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Proceeding Paper

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

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Abstract: 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 min were shown to be ideal conditions for extracting protein from rice grains utilizing the 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

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1. Introduction

Protein drinks are 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 useful to not only an athlete striving to build muscle, but also to a fitness enthusiast aiming to recover after a workout, or simply someone looking to maintain a balanced and nutritious diet to manage their weight 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 suitable 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, Ringwood, UK) 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 °C 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 levels according to CCD (Table 1). Coded value of alpha (α) for four factors in CCD was far from central point by two points. The generalized second-order polynomial model used in the RSM analysis as Equation (1).

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

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.

2.3. Extraction of Rice Proteins

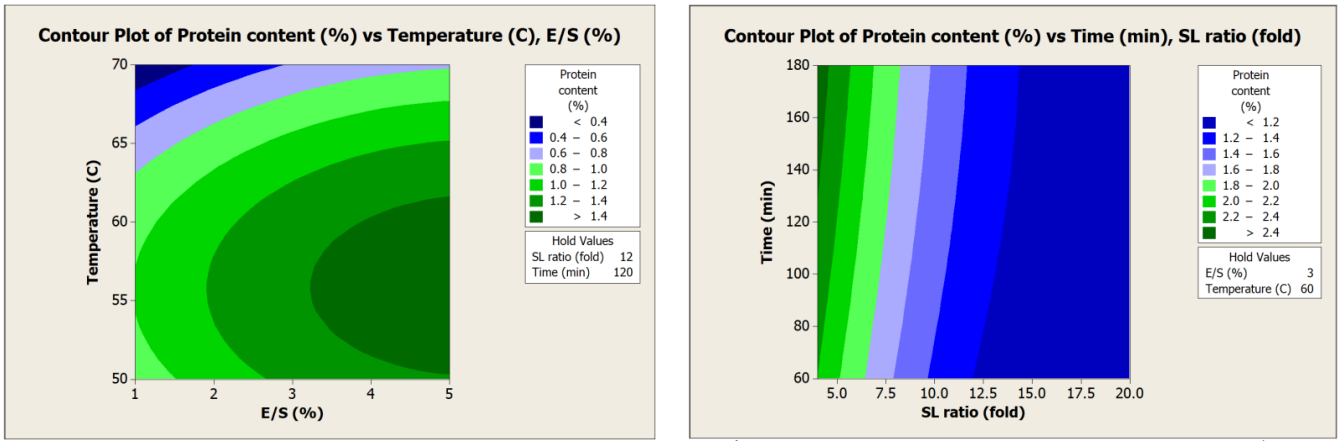
Rice proteins in CDR powders were extracted by Protease G6 (EC 3.4.21.62) derived from Siam Victory Chemical Co., Ltd., Bangkok, Thailand. Protein extraction was conducted according to the conditions, which independent parameters were varied according to Table 1. The pH of extraction was controlled at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

2.4. Statistical 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 influencing on protein concentrations were E/S, SL ratio and temperature ($p \leq 0.05$), while extraction time was indifferent. The relationships between parameters and protein concentration were illustrated by contour plots (Figure 1). Two parameters were plotted against protein concentration, while other variables were fixed constantly at the middle value.



(**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).

According to the contour plots, protein concentration increased when E/S was higher. The range of optimal temperature was around 50–60 °C (Figure 1a). Clearly, a proportion of enzymes is significant to the 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 by breaking down interactions between proteins and the 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 interactions. In addition, protease also facilitates the reformation of extracted proteins [12]. The advantage of enzymatic extraction, which is superior to conventional alkaline extraction, its higher protein solubility and nutritional values [6].

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

8	E/S	SL Ratio	Temperature	Time	Protein Concentration (%)	
	(%)	(Fold)	(°C)	(min)	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

Moreover, the effects of SL ratio and extraction time were opposite (Figure 1b). Protein concentration decreased when a higher proportion of the SL ratio was shown. Except for the previous three parameters, extraction time rarely influenced protein concentration, and protein concentration was almost indifferent among various extraction times. The effect of the SL ratio was recognized as a driving force of mass transfer [13]. The difference in SL ratio directly affects the final concentration of protease in the liquid phase. A mass transfer is effective when the concentration of the liquid phase is higher than that inside the substrate, which induces the penetration of enzymes or osmosis [6]. In terms of the effect of extraction time, this result was similar to the data of Zhang, L. et al. [14], in which further extraction over 90 min 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 the RSM provides an equation model representing the relationship between protein concentrations and parameters in coded units as Equation (2). The determination efficient (R^2) of the model was 0.952, which indicates a fitted model. The lack of fit value (0.144) at $p > 0.05$ also verified that the model equation

could appropriately represent the relationship between protein concentration and related parameters [15].

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.

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

4. Conclusions

Rice paste, a by-product from sports drink 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 terms of amino acid composition and antioxidant activities, one of the crucial features of protein hydrolysates from Protease G6.

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