JST: Engineering and Technology for Sustainable Development
Volume 31, Issue 4, October 2021, 001-006

Enrichment of Protein Content in Rice-Based Dried Distillers' Grain from Food-Ethanol Factory by Chemical Method

Làm giàu hàm lượng protein trong bã rượu gạo từ nhà máy cồn thực phẩm bằng phương pháp hoá học

Nguyen Tien Thanh**, Nguyen Thi Thao, Chu Ky Son
School of Biotechnology and Food Technology, Hanoi University of Science and Technology, Hanoi, Vietnam
**Email: thanh.nguyentien@hust.edu.vn*

Abstracts

Dried distillers' grain (DDG) from rice-based alcohol factories contains relatively high protein (ca. 79% of dry matter). However, this vegetative protein source has only been used for animal feeding. To enhance the value of this by-product, i.e. toward application for the food industry, this study has applied different approaches for the enrichment of protein content in this by-products. These approaches were either using solvents to directly extract and precipitate protein or removing non-protein components in rice-based DDG. The results showed that the direct extraction and precipitation of protein was not effective as the removal of non-protein components. The use of NaOH 10 mM has increased protein content up to 87% of dry matter by washing out the non-protein components such as starch from DDG. Preliminary, the protein-enriched DDG was used up to 15% as an ingredient for cookies without negative effect on the taste or color of this product.

Key words: Rice-based dried distillers' grain, DDG, protein enrichment, ethanol

Tóm tắt

Bã rượu sấy khô DDG từ các nhà máy cồn thực phẩm từ gạo có hàm lượng protein tới 79,28% chất khô. Tuy nhiên, nguồn protein này đang bị lãng phí do mới sử dụng cho chăn nuôi. Để nâng cao giá trị và ứng dụng nguồn protein này trong thực phẩm, nghiên cứu đã áp dụng các phương án tăng hàm lượng protein bao gồm trích ly trực tiếp protein hoặc loại bỏ thành phần phi protein bằng NaOH biến thiên nồng độ có kết hợp các tác nhân khác. Kết quả cho thấy hiệu quả của việc xử lý bã rượu bằng NaOH nồng độ cao kém hơn so với cách sử dụng NaOH loãng để loại bỏ thành phần phi protein. Cụ thể, với NaOH 10 mM cho phép tăng hàm lượng protein trong bã rượu đến 87% chất khô. Qua đánh giá sơ bộ, bã rượu gạo làm giàu protein có thể sử dụng tới 15% trong sản phẩm bánh quy mà không gây ảnh hưởng xấu tới mùi và vị của bánh.

Từ khoá: Bã rượu gạo sấy khô, DDG, làm giàu protein, ethanol

1. Introduction

In 2017, in Vietnam, the total industrial alcohol production reached over 70 million liters. Most beverage-alcohol factories use rice or broken rice as the main ingredient. During processing, rice starch is gelatinized, liquefied, and saccharified to fermentable saccharides which are then used by yeast to form ethanol. The distillation process is carried out to obtain ethanol from the fermentation mixture. The solid residue in the post-distillation liquid is decanted, dried (thereby called dried distillers' grain, DDG), and generally used for livestock purposes. DDG amount is estimated at approximately 30% of raw ingredient load (for fermented ethanol of 14% of volume). Each year, beverage alcohol factories in Vietnam produce about 12,000 tons of DDG with a moisture content of 10%. Recently, a study reported that protein content in rice-based DDG was relatively high, 70 - 80% of dry matter [1]. Rice protein has been confirmed the functional properties [2], therefore rice-based DDG can be considered as a valuable plant protein source. However, rice-based DDG is currently only used for livestock [1].

Over the world, many types of research have focused on extraction and improvement of the protein level in DDG, however mostly based on corn and wheat materials [3,4]. For that purpose, DDG was defatted with hexane, then mixed with the solvent to extract protein. The soluble protein fraction was further precipitated by adjusting the pH of extracting solvent. Many types of solvents have been applied based on the solubility of protein fraction in DDG. For example, zein is soluble very well in ethanol 60 - 70%, meanwhile, glutenin dissolves most in alkaline - ethanol solution [3]. The use of reducing agents also enhances the efficiency of protein extraction because it affects disulfide bonds that change the solubility of protein [4]. Furthermore, protease can be used to support the extraction of protein from DDG [3].

To date, there has been no effort on extraction or enrichment of protein in rice-based DDG. This study

ISSN: 2734-9381
https://doi.org/10.51316/jst.153.etsd.2021.31.4.1
Received: November 11, 2020; accepted: May 22, 2021

was the first attempt to improve the protein content in rice-based DDG by chemical methods towards application in the food sector and enhance the value of this abundant by-product from the beverage-ethanol industry.

2. Experiments

The rice-based DDG was obtained from a beverage ethanol factory in the Northern of Vietnam. The wet distillers' grain was separated from the decantation step using a screw expeller and dried at 50 °C to reduce the moisture below 10%, packaged and stored at -20 °C until being used.

To remove fat in DDG, hexane with a volume of 5 times the weight of DDG was added in and stirred at 0 °C (in an ice bath) for 1 hour. The mixture was centrifuged at 6000 ^{x} g /15 min to remove hexane, the residual solid was left at room temperature for 12 h in a fume hood to evaporate excess hexane.

2.1. Extract Protein from DDG

The rice-based DDG was mixed with different solvents (Table 1) at the ratio of 1:30 weight/volume. The mixtures were stirred at 600 rpm and maintained at 70 °C in a water bath (Memmert WNB 22, Germany). After 2 hours, mixtures were centrifuged at 6000 ^{x} g /15 min to separate supernatants and residue solids. The supernatants were adjusted pH to 4.0 with HCl to precipitate the extracted components. Precipitates were collected by a similar centrifugation step. The residue solids and the precipitates were washed with water, dried, and used for measurement of main components including protein, starch, and fiber (Fig. 1).

2.2. Extraction of Non-Protein Components in Rice Based DDG

In this experiment, the non-protein components such as starch were dissolved from DDG. Dilute NaOH solutions of 5 mM - 25 mM were mixed with DDG. The extraction process was performed in the same protocol as described above (Fig. 1). However, the supernatants were discarded and only the residue solids were recovered by centrifugation.

Table 1. List of solvents used in the experiments

Solvents	Purpose
NaOH 0.1-1 M NaOH 0.5M - Ethanol 50% NaOH 0.5M - $NaHSO_3$ 0.05 M	Extraction of protein
NaOH 5 - 25 mM	Extraction of non-protein components

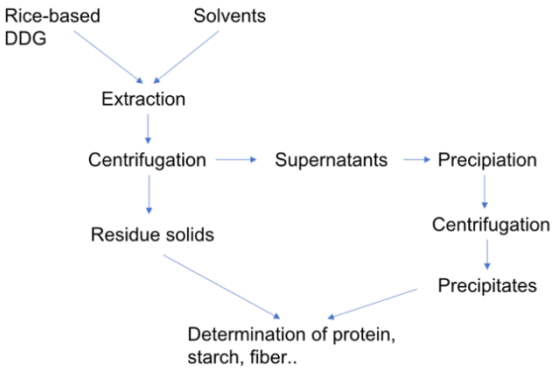


Fig. 1. The procedure of protein extraction from rice-based DDG by solvents

Protein, starch, and fiber content in initial DDG and post extraction solids or precipitates were analysed. Protein was determined using the Kjeldahl method (TCVN 4328 - 1: 2007). Starch content was determined via the amount of reducing sugar (reacting with DNS) obtained after hydrolysis by HCl 2%. The fiber was determined by the method of Alkom (AOCS Ba 6a - 05). The moisture content was measured by using MA35 moisture analyser (Satorius, Germany).

The amino acid profile of protein in DDG was analysed by the HPLC method as described previously [1]. DDG samples (20-40 mg of the sample) were hydrolysed in a vapor phase of 1 ml HCl 6 M, 0.5% phenol for 24 h at 120 °C. Afterward, the hydrolyzed DDGs were responded in deionized water, neutralized with NaOH to pH 7.0 and brought up to 10 ml of total volume. These solutions were filtered through 0.45 µm membrane before applying for HPLC analysis. The HPLC analysis of amino acid was performed with Agilent 1200 series (Germany) with DAD detector. Amino acid samples were derivatized with OPA reagents in an autosampler before being injected for separation in C18 Elipse Zorbax 5µm, 4,6 × 150 mm (Agilent, US). The gradient elution was performed with buffer A Sodium phosphate 40 mM, pH 7.8 and buffer B consisting of methanol, acetonitrile and deionized water (45 : 45 : 10). The buffer A was changed during elution as following 100% (0 - 1.9 min); to 50% (1.9 - 15.5 min), to 43% (15.5 - 21 min), to 0% (21 - 22 min), 0% (22 - 26 min); to 100% (26 - 27 min); 100% (27 - 31 min). The analysis time for each sample was 31 min at a flow rate of 1 min/ml. The column was maintained at 30 °C for separation.

The free amino acid in DDG was dissolved by HCl 0.1 N and analysed by HPLC as mentioned. Protein recovery yield was determined based on the ratio of protein (calculated with weight of samples and protein content in samples) in samples after treatment and initial DDG.

2.3. Preliminary Application of DDG to Cookies

The original and treated rice-based DDG were added to the cookie recipe, aiming to replace a portion of flour or improve the protein content in cookie products. From a general formulation of cookie products including wheat flour (200 g), butter (130 g), sugar (160 g), salt (1.4 g), baking soda (3 g), egg (2 pieces), and Vani (2 ml), DDG was added with a replacement rate of 15% for wheat flour. Cookie products were preliminarily assessed by asking 10 people whether able to see the differences of organoleptic properties such as color and taste compared to the control cookies (without the addition of DDG samples) when tasting.

3. Results and Discussions

3.1. Extraction of Protein from DDG

3.1.1. Extraction of protein by alkaline solution

The protein in cereals and rice in particular consists of 4 components with different proportions including albumin, globulin, prolamin (gliadin/oryzin/zein), and glutelin. In rice, glutelin makes up the majority with 75 - 90% of total protein content (7 - 8% of dry matter of grain [5]). The remaining three components were equivalent and vary in a range of 2 - 10% of total protein. Glutelin was proven as good solubility in alkaline solvent, therefore NaOH was used to extract protein from rice-based DDG.

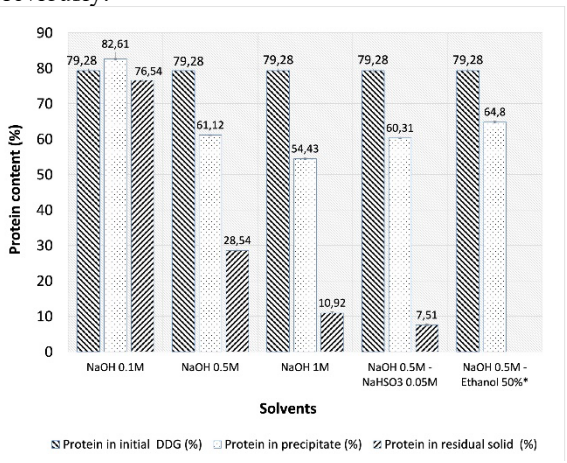
Initially, NaOH varying concentrations of 0.1, 0.5, and 1M were applied. The protein content in the residual solid and precipitates was analysed (Fig. 2). Varying concentration of NaOH from 0.1 M to 1M has resulted in the decreasing of the protein content in the residual solids, especially with 1 M NaOH, a relatively low protein content (10.92%) in residue solid was observed (Fig. 2), this means the extraction protein by NaOH was properly happened. The more concentrated NaOH, the more protein was extracted (the lower protein content in residue solids). However, protein content in the precipitates was also increased as expected (Fig. 2). The precipitate from extraction using NaOH 1M consists of 54.43% protein only, much lower than protein in initial DDG (79.28%). At a lower concentration of 0.1M, protein in precipitate was higher than in initial DDG (82.61% compared to 79.28%) but protein extraction efficiency was low, thus the remaining protein content in the residual solid was rather high of 76.53%.

This proves that the higher the concentration of NaOH used, the more protein was extracted from the DDG but also the non-protein components co-dissolved. The non-protein components could be starch as Jivan et. al. reported that the solubility of potato starch increased to 51% or 74% in alkaline solution [6]. It was possible that the pH 4 inducing the precipitation was also not "selective" for protein in this case even though this pH 4 was reported as optimal pH for rice bran protein precipitation [7].

The observation in this study with 1M NaOH solution is in agreement with results from other authors. However, in terms of efficiency compared to the initial protein content (~79.20% dry matter), the use of NaOH 0.1 - 1 M did not result in a significant improvement of the protein content in the rice-based DDG.

3.1.2. Extraction of protein by alkaline solutions combined with reducing agents

Reducing agents can break down disulfide bonds thus increase the solubility of protein [3,4]. In this study, $NaHSO_3$ 0.05 M as a reducing agent was used in combination with NaOH 0.5 M. The resulted protein content in residual solid was 7.51% which was much lower than value obtained of 28.54% with NaOH 0.5 M individually (Fig. 2). This meant the enhancing effect of reducing agents on the extraction of protein. However, protein content in precipitation from this extraction was 60.31%, slightly lower to precipitate obtained with NaOH 0.5M (without reducing agent) (61.12 % Fig. 2). Perhaps, the pH 4 applied for precipitation was not "selective" as we mentioned previously.



** The protein content in residual solid was not determined.*

Fig. 2. Extraction of protein by using NaOH-based solution.

3.1.3. Extraction of protein by alkaline - ethanol solution

According to other authors, the combination of alkaline solution and ethanol will enhance the efficiency of extracting protein from DDG[3]. In this study, we extracted protein from rice-based DDG with a mixture of NaOH 0.5 M and ethanol 50%. The protein content of the precipitates was 64.8% which was slightly higher than that using solely NaOH 0.5 M (Fig. 2). In fact, ethanol might not significantly affect this case because ethanol can dissolve most zein which presents in rice protein at a low level (< 1 - 5%) compared to in corn protein (50 - 55% of total protein).

In summary, the direct extraction of protein from rice-based DDG by NaOH did not improve the protein content in DDG. Therefore, the indirect enrichment of protein in DDG was performed by the removal of non-protein components.

3.2. Extraction of Non-Protein Components

To eliminate non-protein components, the rice-based DDG was treated with a low concentration of NaOH solution (5mM - 25mM) at 60ºC for 2h. The composition of the residual solids after treatment are shown in Table 2.

The results show that NaOH at a low concentration in the range of 5 mM - 25 mM can dissolve protein, starch, fiber to different extend. Among, starch was observed as better soluble, thus presented at reduced content in the residual solids. The fiber content was almost unchanged when varying the NaOH concentration below 20 mM (Table 2). Protein content, as expected, was increased 3 - 6% compared to the value in the initial DDG. NaOH at 10 mM was shown the best for the enrichment of protein which amounts to 85.45% and 87.8% in the residual solids when treated with non-defatted and defatted rice-based DDG, respectively. The recovery protein yield was mostly higher than 90%, except NaOH of 25 mM resulting in 85.62% of recovery yield. This observation was according to the results obtained with a higher concentration of NaOH as previously discussed.

Table 2. Composition of residual solids after treatment of rice-based DDG with dilute NaOH solutions

NaOH (mM)	Composition of residual solids (%)		
	Protein (recovery yield %)	Starch	Fiber
Initial DDG	79.28 ± 0.52	8.72 ± 0.09	4.20 ± 0.34
5	82.68 ± 0.16 (90.23)	6.93 ± 0.18	4.24 ± 0.06
10	85.45 ± 0.51 (92.12)	5.24 ± 0.33	4.50 ± 0.08
15	83.63 ± 0.29 (90.15)	7.06 ± 0.05	4.17 ± 0.13
20	83.08 ± 0.87 (91.08)	6.03 ± 0.73	5.38 ± 0.06
25	82.99 ± 0.05 (85.62)	6.78 ± 0.43	2.84 ± 0.04
10*	87.80 ± 0.23 (98.64)	4.05 ± 0.14	4.52 ± 0.15

** defatted DDG*

With defatted DDG, starch in residual solid was reduced most to 4.05%, hence the protein content in the residual solid was increased to 87.8% with a recovery yield of 98.6% (Table 2). This is probably due to the residual starch in the rice-based DDG that exists as an amylose-lipid complex [8]. The separation of fat helps the starch in the amylose-lipid complex be released and dissolved easily into alkaline solvent. Furthermore, temperature and extracting duration were also investigated for extraction with NaOH 10 mM, however, 60 ºC and 2 h were shown as the most suitable (data not shown).

In short words, from such high protein content rice-based DDG, in comparison to the high concentration for direct extraction of protein, the low concentration of NaOH has resulted in better enrichment of protein via extracting of non-protein such as starch or fiber from DDG.

Table 3. Total amino acid profile of initial and treated DDG

Amino acids	% in total amino acid		
	Initial DDG	NaOH 10mM treated DDG	Rice endosperm*
Non-essential AA			
Asp	5.87 ± 0.15	4.33 ± 0.18	9.90 ± 0.72
Ser	2.26 ± 0.19	2.65 ± 0.15	5.03 ± 0.16
Gly	4.85 ± 0.23	5.23 ± 0.07	4.22 ± 0.10
Arg	12.24 ± 0.01	15.61 ± 0.51	8.37 ± 0.16
Ala	3.89 ± 0.11	4.06 ± 0.09	5.33 ± 0.21
Tyr	3.29 ± 0.29	3.12 ± 0.26	5.04 ± 0.10
Cys	2.02 ± 0.08	1.26 ± 0.11	0.62 ± 0.01
Glu	19.7 ± 1.41	19.75 ± 0.37	17.7 ± 0.79
Essential AA			
His	3.20 ± 0.15	3.17 ± 0.09	2.00 ± 0.04
Thr	5.23 ± 0.13	4.52 ± 0.01	3.55 ± 0.10
Val + Met	6.89 ± 0.25	6.44 ± 0.04	6.87± 0.15
Phe	6.45 ± 0.60	5.74 ± 0.21	4.93 ± 0.13
Ile	6.47 ± 0.22	6.39 ± 0.42	3.73 ± 0.14
Leu	13.23 ± 0.02	13.70 ± 0.26	7.37 ± 0.21
Lys	4.4 ± 0.01	4.03 ± 0.04	3.42 ± 0.02

** According to Sekhar et. al. [9], % of total protein*

3.3. Amino Acid Profiles

Results of amino acid analysis by HPLC show that the free amino acids in the initial DDG is at a negligible level. This proves that most amino acids produced in saccharification were used by yeast during fermentation or degraded and changed during the distillation. The profiles of total amino acids in initial DDG and treated DDG by 10 mM NaOH are shown in Table 3. There is a similarity of amino acid profiles between initial and treated DDG, except minor changes in the proportion of some amino acids such as aspartic acid, threonine, arginine..., perhaps due to the dissolving of a little amount of protein from DDG into NaOH 10mM. The amino acid profile of DDG protein is slightly different from that of rice endosperm protein [9] (Table 3)

Preliminary Assessment of the Applicability of DDG to cookies

Replacement of 15% wheat flour (with estimated protein content of 10%) by enriched DDG (87% protein) increased the protein content in this part up to 21.55%, thus elevated the total protein content in cookies. In preliminary sensory evaluation, most of the people asked (10/10) to test could recognise the cookies using the initial DDG from the rest due to the odor of rice-based DDG. However, they (10/10) could not differentiate the cookies using treated DDG from the reference ones in terms of taste, odor, and structure. The cookies containing DDG retained the golden brown color (Fig. 3).

Fig. 3. Cookies added of initial DDG (in the middle) and treated DDG (at the right) compared to reference cookies (without addition, at the left).

This meant the treatment not only increased the protein content but also eliminated the DDG odor and prevented negative effects on cookies.

The addition of corn-based DDG to food products has also been studied by several authors. For example, a group of researchers from the Food Department at South Dakota State University), has supplemented DDGS in different food products including cookies, bread. The authors have used a pretreatment process to rinse, remove odors and grind into a fine powder of DDGS containing 40% fiber and 36% protein [10]. The results showed that the use of DDGS for cookies was not only safe but also elevating the content of nutrients such as protein or fiber in products. The sensory properties of products were also acceptable. No allergic reactions to DDGS supplemented cookies were reported. In our study, the enriched rice-based DDG was shown a high potential for enrichment of protein in several food products such as cookies.

4. Conclusion

Treatment of the rice-based DDG by dilute NaOH solution (5 mM – 20 mM) showed effectiveness in removing non-protein components, thus improved the protein content in the residual DDG. In particular, the 10mM NaOH solution has increased the protein content in defatted DDG up to 87% with a recovery yield of 98.6%. Similarly, to the initial DDG, the protein part in the treated DDG contains full of essential and non-essential amino. The treatment did not change the profile (the ratio to the total protein) of these amino acids. The replacement of treated DDG to wheat flour in cookies formulation up to 15% was not shown a significant effect on sensory characteristics of the cookies.

Acknowledgement

This research is funded by the Hanoi University of Science and Technology (HUST) under project number T2018-PC-009.

References

[1] Taranu *et al.*, Rice and Cassava Distillers Dried Grains in Vietnam: Nutritional values and effects of their dietary inclusion on blood chemical parameters and immune responses of growing pigs, Waste and Biomass Valorization 10 (11) (2019) 3373-3382. https://doi.org/10.1007/s12649-018-0341-7

[2] H. Li, M. Liang, Z. Wang, Y. Zhang, Q. Wu, and L. Yang, Rice protein exerts endogenous antioxidant capacity via methionine sulfoxide reductase and the Nrf2 antioxidant system independent of age, J. Med. Food. 23 (6) (2020) 565-574. https://doi.org/10.1089/jmf.2019.4504

[3] D. J. Cookman and C. E. Glatz, Extraction of protein from distiller's grain, Bioresource Technology 100 (6) (2009) 2012-2017. https://doi.org/10.1016/j.biortech.2008.09.059

[4] A. Chatzifragkou, P. C. Prabhakumari, O. Kosik, A. Lovegrove, P. R. Shewry, and D. Charalampopoulos, Extractability and characteristics of proteins deriving from wheat DDGS, Food Chem. 198 (2016) 12-19. https://doi.org/10.1016/j.foodchem.2015.11.036

[5] T. Kawakatsu and F. Takaiwa, 4 - Rice proteins and essential amino acids, in Rice (Fourth Edition), Ed.: AACC International Press (2019) 109-130 https://doi.org/10.1016/B978-0-12-811508-4.00004-6

[6] M. J. Jivan, M. Yarmand, and A. Madadlou, Preparation of cold water-soluble potato starch and its characterization, Journal of Food Science and Technology 51 (3) (2014) 601-605. https://doi.org/10.1007/s13197-013-1200-y

[7] T.M.P. Nguyen, H.B. Vo, T.N. Tran , H.H. Do**,** Preparation of protein isolated from rice bran, Journal of Biotechnology 37 (4) (2015) 8-16 (in Vietnamese)

[8] S. Srichuwong and J.-l. Jane, Methods for characterization of residual starch in distiller's dried grains with solubles (DDGS), Cereal Chemistry 88(3) (2011) 278-282. https://doi.org/10.1094/CCHEM-11-10-0155

[9] B. P. Sekhar and G. M. Reddy, Amino acid profiles in some scented rice varieties, Theor Appl Genet*,* 62(1) (1982) 35-37.

[10] P. Singha, K. Muthukumarappan, and P. Krishnan, Influence of processing conditions on apparent viscosity and system parameters during extrusion of distiller's dried grains-based snacks, Food Sci. Nutr*.,* 6(1) (2018) 101-110 https://doi.org/10.1002/fsn3.534