concentration processes on properties of longan syrup. J. Food Sci. Technol., **51:** 2062-2069.
[16] P. Khammee, Y. Unpaprom, S. Buochareon, and R. Ramaraj **(2019)**. Potential of bioethanol production from marigold temple waste flowers. The 1st Thailand Biorefinery Conference "The Future of Biorefinery for Thailand 4.0". Suranaree University of Technology, Nakhon Ratchasima, Thailand, 18-23.
[17] A. B. Hossain, A. A. Saleh, S. Aishah, A. N. Boyce, P. P. Chowdhury and M. Naqiuddin **(2008)**. Bioethanol production from agricultural waste biomass as a renewable bioenergy resource in biomaterials. In 4th Kuala Lumpur International Conference on Biomedical Engineering, Springer, Berlin, Heidelberg, 300-305.
[18] H. S. Oberoi, P. V. Vadlani, A. Nanjundaswamy, S. Bansal, S. Singh, S. Kaur and N. Babbar (2011). Enhanced ethanol production from Kinnow mandarin (*Citrus reticulata*) waste via a statistically optimized simultaneous saccharification and fermentation process. Bioresour. Technol., **102:** 1593-1601.
[19] S. Ingale, S. J. Joshi and A. Gupte **(2014)**. Production of bioethanol using agricultural waste: banana pseudo stem. Braz. J. Microbiol., 45: 885-892.
[20] A. M. Alshammari, F. M. Adnan, H. Mustafa and N. Hammad **(2011)**. Bioethanol fuel production from rotten banana as an environmental waste management and sustainable energy. Afr. J. Microbiol. Res., **5:** 586-598.
[21] M. Dubois, K. A. Gilles, J. K. Hamilton, P. T. Rebers and F. Smith **(1956)**. Colorimetric method for determination of sugars and related substances. Anal. Chem., **28:** 350-356.
[22] G. L. Miller **(1959)**. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., **31:** 426-428.
[23] P. U. Amadi, M. O. Ifeanacho and E. N. Agomuo **(2018)**. The effects of different heating periods and exclusion of some fermentation conditions on bioethanol production from plantain pseudo-stem waste using the digestive juice of *Archachatina marginata*, garlic and *Saccharomyces cerevisiae*. Biofuels, **9:** 531-539.
[24] J. Gabhane, S. P. William, A. N. Vaidya, K. Mahapatra and T. Chakrabarti **(2011)**. Influence of heating source on the efficacy of lignocellulosic pretreatment–a cellulosic ethanol perspective. Biomass Bioenerg., **35:** 96-102.
[25] N. Manmai, Y. Unpaprom, and R. Ramaraj **(2020)**. Bioethanol production from sunflower stalk: application of chemical and biological pretreatments by response surface methodology (RSM). Biomass Conv. Bioref. doi: 10.1007/s13399-020-00602-7
[26] A. Hadeel, A. B. Hossain, K. Latifa, H. AL. Naqeb, J. Abear and A. Norah **(2011)**. Bioethanol fuel production from rambutan fruit biomass as reducing agent of global warming and greenhouse gases. Afr. J. Biotechnol., **10:** 10157-10165.
[27] T. Shubhra, S. K. Jadhav, S. Mayuri and K. L. Tiwari **(2014)**. Fermentation of waste fruits for bioethanol production. Asian J. Biol. Sci., **7:** 30-34.