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Comparative evaluation of functional properties of protein isolates from Malaysian rice varieties MRQ 76, Bario Merah Ranau and Long Pasia

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ABSTRACT

In Malaysia, a wide diversity of rice varieties is cultivated across distinct agro-ecological zones, but their protein fractions remain functionally underexplored. This comparative study evaluated the functional properties of protein isolates extracted from three Malaysian rice varieties: MRQ 76, Bario Merah Ranau, and Long Pasia. The MRQ 76 variety was obtained from the Malaysian Agricultural Research and Development Institute, while the Bario Merah Ranau and Long Pasia varieties were supplied by Mutiara Timur Rice Mill Sdn. Bhd. Protein extraction was performed using alkaline solubilization–isoelectric precipitation, and the functional properties of the protein isolates were assessed across a range of pH values. The results showed that the MRQ 76 variety exhibited the highest protein content (8.14 ± 0.03%), oil-binding capacity (239.40 ± 4.25%), and foaming stability (88.64 ± 2.75%), while the Long Pasia variety showed superior water-binding capacity (266.0 ± 5.35%) and foaming capacity (77.78 ± 3.85%). However, the protein solubility (pH 5 and 9) and emulsifying capacity (pH 4 and 7) tests showed no significant differences among the three rice varieties. The findings demonstrate variations in the influence of rice varieties on protein functionality, highlighting the MRQ 76 variety as a promising candidate for moisture-retentive food systems, while the Long Pasia variety is suitable for sparkling applications. These findings offer new insights into improving protein isolates and supporting innovation in health-oriented food products.

Contribution/Originality: The paper's primary contribution is to provide the first comparative characterization of the functional properties of protein isolates from improved (MRQ 76) and indigenous (Bario Merah Ranau and Long Pasia) Malaysian rice varieties, demonstrating distinct functional profiles that support their targeted use in different food applications.

1. INTRODUCTION

Due to their hypoallergenic nature, relatively balanced amino acid composition, and techno-functional properties suitable for food applications (Jayaprakash, Bains, Chawla, Fogarasi, & Fogarasi, 2022; Opazo-Navarrete et al., 2025; Suo et al., 2022), rice proteins have received increasing attention as sustainable, plant-based ingredients. Recent developments in rice protein research have highlighted the need for extraction techniques that achieve high protein

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recovery while retaining functional properties. Despite the increasing use of non-conventional alkaline extraction because of its unique advantages, conventional alkaline extraction remains a common technique in most laboratories because it is simple, inexpensive, and provides high extraction yields; however, it may denature proteins, thereby negatively impacting functionality (Roy, Pawar, Singh, Loushigam, & Wagh, 2025; Zheng, San, Xing, & Regenstein, 2024).

Therefore, innovative methods, such as enzyme-assisted extraction, ultrasound-assisted extraction, high-pressure liquid chromatography, and hybrid processes, are employed to reduce protein denaturation, leading to improved protein functionality (Anyiam, Tangjaidee, Zhang, & Rawdkuen, 2025; Yin et al., 2026). However, the functional attributes of rice proteins are determined not only by extraction methods but also by intrinsic factors, such as protein composition (ElShamey, Yang, Yang, Xia, & Zeng, 2025; Li et al., 2023), amino acid profiles, molecular structures, varietal differences, solubility in water or oil, emulsification properties, foaming capacity, and bitter taste.

Nonetheless, recent research has mainly focused on commercial rice varieties (Jukanti et al., 2025; Lee, Choi, & Han, 2022), while data on indigenous Malaysian rice varieties, such as MRQ 76, Bario Merah Ranau, and Long Pasia, are minimal. Thus, comparative data on these varieties are limited, making it difficult to determine whether they would be suitable for specific food applications. Given that functional properties play a significant role in determining food-processing efficiency and final product characteristics, it is crucial to understand these properties to facilitate the proper selection of protein sources.

This study therefore aimed to evaluate the functional properties of protein isolates from MRQ 76, Bario Merah Ranau, and Long Pasia in terms of protein extraction yield, protein concentration, protein solubility, water and oil absorption capacities, foaming properties, and emulsion properties. The results would provide further insight into the functional properties of protein isolates from MRQ 76, Bario Merah Ranau, and Long Pasia.

2. MATERIALS AND METHODS

2.1. Sample Collection

MRQ 76 rice, known as a fragrant rice variety, was purchased from the Malaysian Agricultural Research and Development Institute (MARDI), while Bario Merah Ranau and Long Pasia were sourced from Mutiara Timur Rice Mill Sdn. Bhd. The rice grains were free from dirt and contamination and were kept in a sealed box at room temperature (25°C).

2.2. Rice Grain Characteristics

Grains of MRQ 76 are long and slender owing to their length-to-width ratio, and they have a sweet, natural aroma and a soft texture when cooked. In contrast, Bario Merah Ranau has small, slender, and elongated grains, with a distinctive reddish-brown bran layer. Long Pasia grains are fine, short to medium in size, and elongated in shape. In terms of color, the grains are mostly bright and pearly white.

2.3. Defatting and Protein Isolation

The rice grains were ground into a powder and defatted using the Soxhlet extraction technique with hexane (1:5, w/v) at 50 °C (Cho, Lee, & Han, 2022). This extraction technique was chosen because it allows for the effective and reproducible removal of lipids, thereby improving protein isolation and reducing lipid interference in future functional studies. The defatted flour was suspended in distilled water 5x and agitated for 1 h at 30 °C, with the pH adjusted to 9.0 to increase protein solubility. Subsequently, the suspension was centrifuged at 15,000 ×g for 10–15 min at 4 °C, and the supernatant was separated. Protein precipitation was then achieved by adjusting the pH of the supernatant to 4.5, after which the sample was centrifuged at 15,000 ×g for 10–15 min at 4 °C. The obtained protein pellet was resuspended in 200 mL per sample and stored at −20 °C in closed containers. The extraction yield was calculated using the formula below.

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$$\text{Extraction Yield (\%)} = \frac{W_2}{W_1} x\ 100$$

Where,

W_1 = The original weight of dried sample.

W_2 = The weight of the dried extract.

2.4. Protein Analysis

The total protein content was determined using the Kjeldahl method with an automated analyzer (Kjeltec 8200, Foss, Hillerød, Denmark). A total of 1 g of each sample was weighed into a 250 mL digestion tube. The samples were subjected to acid digestion for 3 h, followed by cooling to allow the complete conversion of organic nitrogen into ammonium sulfate. Subsequently, an alkaline solution was added to convert the ammonium ions into ammonia, which was then distilled and collected quantitatively. The liberated ammonia was finally titrated to determine the nitrogen content, and the protein content was calculated accordingly.

$$\text{Total Nitrogen (\%)} = \frac{[(A \times (V_A - V_B) \times 1.4007)]}{\text{Weight of sample (g)}} \times 100$$

$$\text{Protein (\%)} = \text{Total Nitrogen (\%)} \times 6.25$$

Where,

V_A = Volume of acid in sample.

V_B = Volume of acid for blank.

A = Normality of hydrochloric acid (HCL).

2.5. Functional Properties

2.5.1. Protein Solubility

Protein solubility of the samples was determined following a modified method described by Jeong, Lee, and Cho (2021). Briefly, 0.2 g of extracted rice protein was dispersed in 200 mL of distilled water and stirred for 20 min to ensure proper hydration. The pH of the mixture was then adjusted to the desired values (pH 5 and pH 9), and the final volume was brought to 250 mL. The dispersion was further agitated for 5 min and subsequently centrifuged at 15,000 × g for 10 to 15 min at 4 °C. The protein content in the resulting supernatant was measured, and protein solubility was calculated as follows:

$$\text{Protein Solubility (\%)} = \frac{[\text{Protein content in the supernatant}]}{\text{Total protein content in sample}} x\ 100$$

2.5.2. Emulsifying Capacity

The ability of the rice protein isolates to form an emulsion was assessed following the procedure outlined by Sha, Koosis, Wang, True, and Xiong (2021). A 1% (w/w) protein solution was prepared and slowly emulsified with soybean oil (200 mL), after which the mixture was homogenized for 5 min. The conductivity of the resulting emulsion was measured using a conductivity/pH meter. The emulsifying capacity was then expressed as the volume of oil emulsified per gram of protein (mL/g), calculated as follows:

$$\text{Emulsifying Capacity (mL/g)} = \frac{V_0}{W_E} x\ 100$$

Where,

V_0 = Total volume of oil added until the endpoint (mL).

W_E = Weight of protein used (g).

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2.5.3. Water- and Oil-Binding Capacities

The water- and oil-binding properties of the purified protein were evaluated by dissolving 1 g of the sample in 10 mL of distilled water or soybean oil in separate 50 mL test tubes. The mixture was vigorously shaken to ensure full contact between the protein and solvent. It was then centrifuged at 15,000 × g for 10–15 min at 4 °C, and the supernatants were removed. The remaining pellets were weighed, and the water- and oil-binding capacities were calculated as percentages, as shown below.

$$\% \text{ Water-Binding Capacity} = \frac{(Weight\ pellet\ (g) - Weight\ of\ dry\ sample\ (g))}{Weight\ of\ dry\ sample\ (g)}\ x\ 100$$

$$\% \text{ Oil-Binding Capacity} = \frac{(Weight\ pellet\ (g) - Weight\ of\ dry\ sample\ (g))}{Weight\ of\ dry\ sample\ (g)}\ x\ 100$$

2.5.4. Foaming Properties

The foaming capacity and foam stability of the extracted protein were estimated using the procedure developed by Cano-Medina et al. (2011). Initially, the pH of the protein suspension was adjusted to pH 7. Next, the protein suspension was rapidly homogenized for 2 min to allow air incorporation and foam formation. The volume of foam formed was measured immediately after homogenization to assess foaming capacity and after 30 min to measure foam stability. Both parameters were calculated as follows:

$$\% \text{ Foaming Capacity} = \frac{(V_0 - V)}{V}\ x\ 100$$

$$\% \text{ Foaming Stability} = \frac{V_{30}}{V_0}\ x\ 100$$

Where,

V= Volume of the solution before homogenization.

V_0= Volume of the solution after homogenization.

V_{30}= Volume of the solution 30 min after homogenization.

2.6. Statistical Analysis

Statistical evaluation of the functional properties of protein isolates from various rice varieties was conducted using the statistical software packages Microsoft Excel and IBM SPSS Statistics, version 26 (IBM Corp., Armonk, NY, USA). The data were presented as means ± standard deviations (SD). Before inferential analysis was conducted, normality was assessed using the Shapiro–Wilk test, while homogeneity of variances was assessed using Levene's test. Data that met these assumptions were subjected to one-way analysis of variance (ANOVA) to determine significant differences ($p < 0.05$) among the groups. Where significant differences were found, Duncan's multiple range test was used to compare specific groups.

3. RESULTS AND DISCUSSION

3.1. Extraction Yield

As shown in Table 1, there were significant differences in protein extraction yield between the three types of rice analyzed. The highest yield was observed in Bario Merah Ranau (93.0%), while the second-highest yield was observed in Long Pasia (92.5%), followed by a significantly lower yield for MRQ 76 (63.0%). The results show that varietal type has a significant effect on protein extractability; Bario Merah Ranau and Long Pasia had significantly higher yields than MRQ 76. Bario Merah Ranau and Long Pasia also showed significantly higher crude extract weights (186.0 g and 185.0 g, respectively).

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Table 1. Extraction yield of protein isolates of MRQ 76, Bario Merah Ranau and Long Pasia.

Rice Varieties	Weight of Crude Extract (g)	Extraction Yield (%)
MRQ 76	126.0	63.0
Bario Merah Ranau	186.0	93.0
Long Pasia	185.0	92.5

Based on the above observation, the extraction of proteins appears to depend on varietal traits under similar extraction conditions. The higher protein yields observed in the Bario Merah Ranau and Long Pasia varieties may be attributed to the ease of solubilization and recovery of proteins from these varieties compared with the MRQ 76 variety. This difference could have resulted from interactions between the proteins and other grain components (Jayaprakash et al., 2022). However, this was not investigated in the current experiment. Nevertheless, the higher protein yields in Bario Merah Ranau and Long Pasia suggest that their proteins were more readily recovered using the extraction technique employed. From a functional perspective, increased extraction efficiency is preferable for food applications.

3.2. Total Protein Contents

It was noted from Table 2 that there were significant differences in the total protein content of the isolated proteins from different rice varieties. For instance, MRQ 76 and Long Pasia had comparable protein contents of 8.14 ± 0.03% and 8.10 ± 0.19%, respectively, with no significant difference ($p > 0.05$). On the other hand, Bario Merah Ranau had a significantly lower protein content of 6.14 ± 0.05%, as shown by the results of a one-way ANOVA ($F = 313.73$, $p = 0.01$).

Table 2. Total protein content of protein isolates from MRQ 76, Bario Merah Ranau and Long Pasia varieties.

Rice Proteins (%)			F-statistics (df)	p-value
MRQ 76	Bario Merah Ranau	Long Pasia		
8.14 ± 0.03 [a]	6.14 ± 0.05 [b]	8.10 ± 0.19 [a]	313.73 (2, 6)	0.01

Note: Data are expressed as mean ± SD (n = 3). Means with different superscript letters (a, b) are significantly different according to Duncan's post hoc test ($p < 0.05$), whereas means sharing the same superscript letter are not significantly different ($p > 0.05$).

Given the marked difference in the protein content between rice protein isolates, it can be deduced that the properties of each variety affected the enrichment of proteins during isolation. The elevated protein content in the MRQ 76 and Long Pasia could have resulted from a high amount of protein in the isolates, while the reduced protein content in the Bario Merah Ranau could be attributed to the presence of other constituents besides proteins in the isolated samples (Nicholas, Hazila, Chua, & Rosniyana, 2014; Xinkang et al., 2023). With regard to functionality, an elevated protein content would be desirable because it offers concentrated proteins, but the protein's functionality will depend on its properties (Ghanghas, MT, Sharma, & Prabhakar, 2022).

3.3. Functional Properties of Protein Isolates

3.3.1. Protein Solubility

In Table 3, the protein solubility of protein isolates from the MRQ 76, Bario Merah Ranau, and Long Pasia rice varieties is presented. In all three varieties, protein solubility was quite low at both pH values, with no significant differences observed ($p > 0.05$). At pH 5, the Bario Merah Ranau variety had the highest solubility at 1.42 ± 0.01%, followed by Long Pasia at 1.28 ± 0.15% and the MRQ 76 variety at 0.97 ± 0.29%. At pH 9, the MRQ 76 variety showed the highest solubility value at 1.69 ± 0.01%, followed by the Bario Merah Ranau variety at 1.55 ± 0.01% and Long Pasia at 1.20 ± 0.01%.

In this case, the high similarity in protein solubility among MRQ 76, Bario Merah Ranau, and Long Pasia implies that the rice varieties affected protein solubility to a lesser extent at the tested pH levels. The low protein solubility

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values obtained at pH levels 5 and 9 reveal that the isolated rice proteins exhibited limited dispersion in aqueous systems, affecting their functionality in food products requiring protein solubility. Protein solubility is one of the techno-functional properties that influence the emulsifying and foaming capacities of proteins (De Angelis, Latrofa, Squeo, Pasqualone, & Summo, 2024). Moreover, the lack of significant differences implies that the isolates showed almost equal solubility regardless of variation among the rice varieties.

Table 3. Protein solubility and emulsifying capacity of protein isolates from MRQ 76, Bario Merah Ranau and Long Pasia varieties.

pH	Protein Solubility (%)		
	MRQ 76	**Bario Merah Ranau**	**Long Pasia**
pH 5	0.97 ± 0.29 [a]	1.42 ± 0.01 [a]	1.28 ± 0.15 [a]
pH 9	1.69 ± 0.01 [a]	1.55 ± 0.01 [a]	1.20 ± 0.01 [a]
	Emulsifying Capacity (mL/g)		
	MRQ 76	Bario Merah Ranau	Long Pasia
pH 4	211.15 ± 4.04 [a]	205.76 ± 11.21 [a]	210.81 ± 4.65 [a]
pH 7	206.90 ± 5.32 [a]	201.90 ± 10.45 [a]	203.53 ± 6.89 [a]

Note: Data are expressed as mean ± SD (n = 3). Means within the same row sharing the same superscript letter (a) are not significantly different according to Duncan's post hoc test ($p > 0.05$).

3.3.2. Emulsifying Capacity

In addition, the emulsifying abilities of protein isolates from the MRQ 76, Bario Merah Ranau, and Long Pasia rice varieties are illustrated in Table 3. All the rice varieties were found to have high and comparable emulsifying abilities at pH 4 and pH 7, with no significant differences ($p > 0.05$). At pH 4, MRQ 76 (211.15 ± 4.04 mL/g) and Long Pasia (210.81 ± 4.65 mL/g) showed slightly higher emulsifying abilities than Bario Merah Ranau (205.76 ± 11.21 mL/g). Similarly, at pH 7, MRQ 76 (206.90 ± 5.32 mL/g) had the highest emulsifying ability, followed by Long Pasia (203.53 ± 6.89 mL/g) and Bario Merah Ranau (201.90 ± 10.45 mL/g).

Relative to one another, the emulsifying abilities of MRQ 76, Bario Merah Ranau, and Long Pasia appear to be consistent with their respective protein solubility at the two pH levels tested. While protein solubility plays a role in making protein molecules available to migrate to and interact with the oil–water interface (Xiao et al., 2025), the lack of notable differences implies that the three isolates have similar emulsifying capabilities. The moderately high emulsifying-capacity values imply that the proteins were capable of effectively adsorbing at the interface and forming emulsions (Zhang et al., 2025). From a techno-functional point of view, the similarities in emulsifying ability imply that the protein isolates have the potential to perform similarly in food products where emulsifying functionality is required.

3.3.3. Water- and Oil-Binding Capacities

Oil-binding capacity (OBC) and water-binding capacity (WBC) of protein isolates obtained from MRQ 76, Bario Merah Ranau, and Long Pasia are shown in Table 4. Significant differences ($p < 0.05$) were found in OBC among the three varieties, with the highest value recorded for MRQ 76 (239.40%), followed by Bario Merah Ranau (226.70%) and Long Pasia (220.63%). On the other hand, WBC varied from 246.87% to 266.00%, but there were no significant differences ($p > 0.05$) among the varieties, although slight increases were observed from MRQ 76 to Long Pasia. It can be concluded that varietal differences had a greater effect on oil-binding capacity than on water-binding capacity.

The mechanism of oil binding seems to be mainly influenced by the presence of hydrophobic amino acid residues and the flexibility of protein molecules, which enable hydrophobic interactions with nonpolar substances (Miedzianka et al., 2023). Although the structural properties of the proteins were not investigated in the current research, the differences between the varieties might have been due to natural differences in their protein composition and structure. In contrast, the absence of differences in WBC values suggests that the three proteins have similar abilities to bind water through hydrogen bonds and polar interactions (Miedzianka et al., 2023; Santos et al., 2025).

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This finding indicates that the hydrophilic nature of the protein isolates was fairly uniform despite variations in the genetic makeup of the rice. The consistently high WBC values across the various genotypes demonstrate that these protein isolates can help retain moisture in foods, while the high OBC value of MRQ 76 may contribute to flavor retention and food texture (Kurek et al., 2022). Ultimately, this will contribute to existing knowledge about underexploited rice proteins and their applications in food products.

Table 4. The OBC, WBC and foaming properties (FC, FS) of protein isolates from MRQ 76, Bario Merah Ranau and Long Pasia varieties.

Functional Property	Oil- and Water-Binding Capacities (%)		
	MRQ 76	**Bario Merah Ranau**	**Long Pasia**
OBC	239.40 ± 4.25[a]	226.70 ± 3.80[b]	220.63 ± 3.45[c]
WBC	246.87 ± 5.10[a]	255.73 ± 4.65[a]	266.0 ± 5.35[a]
	Foaming Properties (%)		
	MRQ 76	Bario Merah Ranau	Long Pasia
FC	11.90 ± 1.25[b]	23.81 ± 2.10[b]	77.78 ± 3.85[a]
FS	88.64 ± 2.75[a]	84.55 ± 3.10[a]	59.79 ± 4.20[b]

Note: Data are expressed as mean ± SD (n = 3). Means within the same row with different superscript letters (a, b, c) are significantly different according to Duncan's post hoc test ($p < 0.05$), whereas means sharing the same superscript letter are not significantly different ($p > 0.05$).

3.3.4. Foaming Properties

Foaming capacity (FC) and foam stability (FS) values for protein isolates from MRQ 76, Bario Merah Ranau, and Long Pasia are tabulated in Table 4. There are statistically significant differences ($p < 0.05$) in these properties among the varieties. The FC value for Long Pasia was much higher (77.78%) than those for MRQ 76 (11.90%) and Bario Merah Ranau (23.81%). On the other hand, the FS values were inversely related to the FC values, with MRQ 76 (88.64%) and Bario Merah Ranau (84.55%) showing significantly higher FS values than Long Pasia (59.79%).

Further, the higher FC of Long Pasia protein isolate may be due to its higher WBC, which would help hydrate the proteins and facilitate their interaction with the air–water interface during the foaming process (Koshland & Haurowitz, 2026), as observed in the previous results. Greater water interaction may promote protein dispersion and hydration, facilitating the movement of protein molecules to the air–water interface during the foaming process (Hollensteiner, Senck, Heupl, & Augat, 2025). Nevertheless, high FC does not imply higher FS, since high flexibility or weak interfacial film strength can cause rapid foam breakdown.

However, MRQ 76 and Bario Merah Ranau showed higher FS values, suggesting greater foam structure stability. The higher OBC value could contribute to stronger protein–interface interactions that lead to foam stability (Zhang et al., 2024). Although more research is needed to determine the specific mechanism behind these observations, the variation in FC and FS indicates that the functional properties of rice protein isolates depend on the rice variety. Therefore, Long Pasia could be utilized when high foaming capacity is needed, whereas MRQ 76 and Bario Merah Ranau could be utilized when foam stability is required.

4. CONCLUSION

A functional assessment of rice protein isolates from the MRQ 76, Bario Merah Ranau, and Long Pasia rice varieties revealed techno-functional differences, even though the isolates had similar levels of protein solubility and emulsifying ability. Protein isolation yields were higher for the Bario Merah Ranau and Long Pasia rice varieties, while protein content was relatively high in the MRQ 76 and Long Pasia varieties. Significant differences in OBC, WBC, foaming ability, and foam stability indicated varietal differences in functional behavior. These results indicate that Malaysian rice protein isolates are suitable for use as functional food ingredients based on their specific technological characteristics. Nonetheless, the present research focused only on the functionality of the rice isolates and did not examine the structural aspects that contribute to differences in functional behavior. Thus, further studies

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should characterize the structure of the protein isolates, including protein fractionation, amino acid composition, molecular weight, and conformation.

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