biology and life sciences forum

MDPI

Proceedings

Developing a Nutrient-Rich Rice Protein Drink for Athletes using Protease G6 Enzyme†

Sukan Braspaiboon[1], Sukhuntha Osiriphun[*2], Prasit Peepathum[3], Witid Mitranun[3], Wachira Jirarattanarangsri[4], Suthat Surawang[5], Thunnop Loakuldilok[1] and Nut Koonrungseesomboon[6]

[1] Faculty of Agro-Industry, Chiang Mai University, Mueang, Chiang Mai, 50100, Thailand; sukan.bras@cmu.ac.th, thunnop.l@cmu.ac.th
[2] Agro-Industry, Chiang Mai University; sukhuntha@hotmail.com
[3] Department of Sports Sciences, Faculty of Physical Education, Srinakharinwirot University, Bangkok 10117, Thailand; prasitpeepathum@yahoo.com, mitranunwitid@hotmail.com
[4] Division of Food Science and Technology, Agro-Industry, Chiang Mai University, Muaeng, Chiang Mai, 50100, Thailand; wachira.J@cmu.ac.th
[5] Division of Product Development Technology, Agro-Industry, Chiang Mai University, Muaeng, Chiang Mai, 50100, Thailand; suthat.s@cmu.ac.th
[6] Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand; Nut.koonrung@cmu.ac.th
* Correspondence: sukhuntha@hotmail.com

The purpose of this study was to determine the extraction of hydrolysate protein from waste materials (rice grain and rice beverages) in order to increase the value of domestic raw materials. The goal was to create protein beverage products containing rice protein hydrolysates that are customized to the needs of athletes for post-workout muscle restoration. Carbohydrates were extracted from rice paste using an amylase enzyme, followed by protein extraction using the Protease G6 enzyme. The E/S SL ratio, temperature, and time were investigated, with the extraction taking place at a pH of 7.0. The Central Composite Design approach was used in the experimental design to change the extraction conditions. The protein concentration and the concentration levels were determined. The concentration data were then submitted to 95 percent confidence level Analysis of Variance (ANOVA) to find significant differences. To visualize the relationship between protein concentration and the interaction between the E/S SL ratio, temperature, and extraction duration, a contour plot was generated. The results showed that increasing enzyme proportions and temperatures between 50 and 60 degrees Celsius boosted protein concentration. Lower E/S SL ratios and longer extraction times enhanced protein concentration. An E/S, SL ratio of 5%, a temperature of 52 degrees Celsius, and an extraction time of 180 minutes were shown to be ideal conditions for extracting protein from rice grains utilizing Protease G6 enzyme. The final protein content was 3.14 g/100 ml. These findings suggested that Protease G6 can be a viable alternative for developing rice protein beverages for athletes and health-conscious individuals.

Keywords: Rice Protein; Protein Drinks; Protease G6

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Published: date

1. Introduction

Protein drinks have emerged as popular in today's health-conscious society. These nutritional beverages are designed to provide a convenient and efficient way to supplement one's diet with essential proteins [1]. These can be served not only an athlete striving to build muscle, but also a fitness enthusiast aiming to recover after a workout, or simply someone looking to maintain a balanced and nutritious diet to weight management or promote overall well-being [2].

In recent years, the quest for healthier dietary options has led to a surge of interest in using rice as an alternative to sugar. This exploration aligns with the global shift towards

healthier lifestyles and dietary choices. One significant motivation for this endeavor is the desire to combat the health risks associated with excessive sugar consumption, such as obesity and related chronic illnesses [3]. In addition, governments in several countries have introduced sugar taxes to discourage the consumption of sugary beverages and address public health concerns [4]. To utilize rice as a sugar alternative in beverage production, manufacturers aim to reduce the sugar content but still contain short polysaccharides to provide sufficient energy for consumers during doing physical activities [5]. This not only benefits consumers but also promotes healthier choices in the market.

The production process of the alternative sports drink can yield valuable by-products, including rice paste. This paste differs from traditional rice products because of a lower carbohydrate content, while its protein content is boosted [6]. This unique composition makes rice paste an attractive ingredient for innovative beverage production, especially protein drinks. Athletes and fitness enthusiasts often seek protein-rich beverages to aid in muscle recovery and overall performance. Rice protein is an excellent option for those seeking plant-based alternatives to traditional animal-based protein sources like meat or dairy [7]. It provides a multitude of benefits, such as hypoallergenic ingredients, and a well-balanced amino acid profile. In recent years, the popularity of rice protein has soared, driven by the growing demand for plant-based diets and dietary supplements [8].

Protease G6 is categorized as an alkaline serine endoprotease, which is one of the commercial proteolytic enzymes popularly used for hydrolysis. Protein hydrolysate digested by this can provide a wide range of functional properties, especially antioxidant activities and inhibition of lipid oxidation. Thus, Protease G6 is appropriated to extract protein from rice paste [9].

According to the rationale mentioned above, this research aimed to utilize rice paste to produce rice protein drinks for athletes through protein extraction executed by Protease G6. The parameters related to extraction, such as enzyme per substrate (E/S), ratio of liquid per solid (SL ratio), temperature, and time, were all investigated against protein concentration. The Central Composite Design (CCD) approach was applied in experimental design to explore the optimal condition of extraction.

2. Materials and Methods

2.1. Rice paste

Rice (Sao Hai cultivar) was grinded by FT2 Hammer Mill machine (Armfield, England) before carbohydrates of rice flour were digested by α-amylase to produce sports drinks [6]. Rice paste, by-product of the production, was collected and dried at 60 °C for 24 h. This dried material called CDR powder was stored in aluminum bags at 4 ºC until use.

2.2. Experimental designs

Response surface methodology (RSM) was applied to investigate the effect of enzymatic extraction on concentrations of rice proteins. Independent parameters, consisting of enzyme concentration (E/S), ratio of liquid per solid (SL ratio), temperature and extraction time, were varied into five level according to CCD (Table 1). Coded value of alpha (α) for four factors in CCD was far from central point for two points. The generalized second-order polynomial model used in the RSM analysis as eq. (1).

2.3. Extraction of rice proteins

Rice proteins in CDR powders were extracted by Protease G6 (EC 3.4.21.62) derived from Siam Victory Chemical Co. Ltd., Thailand. Protein extraction was conducted according to the conditions, which independent parameters were varied according to Table 1. The pH of extraction was controlled at pH 7.0 by 20 mM Tris-HCl. The extracted protein in solutions were quantified by Kjeldahl method and represented by percentages of protein concentration.

2.4. Statistic analysis

The mean values and standard deviation were representative of all measurements. ANOVA were applied to identify difference among all values, which was significant at $p \leq 0.05$. Experimental designs and contour plots were generated using Minitab 16 statistical software.

3. Results and Discussion

Protein concentrations from rice paste extracted by Protease G6 were expressed by 31-treatments according to Table 2. Parameters significantly influence on protein concentrations were E/S, SL ratio and temperature ($p \leq 0.05$) while extraction time was indifferently. The relationship between parameters and protein concentration were illustrated by contour plots (Fig. 1). Two parameters were plotted against protein concentration while other variables were fixed constantly at the middle value.

According to the contour plots, protein concentration was increased when E/S was higher. The range of optimal temperature was around 50-60 °C (Fig 1a). Obviously, a proportion of enzyme is significant to extracted protein yield. It reflects the amount of enzyme unit per substance. An increase in enzyme concentration leads to an acceleration of protein digestion [10]. Proteases function of breaking down interactions between proteins and polysaccharide matrix [11]. Protein in rice is attached to starch granules. Thus, the process of amylase digestion is a good pre-treatment to destroy interaction between interaction. In addition, protease also inhabits the reformation of extracted proteins [12]. The advantage of enzymatic extraction, which is superior to conventional alkaline extraction, is the higher protein solubility and nutritional values [6].

Moreover, the effect of SL ratio and extraction time were opposite (Fig 1b). Protein concentration was decreased when the higher proportion of SL ratio was shown. Except for the previous three parameters, extraction time rarely influenced protein concentration, which protein concentration was almost indifferent among various extraction time. The effect of SL ratio was recognized as a driving force of mass transfer [13]. The difference of SL ratio directly affects the final concentration of protease in liquid phase. A mass transfer is effective when the concentration of liquid phase is higher than inside substrate, which induces a penetration of enzyme or osmosis [6]. In the effect of extraction time, this result was similar to data of Zhang, L., et al. [14], which the further extraction over 90 minutes cannot provide a higher yield of anthocyanin. This phenomenon is caused by the reaction equilibrium and concentration difference between solution and substrate.

The regression equation generated by RSM provides an equation model representing the relationship between protein concentrations and parameters in coded units as eq. (2). The determination efficient (R^2) of the model was 0.952, which indicates the fitted model. The lack of fit value (0.144) at $p > 0.05$ also verified that the model equation could represent appropriately the relationship between protein concentration and related parameters [15].

The maximum protein concentration predicted by the model (eq. 2) was calculated as 3.193% at composite desirability = 1. The extraction condition of Protease G6 providing the highest protein concentration were 5% of E/S, 4 folds of SL ratio, temperature at 52 °C, and 180 minutes of extraction time. Extraction according to this condition was executed to verify the accuracy of prediction. The result showed that the protein concentration was 3.14%, calculated as 0.05% of different interval to the predicted value.

4. Conclusion

Rice paste, a by-product from sports drinks production, provides a potential source of plant-based protein extraction. Protease G6 displayed a capability in protein extraction from rice paste to the solution at a specific condition, which can be developed into a commercial rice protein drink for athletes more efficiently than conventional alkaline extraction in terms of protein solubility and nutritional value. However, this protein hydrolysate

must be further studied in amino acid composition and antioxidant activities, one of the crucial features of protein hydrolysates from Protease G6.

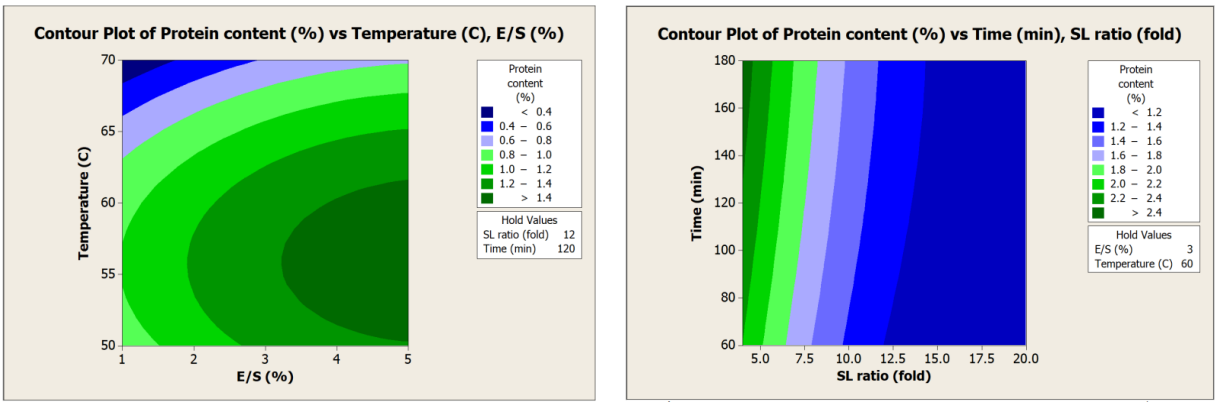
5. Figures, Tables and Mathematical equation

Table 1. The variation of coded and real values of factors conducted by Protease G6.

Coded value	-2	-1	0	1	2
E/S (%)	1	2	3	4	5
SL ratio (fold)	4	8	12	16	20
Temperature (°C)	50	55	60	65	70
Time (min)	60	90	120	150	180

Table 2. Protein concentration extracted by Protease G6 in different conditions (31 treatments).

8	E/S (%)	SL ratio (fold)	Temperature (ºC)	Time (min)	Protein concentration (%)	
					Experimental	Predicted
1	2	8	55	90	1.38 ± 0.13	1.50
2	4	8	55	90	1.88 ± 0.03	1.87
3	2	16	55	90	1.08 ± 0.11	0.98
4	4	16	55	90	1.17 ± 0.08	1.09
5	2	8	65	90	1.21 ± 0.03	1.21
6	4	8	65	90	1.54 ± 0.05	1.59
7	2	16	65	90	0.83 ± 0.03	0.80
8	4	16	65	90	0.96 ± 0.04	0.92
9	2	8	55	150	1.71 ± 0.01	1.70
10	4	8	55	150	2.04 ± 0.06	2.10
11	2	16	55	150	1.13 ± 0.03	1.10
12	4	16	55	150	1.29 ± 0.05	1.24
13	2	8	65	150	1.13 ± 0.11	1.23
14	4	8	65	150	1.58 ± 0.06	1.64
15	2	16	65	150	0.79 ± 0.05	0.75
16	4	16	65	150	1.00 ± 0.10	0.90
17	1	12	60	120	0.96 ± 0.02	0.94
18	5	12	60	120	1.42 ± 0.04	1.46
19	3	4	60	120	2.58 ± 0.21	2.38
20	3	20	60	120	0.88 ± 0.23	1.11
21	3	12	50	120	1.21 ± 0.04	1.24
22	3	12	70	120	0.63 ± 0.01	0.61
23	3	12	60	60	1.17 ± 0.03	1.20
24	3	12	60	180	1.38 ± 0.01	1.37
25	3	12	60	120	1.21 ± 0.10	1.31
26	3	12	60	120	1.29 ± 0.02	1.31
27	3	12	60	120	1.42 ± 0.10	1.31
28	3	12	60	120	1.33 ± 0.03	1.31
29	3	12	60	120	1.21 ± 0.11	1.31
30	3	12	60	120	1.32 ± 0.01	1.31
31	3	12	60	120	1.43 ± 0.09	1.31



(**a**) (**b**)

Figure 1. Contour plots of parameters against protein concentration (%) extracted by Protease G6 (**a**) E/S and temperature (°C); (**b**) SL ratio (fold) and extraction time (min).

The generalized second-order polynomial model

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_{ii}^2 + \sum \sum_{i<j=1}^{k} \beta_{ij} X_i X_j \quad (1)$$

Where X_i and X_j are the independent parameters and k is a number of input variable (k=4). Regression coefficients of B_0, B_i, B_j and B_{ij} are for intercept, linear, quadratic and interaction coefficients, respectively.

The equation model for prediction

$$Y=1.30722+0.130X_1-0.318X_2-0.158X_3+0.043X_4-0.0269X_1^2+0.109X_2^2-0.095X_3^2-0.006X_4^2-0.065X_1X_2+0.03X_1X_3+0.08X_1X_4+0.029X_2X_3-0.018X_2X_4-0.044X_3X_4 \quad (2)$$

Where Y was protein concentration, while X parameters were E/S (X_1), SL ratio (X_2), temperature (X_3) and time (X_4), respectively.

Acknowledgments: The authors greatly thank the Faculty of Agro-Industry, Chiang Mai University and to provide the analytical instrument for analysis.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Arenas-Jal, M., et al. Trends in the food and sports nutrition industry: A review. *Crit. Rev. Food Sci.* **2020**, 60(14), 2405-2421.
2. Ahern, N., Arendt, E.K., and Sahin, A.W. Protein Soft Drinks: A Retail Market Analysis and Selected Product Characterization. *Beverages* **2023**, 9, 73.
3. Magriplis, E., et al. A. Dietary Sugar Intake and Its Association with Obesity in Children and Adolescents. *Children* **2021**, 8, 676.
4. Young, A., James, K., and Hassan, A. The role of regressive sugar tax in the soft drink industry levy (SDIL): A Marxist analysis. *Crit. Perspect. Account* **2022**, 88, 102326.
5. Chen, Y. J., et al. Anti-fatigue effect of a dietary supplement from the fermented by-products of Taiwan tilapia aquatic waste and *Monostroma nitidum* oligosaccharide complex. *Nutrients* **2021,** 13(5), 1688.
6. Braspaiboon, S., et al. Comparison of the effectiveness of alkaline and enzymatic extraction and the solubility of proteins extracted from carbohydrate-digested rice. *Heliyon* **2020**, 6.11.
7. Lee, J. S., et al. Physico-chemical characteristics of rice protein-based novel textured vegetable proteins as meat analogues produced by low-moisture extrusion cooking technology *LWT* **2022**, 157,113056.
8. Wang, N., et al. Potential health benefits and food applications of rice bran protein: Research advances and challenges. *Food Rev. Int.* **2023**, 39(6), 3578-3601.
9. Gong, X., et al. Investigation of nutritional and functional effects of rice bran protein hydrolysates by using Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines: A review. *Trends Food Sci. Technol.*, **2021**, 110, 798-811.
10. Mwaurah, P. W., et al. Novel oil extraction technologies: Process conditions, quality parameters, and optimization. *Compr. Rev. Food Sci. Food Saf.* **2020**, 19(1), 3-20.

11. Chen, B., et al. Extraction, Structural Characterization, Biological Functions, and Application of Rice Bran Polysaccharides: A Review. *Foods*. **2023**; 12. 639.
12. Kumar, M., et al. Advances in the plant protein extraction: Mechanism and recommendations. *Food Hydrocolloids* **2021**. 115, 106595.
13. Kamal, H., et al. Extraction of protein from food waste: An overview of current status and opportunities. *Compr. Rev. Food Sci. Food Saf.* **2021**, 20(3), 2455-2475.
14. Zhang, L., et al. Ultrasonic-assisted enzymatic extraction and identification of anthocyanin components from mulberry wine residues. *Food Chem.* **2020**, 323, 126714.
15. Chakraborty, S., Uppaluri, R., and Das, C. Optimization of ultrasound-assisted extraction (UAE) process for the recovery of bioactive compounds from bitter gourd using response surface methodology (RSM). *Food and Bioprod. Process.* **2020**, 120, 114-122.