Vietnam Journal of Science and Technology ***61*** *(5) (2023) 787-797*

doi:10.15625/2525-2518/17252

High gravity enzymatic hydrolysis of non–gelatinized starch from black - purple rice

Huong Do Thi Thanh, Tien Thanh Nguyen*

School of Biotechnololgy and Food Technology, Hanoi University of Science and Technology, No. 1 Dai Co Viet, Hai Ba Trung, Ha Noi, Viet Nam

*Email: *thanh.nguyentien@hust.edu.vn*

Received: 1 July 2022; Accepted for publication: 16 September 2022

Abstract. Thanks to novel recombinant starch degrading enzymes which can directly hydrolyze raw starch at ambient temperature, the technology of hydrolysis of uncooked starch has recently been developed andproven to be effective. In combination with high substrate concentration (high gravity) approach, this technology not only saves the thermal energy for starch gelatinization but also reduces the impact of heat on valuable components in starch–containing raw materials. Black–purple rice is a specialty of Northwest Viet Nam, which contains high level of anthocyanin, a biologically active ingredient. In this study, commercial enzymes were applied to hydrolyze non–gelatinized black–purple rice to obtain anthocyanin–containing sugar solution. Factors affecting the starch conversion such as enzyme/substrate ratio, hydrolysis duration, temperature, and the presence of supporting enzymes in the hydrolysis process were investigated. The results showed that in the presence of Stargen 002, Viscozyme L, and Cellulast 1.5 L, more than 86 % of starch in a total of 320 g/L black-purple rice were converted into glucose after 72 hours at 50 °C. From black-purple rice, two products were received, including a glucose–rich hydrolysate (245.92 g/L) containing anthocyanin (30 mg/L) and a solid by–product rich in protein, which were very potential for food application.

Keywords: Anthocyanin, non–gelatinzed starch, hydrolysis, black–purple rice, very high gravity.

Classification numbers: 1.4.2

1. INTRODUCTION

Being cultivated mostly in the Northwest mountain area of Viet Nam, such as Dien Bien, Hoa Binh, Son La, and Lai Chau, black–purple rice (*nep cam* rice) is a nutritious sticky rice that turns dark purple from dark black when cooked [1]. Its black color is due to its high anthocyanin concentration of up to 327.6 mg/100 g, higher than most other grains [2, 3]. Fiber and antioxidants in black–purple rice are comparable to blueberries or raspberries. Black purple rice contains about 80 % dry weight starch and also contains more protein (6 - 8 % dry weight) and lipids than other rice varieties [4]. Black–purple rice is mostly consumed and used for wine production or as supplement in diets.

Starch in grain can be enzymatically converted to mono–, di– and oligosaccharides. Conventionally, starch must be gelatinized and liquified in the presence of water at high temperature (90 -100 ^{o}C) and liquefaction enzyme to promote enzymatic saccharification (at 60 - 65 ^{o}C) afterward. Recently, thanks to novel recombinant starch–degrading enzymes which can directly hydrolyze raw starch at ambient temperature, the technology of hydrolysis of uncooked or non–gelatinized starch has recently been developed and proven to be effective [5], especially in combination with high substrate (or high gravity) approach [6, 7]. This technology not only saves the thermal energy for starch gelatinization but also reduces the impact of heat on valuable components in starch–containing raw materials. Many research groups have recently published work on enzyme–assisted starch hydrolysis under gelatinization temperatures, which demonstrates its benefits. A review of the literature on granular starch hydrolysis with commercial and non–commercial enzyme preparations reveals that hydrolysis efficiency can range from 12 to 98 % [8].

In this study, in order to valorize black-purple rice, we used commercial enzyme preparations to hydrolyze the raw starch from this material to produce an anthocyanin–containing glucose solution. Different parameters such as dry matter concentration, amylase enzyme/substrate ratio, hydrolysis duration, temperature and the addition of auxiliary enzymes affecting hydrolysis efficiency of raw starch to glucose were investigated. In addition, the solid by–product remaining after hydrolysis was also evaluated for its composition in this study.

2. MATERIALS AND METHODS

2.1. Materials

Dien Bien black–purple rices were purchased, finely ground (< 0.5 mm) and kept in damp bags at 4 - 8 ^{o}C for use in experiments. Commercial enzyme preparations which contain differenent enzymes (or enzymatic activities) were used such as Stargen 002 (alpha amylase and glucoamylase), Optimash TBG (beta–glucanase, xylanase and cellulase) (Genecor, USA), Cellulast 1.5 L (cellulase), Viscozyme L (a blend of beta-glucanases, pectinases, hemicellulases and xylanases) (Novozymes, Denmark).

2.2. Experiment design

2.2.1 Effect of enzyme/rice ratio

Black–purple rice flour was mixed in acetate buffer (pH = 4.2) at a dry matter content of 30 % (w/w). Stargen 002 (611.5 ± 11.5 GAU/g, with substrate p–Nitrophenyl–alpha–D–glucopyranoside) was added at doses of 1, 2, 3, and 4 mL/kg dry weight of rice. The hydrolysis was carried out at 30 °C under stirring at 150 rpm. Samples were collected at intervals during hydrolysis. The supernatants were obtained by centrifugation at 10,000 rpm/10 min and measured for glucose and anthocyanin.

2.2.2 Effect of dry matter content

Similarly, the hydrolysis of starch by Stargen 002 in black–purple rice flour was performed at 30 ^{o}C, 150 rpm in acetate buffer (pH 4.2) at various dry matter contents of 20, 25, 30, and 35 % (w/w). Samples were collected and treated as above.

2.2.3 Effect of temperature

Temperature is one of the most important factors impacting the enzyme catalytic reaction. In this experiment, the hydrolysis was performed at different temperatures of 30, 40, and 50 °C. Dry matter of 30 % (w/w) and 2 mL/kg rice of Stargen 002 were used. The samples were collected and treated as described above.

2.2.4 Effect of auxiliary enzymes

In this experiment, the supporting effect of commercial auxiliary enzymes such as cellulase, beta–glucanase, and xylanaseto amylase was elucidated. The hydrolysis was performed similarly to Stargen 002 in the presence of other enzymes such as Cellulast 1.5L 0.2 mL/kg; Viscozyme L 0.05 w/w; Optimash 0.1 mL/kg. The hydrolysis was caried out for 72 hours at 50 °C, 150 rpm. The samples were collected and treated as described above.

2.3. Composition analysis

Proximate parameters of rice and solid residue after hydrolysis were determined by standard methods, such as moisture (drying method with MA15, Satorius), total protein (Kjeldahl method TCVN 8125 : 2009), fat (Soxhlet), crude fiber (Alkom bag following AOCS Ba 6a–05 method), and ash (TCVN 9474–2012). Starch was determined based on the glucose obtained after hydrolysis in 2 % HCl.

The glucose concentration in the hydrolysate was determined using a GOPOD Kit following the supplier's guidance (Megazyme, USA). The glucose yield was determined by the ratio of the measured glucose concentration to the theoretically calculated glucose concentration from the initial starch content as the following formula:

$$Y_G(\%) = \frac{[Glu]}{1.111 \cdot S_t \cdot [DM]} \cdot 100$$

with Y_G: glucose yield (%); [Glu]: glucose concentration in the hydrolysate (g/L); St: content of the starch rice (% g/g dry matter); [DM]: initial content of the rice dry matter (g/L); The coefficient 1.111 is the conversion factor from starch to glucose.

The anthocyanin concentration was measured using the differential pH method (TCVN 11028–2015).

The product profile in the hydrolysate was analyzed using an Agilent 1200s HPLC. Hydrolysates were filtered through 0.2 μm membrane and applied (20 μL) into an Aminex 87H Column (Biorad, USA). The chromatographic separation was performed at 60 ^{o}C with a flow rate of 0.5 mL/min of 5 mM H_2SO_4 mobile phase. Saccharide products were observed with an RID detector.

The experiments were repeated three times. The data were treated using Excel (Microsoft) and expressed as mean and standard deviation.

3. RESULTS AND DISCUSSION

3.1. Proximate composition of black-purple rice

Proximate composition of black-purple rice was analyzed (Table 1). Black–purple rice has a high starch content of around 80 % dry matter, comparable to some varieties of black and red rice (79 - 83 %) [4], it is lower than regular white rice (about 89 %) [9]. Fiber and fat were

determined to be about 3 %, and ash was about 1 %. These values were similar to those of other black rice varieties in Southeast Asia [4]. In addition, the protein content was around 10 %, which is higher than other rice varieties. Currently, protein from grains or plants is receiving a lot of attention and black-purple rice with such high protein content could be a potential source of plant protein.

Table 1. Proximate composition of black purple rice.

Composition	g/100 g flour	% Dry matter
Moisture	13.06 ± 0.25	–
Starch	70.01 ± 0.48	80.53 ± 0.55
Protein	8.62 ± 0.23	9.92 ± 0.27
Fiber	3.10 ± 0.38	3.56 ± 0.44
Ash	1.05 ± 0.14	1.21 ± 0.16
Fat	2.96 ± 0.30	3.41 ± 0.35

3.2. Effect of Stargen 002/rice flour ratio

Stargen 002, capable of hydrolyzing raw starch, contains alpha–amylase and gluco–amylase synthetized by *Aspergillus niger* and *Aspergillus kawachi*. These enzymes are adsorbed on the surface of starch grain, creating pores on this surface where glucose is released [10]. Stargen 002 were added in hydrolysis reaction with 30 % dry matter of rice at different doses, from 1 to 4 mL/kg of rice flour. During hydrolysis for 172 h at 30 ^{o}C, glucose was observed to be released and used to calculate the glucose yield (Figure 1).

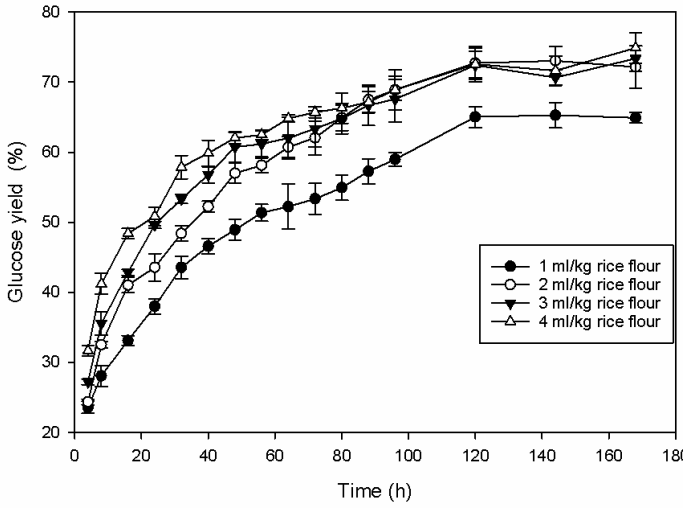

Figure 1. Time curve of glucose yield in the presence of varied Stargen 002 doses.

It can be seen that in the first 48 h, starch was rapidly degraded, so the glucose generation yield increased sharply. After that, the hydrolysis was gradually slower until the hydrolysis time of 120 h, the glucose generation yield was almost constant afterward and ranged from 72 to 73 %

for Stargen 002 doses of 2 - 4 mL/kg, except for the 1 ml/kg dose, the glucose yield (approximately 65 %) was lower than others (Figure 1, Table 2).

Table 2. Glucose concentration in hydrolysate at 120 h of hydrolysis with different Stargen 002 doses.

Stargen 002/rice ratio (mL/kg)	Glucose concentration (g/L)
1	185.03 ± 4.23
2	206.87 ± 6.11
3	205.99 ± 5.72
4	204.39 ± 7.21

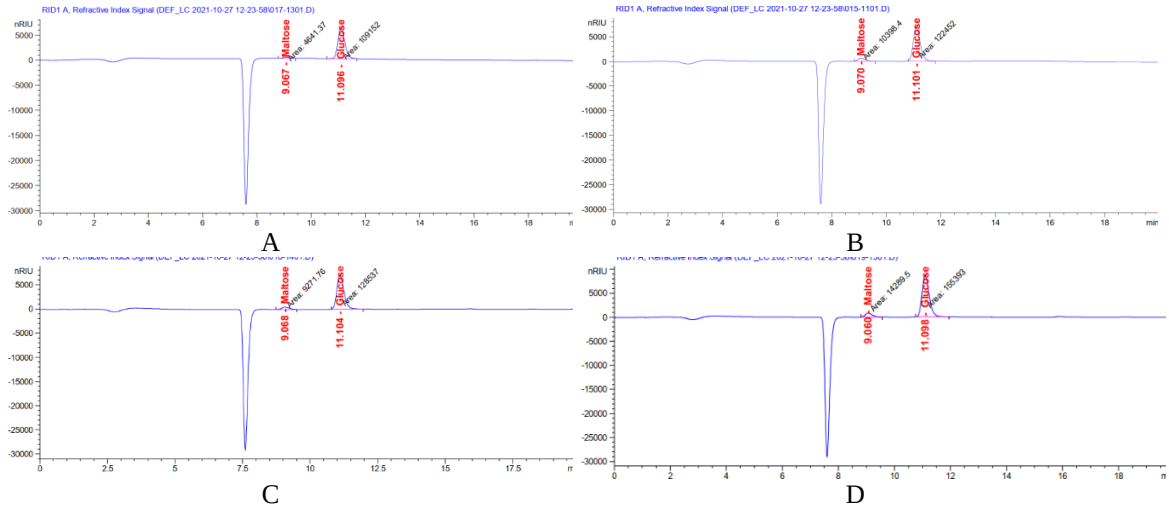

Figure 2. HPLC chromatograph of hydrolysate at 12 h (A); 24 h (B); 48 h (C); 72 h (D).

According to the supplier of Stargen 002, the dose of 0.7 - 1.4 mL/kg raw material is recommended in SLSF for ethanol production. However, without yeast, more enzyme (2 mL/kg substrate in this case) should be used for better starch conversion.

Interestingly, only maltose and glucose in hydrolysate samples during hydrolysis could be detected by HPLC (Figure 2) even at 12 h, at the early stage of the process. Furthermore, glucose was dominant, suggesting that the glucoamylase activities of Stargen 002 effectively converted most available intermediate products generated by α–amylase in Stargen 002 to glucose. The absence of intermediate products such as dextrines suggested that the starch hydrolysis yield was therefore almost equivalent to the glucose yield.

3.3. Effect of dry matter content

In this experiment, Stargen 002 was used at a dose of 2 mL/kg of rice to hydrolyze starch with varied dry matter concentrations from 20 to 35 % in the pH 4.2 citrate buffer. The temperature of the reaction was maintained at 30 °C. The glucose yields were observed (Figure 3). The patterns of glucose yield curves were similar when varying the solid concentration. The glucose yields also reached a maximum and remained constant from 120 h of hydrolysis for all

reactions. There was no significant difference of maximum yields between 20 and 25 % of dry matter, both were in the range of 81 - 82 %. However, when dry matter concentration increased to 30 and 35 %, the maximum glucose generation yield decreased to 72.73 % and 61.02 % (at 120 h), respectively (Table 3).. This result was consistent with previous studies on the effect of substrate concentration on hydrolysis efficiency. The prerequisite for the enzyme to hydrolyze non–gelatinized starch is its ability to adsorb onto the starch surface. When studying the kinetics of enzyme adsorption, TC Nguyen *et al.* (2020) concluded that increasing substrate concentration significantly reduced the enzyme's adsorption on the substrate (the adsorption decreased from 84.8 % at 10 % substrate to only 65.6 % at 30 % substrate) [11]. At high substrate concentration, the viscosity increases, the space between starch granules shrinks, and the swelling rate decreases, reducing the enzyme's ability to contact the substrate, thereby reducing the process performance [11]. On the other hand, such a high glucose concentration generated in hydrolysis of 206.87 g/L (~ 1.15 mol/L) could lead to feedback inhibition of enzyme activity. Under this high–glucose condition, glucoamylase is likely to form reversion products [12].

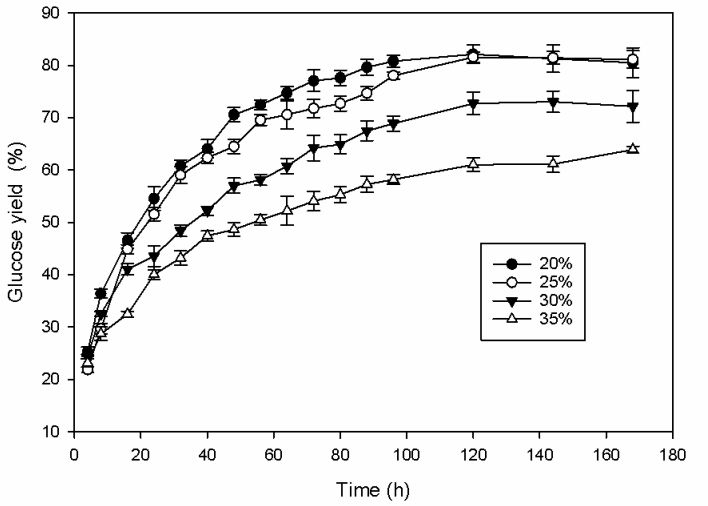

Figure 3. Time curve of glucose yield in the presence of varied dry matter contents.

Despite adverse effects on hydrolysis, the use of very high gravity (VHG) of dry matter of at least 300 g/L has shown extensive application potential, especially in the production of ethanol from cereals [8]. It could save equipment investment and increase productivity. In a recent study, [10] performed VHG simultaneous liquefaction, saccharification, and fermentation (VHG–SLSF) to produce ethanol by yeast. High substrate concentrations up to 300 g/L did not show any adverse effect because the authors used yeast to simultaneously convert glucose to ethanol, thus increasing the starch conversion up to 86.3 % [10], which was much higher than the yield obtained in this study at the same dry matter concentration (72.73 %). In a similar hydrolysis process with Khang Dan rice flour (without the addition of yeast), a lower hydrolysis yield of 54 % was achieved at 120 h (detailed experiments are not shown). This confirmed the adverse effect of product accumulation on enzyme hydrolysis. Moreover, the higher amylopectin content in black–purle rice perhaps leads to better hydrolysis [13].

Table 3. Glucose concentration in hydrolysate at 120 h of hydrolysis with different dry matter contents.

Dry matter content (% w/w)	Glucose concentration (g/L)
20	149.65 ± 3.25
25	188.49 ± 2.29
30	206.87 ± 6.11
35	203.39 ± 4.23

3.4. Effect of hydrolysis temperature

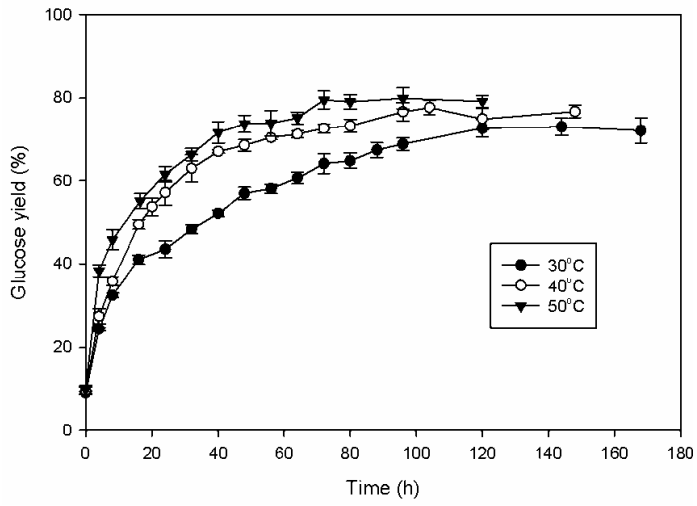

Figure 4. Time curve of glucose yield at different temperatures.

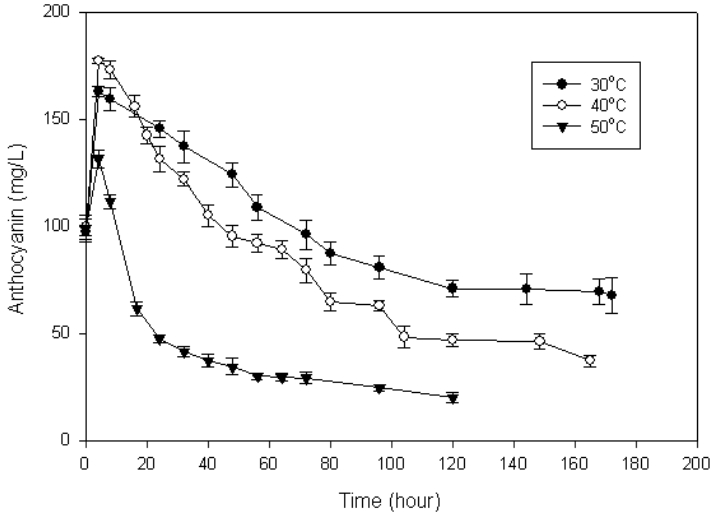

Figure 5. Anthocyanin concentration in hydrolysates.

Similarly, the glucose generation from starch at different temperatures (under gelatinization temperature of starch) such as 30, 40 and 50 ^{o}C were observed (Figure 4). The hydrolysis was performed in the pH 4.2 buffer with a dry matter content of 30 % (w/w). Stargen 002 was used at a dose of 2 mL/kg rice flour.

Obviously, the hydrolysis was faster at higher temperature. In comparison to the temperature of 30 ^{o}C, in the reaction at 40 ^{o}C or 50 ^{o}C, the maximum glucose generation yields were reached earlier at 96 h or 72 hours, respectively (Figure 4). Furthermore, the maximum yields were also slightly higher, i.e 76.62 % and 79.48 % with temperature of 40 ^{o}C and 50 ^{o}C, respectively (Figure 4). It is should be noted that the used temperatures was much lower than the gelatinization temperature of rice starch (80 - 85 ^{o}C). Thus, increasing the temperature has just accelerated the enzyme catalysis rate only. In fact, the manufacturer recommends that Stargen 002 work better at 48 ^{o}C.

Table 4. Anthocyanin and glucose concentration in hydrolysate at different temperatures.

Temperature (^{o}C)	Time (h)	Glucose concentration (g/L)	Anthocyanin (mg/L)
30	120	206.87 ± 6.11	71.14 ± 4.12
40	96	217.94 ± 4.99	63.12 ± 2.34
50	72	226.08 ± 4.59	29.22 ± 2.67

Anthocyanin is a bioactive compound and coloring agent. In this experiment, the extraction and variation of anthocyanin in hydrolysate was also observed during the hydrolysis (Figure 5). Anthocyanin was extracted and accumulated to 160 - 180 mg/L after 4 h at 30 ^{o}C or 40 ^{o}C. However, anthocyanin is known to be unstable to heat and light [14], therefore at high temperatures such as 50 ^{o}C, anthocyanin was partially degraded and was present in the hydrolysate at less levels than at lower temperatures during the hydrolysis. Anthocyanin was rapidly decomposed during the hydrolysis at all temperatures used (Figure 5). Corresponding to the highest glucose yields, the anthocyanin concentrations were 29.22 mg/L (72 h at 50 °C), 63.12 mg/L (96 h at 40 °C), and 71.14 mg/L (120 h at 30 ^{o}C) (Table 4). This observation was in agreement with Pham T.N. *et al.* (2019) who investigated the extraction of anthocyanin from *Clitoria Ternatean l.* [15]. During the hydrolysis at 50 ^{o}C, even though anthocyanin was degraded and remained at a lower level, the risk of commination could be reduced and the time of hydrolysis was saved.

3.5. Effect of auxiliary enzymes

Starch granules in kernel is wrapped by a matrix of beta–glucan and protein. This surrounding layer prevents the starch from being attacked by enzymes. Therefore, the degradation of this matrix perhaps results in a positive effect on starch conversion. In this study, different "accessory" hydrolases including cellulase and xylanase were used to evaluate their enhancing effect on Stargen 002 performance. The auxiliary enzymes were used at recommended doses: Optimash TBG: 0.1 mL/kg of material; Viscozyme L: 0.05 w/w; Cellulast 1.5 L: 0.2 mL/kg of material. The experiments were conducted at a dry matter content of 30 %, a Stargen 002 dose of 2 mL/kg, and pH 4.2. The temperature was maintained at 50 °C and the glucose yields were determined at 72 h (Table 5).

Table 5. Glucose concentration in hydrolysate and glucose yield at 72 h in the presence of supporting enzymes.

	Stargen 002	Stargen 002 Optimash TBG	Stargen 002 Viscozyme	Stargen 002 Cellulast	Stargen 002 Viscozyme Cellulast
Glucose concentration (g/L)	226.08 ± 4.59	228.75 ± 8.69	235.67 ± 2.19	239.46 ± 5.68	245.92 ± 3.46
Glucose generation yield (%)	79.48 ± 2.18	80.42 ± 3.06	82.85 ± 0.77	84.19 ± 1.99	86.46 ± 0.86

It is shown that when combining Stargen 002 with other enzymes, the glucose generation yield increased by 3.37 % (with Viscozyme) or 4.71 % (with Cellulast) at 72 h as compared to using solely Stargen 002. The addition of Optimash TBG, which was perhaps less enzymatic activities than others, did not significantly increase the yield of glucose (Table 5). In contrast, the usage of both Viscozyme and Cellulast might supply more enzyme types and activity to degrade more linkages in matrix, thus when combining with Stargen 002, the generation yield of glucose reached 86.46 % (nearly 7 % higher than the case of using Stargen 002) (Table 5). The enhancing effect of auxiliary enzymes on amylases in the degradation of non–geletinized starch in casava pulp was also reported by Rattanachomsri *et al.* (2009) [16]. Their findings also demonstrated that a combination of several accessory enzymes, including commercial cellulases, β–glucosidase, pectinases, β–glucanase/hemicellulose with glucoamylase and α–amylase increased fermentable sugar as compared to using solely amylase [16]. The addition of Viscozyme and Cellulast might not affect anthocyanin extraction from rice flour, therefore the concentration of anthocyanine in hydrolysate was measured to be approximately 30 mg/L.

To evaluate the value of the solid fraction after starch hydrolysis, the composition of thesolid residue from 72 h hydrolysis in the presence of Stargen 002, Viscozyme and Cellulast 1.5 L was analyzed (Table 6).

Table 6. Composition of the solid fraction after hydrolysis.

Composition	% w/w dry matter
Starch	39.37 ± 1.93
Protein	31.33 ± 0,51
Fiber	14.28 ± 0.36
Ash	0.64 ± 0.03

Even though the majority of starch was converted into glucose (glucose generation rate of 86.46 %), the amount of starch residue remaining in solid fraction was rather high. It suggests that further investigation should be done to convert starch more efficiently. On the other hand, protein, which was not converted during hydrolysis, increased from 10 % in rice to 31.33 % in solid fraction, comparable to protein in soya bean. This solid fraction with such protein content holds promise as a source of plant protein in food applications.

4. CONCLUSIONS

In this study, the enzymatic hydrolysis of non–gelatinized black–purple rice starch at high gravity of 30 % (w/w) was investigated by varying the impact parameters. The results showed

that 86.46 % of glucose in starch could be released after 72 h of hydrolysis at 50 ^{o}C in the presence of 2 mL/kg rice of Stargen 002, Viscozyme and Cellulast. From black– purple rice, two products were received, including a glucose–rich hydrolysate (245.92 g/L) containing anthocyanin (30 mg/L) and a solid fraction containing 31.33 % protein, with great potential for food applications.

Acknowledgements. The support from project ĐTDL.CN–07/20 (Ministry of Science and Technology, Viet Nam) was acknowledged.

CRediT authorship contribution statement. Huong Do ThiThanh: conducting experiments and drafting the manuscript. Tien–Thanh Nguyen: supervision and submission.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Yao S. L., Xu Y., Zhang Y. Y., and Lu Y. H. - Black rice and anthocyanins induce inhibition of cholesterol absorption in vitro, Food Funct. **4** (11) (2013) 1602-1608. doi:10.1039/ C3FO60196J.
2. Oikawa T., Maeda H., Oguchi T., Yamaguchi T., Tanabe N., Ebana K., Yano M., Ebitani T., and Izawa T. - The birth of a black rice gene and its local spread by introgression, Plant Cell **27** (9) (2015) 2401-2414. doi: 10.1105/tpc.15.00310.
3. Abdel–AalE.S. M., Young J. C., and Rabalski I. - Anthocyanin composition in black, blue, pink, purple, and red cereal grains, J. Agr. Food Chem. **54** (13) (2006) 4696-4704. doi:10.1021/jf0606609.
4. Sompong R., Siebenhandl–Ehn S. Linsberger–Martin G., and Berghofer E. - Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka, Food Chem. 1**24** (1) (2011) 132-140. doi:https://doi.org/ 10.1016/j.foodchem.2010.05.115.
5. Zhong Y., Xu J., Liu X., Ding L., Svensson B., Herburger K., Guo K., Pang C., and Blennow A. - Recent advances in enzyme biotechnology on modifying gelatinized and granular starch, Trends Food Sci. Tech. **123** (2022) 343-354. doi:https://doi.org/ 10.1016/j.tifs.2022.03.019.
6. Devantier R., Pedersen S. and Olsson L. - Characterization of very high gravity ethanol fermentation of corn mash, Effect of glucoamylase dosage, pre–saccharification and yeast strain, Appl. Microbiol. Biot. **68** (5) (2005) 622-629. doi: 10.1007/s00253–005–1902–9.
7. Pereira F. B., Guimarães P. M. R., Teixeira J. A., and Domingues L. - Optimization of low-cost medium for very high gravity ethanol fermentations by *Saccharomyces cerevisiae* using statistical experimental designs, Bioresource Technol. **101** (20) (2010) 7856-7863. doi: https://doi.org/10.1016/j.biortech.2010.04.082.
8. Cinelli B. A., Castilho L. R., Freire D. M. G., and Castro A. M. - A brief review on the emerging technology of ethanol production by cold hydrolysis of raw starch, Fuel **150**(2015) 721-729. doi: https://doi.org/10.1016/j.fuel.2015.02.063.
9. Tien T. N., Nguyen T. C., Nguyen C. N., Nguyen T. T., Pham T. A., Pham N. H., and Chu–Ky S. - Protease increases ethanol yield and decreases fermentation time in no-cook

process during very-high-gravity ethanol production from rice, Process Biochem. **117** (2022) 10-18. doi: https://doi.org/10.1016/j.procbio.2022.03.005.

10. Chu–Ky S., Pham T. H., Bui K. L. T., Nguyen T. T., Pham K. D., Nguyen H. D. T., Luong H. N., Tu V. P., Nguyen T. H., Ho P. H., and Le T. M. - Simultaneous liquefaction, saccharification and fermentation at very high gravity of rice at pilot scale for potable ethanol production and distillers dried grains composition, Food Bioprod. Process **98** (2016) 79-85. doi: https://doi.org/10.1016/j.fbp.2015.10.003.
11. Nguyen T. C., Chu–Ky S., Luong H. N. and Nguyen H. V. - Effect of pretreatment methods on enzymatic kinetics of ungelatinized cassava flour hydrolysis, Catalysts **10** (7) (2020). doi: 10.3390/catal10070760.
12. Kim M. S., Park J. T., Kim Y. W., Lee H. S., Nyawira R., Shin H. S., Park C. S., Yoo S. H., Kim Y. H., Moon T. W., and Park K. H. - Properties of a novel thermostable glucoamylase from the hyperthermophilic archaeon *Sulfolobus solfataricus* in relation to starch processing, Appl. Environ. Microb. **70** (7) (2004) 3933-3940. doi:10.1128/AEM. 70.7.3933–3940.2004.
13. Wang P., Singh V., Xue H., Johnston D. B., RauschK. D., and Tumbleson M. E. - Comparison of raw starch hydrolyzing enzyme with conventional liquefaction and saccharification enzymes in dry–grind corn processing, Cereal Chem. **84** (1) (2007) 10-14. doi:https://doi.org/10.1094/CCHEM–84–1–0010.
14. Prior R. L. and Wu X. - Anthocyanins: Structural characteristics that result in unique metabolic patterns and biological activities, Free Radical Res. **40** (10) (2006) 1014-1028. doi: 10.1080/10715760600758522.
15. Pham T. N., Lam T. D, Nguyen M. T, LeX. T., Vo D.V. N., Tran Q. T., Vo T. S. - Effect of various factors on extraction efficiency of total anthocyanins from Butterfly pea (*Clitoria ternatea L*. Flowers) in Southern Viet Nam, IOP Conference Series: Mater Sci. Eng. **544** (2019) p012013. doi: 10.1088/1757–899X/544/1/012013.
16. Rattanachomsri U., Tanapongpipat S., Eurwilaichitr L., and Champreda V. - Simultaneous non-thermal saccharification of cassava pulp by multi-enzyme activity and ethanol fermentation by *Candida tropicalis*, J. Biosci. Bioeng. **107** (5) (2009). doi:https://doi.org/ 10.1016/j.jbiosc.2008.12.024.