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Research Paper

Optimizing Protein Extraction from Cowpea (Vigna unguiculata) and Pigeon Pea (Cajanus cajan) via Central Composite Design (CCD)

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Abstract

Plant-based proteins have gained popularity over the last decade due to their sustainability and favorable environmental impact. Proteins from underused legumes can be converted into value-added compounds, helping to sustain protein demand. This study sought to develop a standardized and improved procedure for producing plant protein concentrate from cowpea and pigeon pea. Protein extraction parameters were improved using the Response Surface Methodology with a Central Composite Design (RSM-CCD). The factors optimized were solid-liquid ratio, pH basic, and pH acidic, each varied within ranges defined by the CCD model to evaluate their effect on protein yield and content. The protein extraction processing parameters derived from the optimization trials were used to scale up protein powder production. The upgraded production yield and protein content for cowpea protein powder are 6.77% and 72.6%, respectively, while pigeon pea protein powder yields 6.01% and 63.8%. Statistical analysis confirmed the model adequacy (cowpea: R^2=0.9399 for % protein, R^2=0.9250 for % yield; pigeon pea: R^2 = 0.7836 for % protein, R^2 = 0.7704 for % yield; $p < 0.05$). Improved yield and protein content can enhance the development of low-cost, sustainable, and culturally inclusive (Halal/vegetarian) protein ingredients, supporting both the food industry and nutrition science by providing alternative protein sources that reduce reliance on imported soy and animal proteins. This study demonstrates that RSM-CCD is a robust and efficient approach for optimizing plant protein extraction parameters, offering valuable insights for functional food formulation and industrial-scale protein production.

Keywords: *Plant Protein, Protein Extraction, Cowpea, Pigeon Pea, Legume, Central Composite Design, RSM*

INTRODUCTION

Plant-based foods have gained popularity in recent years due to customer demand for alternatives to animal-derived products. This trend is driven by the heightened understanding of the nutritional benefits of plant-based foods compared to animal-based options, negative perceptions of animal husbandry techniques, and the impact of livestock on our ecosystem (Aimutis, 2022). Consumers are particularly apprehensive of the elevated cholesterol levels in animal-based diets, lactose intolerance, and increasing instances of allergenicity associated with animal protein. The rising popularity of plant-based diets has led to a growing need for protein concentrates and isolates that perform in plant-based formulations and possess characteristics akin to animal proteins. Nevertheless, a limited number of consumers comprehend plant-based diets, as most associate the term with vegetarianism and veganism (Aimutis, 2022).

Food formulators were tired of the cyclical price and supply demands of dairy; therefore, the demand for soy protein isolates preceded that for plant-based ones. Companies that produced dairy protein concentrates and isolates introduced a slew of new and improved protein products at the start of the century. The utilization of intact and hydrolyzed soy, whey, and milk protein concentrates and isolates enabled food formulators to incorporate these products' functional characteristics into recipes for healthful, nutritious, and clean-label foods. People believed that high-protein diets were healthier and more nutritious. As a result, firms are encouraged to

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manufacture protein-rich foods. This has led to fluctuating pricing and quantities of high-protein dairy products, as is typical when supply and demand are at play. Then, food businesses altered their recipes to include soy protein isolates, which served many of the same functions as dairy proteins (Kinsella, 1979). Similarly, this put pressure on the availability and prices of soy proteins, opening up new markets for products created with plant-based protein.

The global need for protein is rising, necessitating the development of novel sources of food protein. Animal proteins are expensive in terms of market price, land requirements, and environmental impact. Furthermore, consumer faith in animal proteins has diminished as a result of food safety concerns over diseases like bovine spongiform encephalopathy and the use of animal hormones (González-Pérez & Arellano, 2009; Lin et al., 2017). Animal protein requires 8-10 times more energy per kilogram than vegetarian protein in industrial settings (González-Pérez & Arellano, 2009; Lin et al., 2017). Furthermore, rising raw material and energy prices are driving the market to produce low-cost, high-quality protein foods. However, to efficiently replace animal proteins, technical advancements are required. Understanding the link between protein structure and functional properties is critical for carrying out these enhancements successfully and efficiently (Sim et al., 2021; Tang et al., 2024).

In the Philippines, the agriculture sector produces a variety of legumes, including underutilized crops such as cowpea and pigeon pea, which are traditionally grown by smallholder farmers. These crops are rich in protein but remain underexploited in food manufacturing, despite their potential to support food security, reduce import dependency on soy, and cater to culturally inclusive markets such as Halal and vegetarian consumers. Moreover, Islam generally permits Muslims to consume any kind of plant food as long as it doesn't hurt people. Al-asl fi al-ashya' al-ibahah (Permissibility is the original state of things) and al-asl fi al-at'imah al-hill (Halal is the original state of all foods) are the Islamic legal maxims upon which this principle is based. These two maxims stem from the following statements made by Prophet Muhammad (PBUH): "Whatever God has made halal is halal, and whatever that He made haram is haram, and whatever concerning which He has remained silent is forgiven" (Quran, 45:13) and God's statement in Chapter 45 (sura al-Jathiyah), verse 13: "And He has subjected to you whatever is in the heavens and whatever is on the earth." In the Prophet's words, "forgiveness" means "it is permitted until proven prohibited." Accordingly, Muslim scholars concur that genetically modified food may be certified halal provided it poses no risk to humans and it is not derived from haram (illegal) animals or human genes (Hamdan et al., 2023).

Despite the global and local potential, there is limited research on optimizing protein extraction from underutilized legumes such as cowpea and pigeon pea using Response Surface Methodology (RSM) with a Central Composite Design (CCD). Previous studies on legume protein extraction often focus on soybean or other more commercially exploited legumes, with fewer addressing the process optimization of these crops for improved yield and quality (Mune Mune et al., 2008; Pazmino et al., 2018; Klupšaitė & Juodeikienė, 2015; Onyango, 2022). This gap limits the ability of the food industry to adopt these legumes as competitive protein sources in functional foods.

Therefore, this study aims to optimize protein extraction conditions for cowpea and pigeon pea using RSM-CCD to enhance yield and protein content for food applications. The practical significance lies in contributing to the development of low-cost, sustainable plant protein ingredients for the food and nutrition sector, with potential applications in addressing protein-energy malnutrition, supporting local farmers, and promoting culturally inclusive diets.

RESEARCH METHOD

Material Source

Cowpea and pigeon pea were acquired from the Pangasinan Organic Seed Growers and Nursery Multi-Purpose Cooperative with the assistance of the Bureau of Plant Industry, Pangasinan. These legumes were chosen due to their local availability, underutilization in industrial food applications, and high protein content, making them suitable candidates for sustainable protein development in the Philippine agricultural sector.

Laboratory Reagent Acquisition. Sodium hydroxide pellets (Merck) and hydrochloric acid (Lab Scan) were purchased from Belman Laboratories. Deionized water was purchased from Alyson's Chemical. All reagents used were of analytical grade to ensure consistency and reproducibility in the extraction process.

Study Design and Approach

The research employed a quantitative experimental design focused on optimizing protein extraction parameters through multivariate analysis. Response Surface Methodology with Central Composite Design (RSM-CCD) was selected due to its ability to evaluate the interactive effects of multiple factors on efficiency while minimizing the number of experimental runs required (Lin et al., 2021). A total of 20 runs per legume type were generated by Design Expert software (Version 12.0, Stat-Ease Inc., Minneapolis, MN, USA), incorporating factorial points, axial points, and center points to fit a second-order polynomial model. All extraction trials were performed in triplicate to ensure reproducibility, and results were reported as mean ± standard deviation.

Sample Preparation

Cowpea and pigeon pea flour were processed according to the modified method of Ojukwu et al. (2012). Pre-weighed cowpea and pigeon flour were soaked in a 1:2 solid-to-water ratio for 24 hours. After soaking, the legumes were filtered and rinsed three times with running water. After rinsing, the legumes were soaked in pre-boiled water for 30 minutes to inactivate the trypsin inhibitor enzymes, followed by another rinsing with running water. The legumes were then dried for 16 hrs. at 60°C using a cabinet dryer and afterward let cool to room temperature. After drying, the legumes were ground into flour using a hammer mill, weighed, and packed in a foil-laminated pouch.

Optimization of protein extraction parameters

Design Expert software was used to generate the CCD model to determine the optimum extraction. The three independent variables (factors) evaluated were: solid-liquid ratio (X1), mass of legume flour to extraction water volume; pH (X2), adjusted using NaOH to reach target alkalinity levels; and pH acidic (X3), adjusted using HCl to precipitate proteins near their isoelectric point. The response variables were % protein content and % yield of the extracted protein concentrate. The CCD allowed for estimation of linear, quadratic, and interaction effects between the factors, and the optimal parameter set was identified based on desirability criteria for maximizing both yield and protein content.

Protein Extraction Procedure

Cowpea and pigeon pea proteins were extracted using the alkali extraction technique (Arun & Kaul, 2013; Shevkani et al., 2015). Legume flour was mixed with the targeted solid-liquid ratio of deionized water and stirred until fully suspended. The mixture was then alkalized by adding 1M NaOH until the targeted pH was reached, and stirred constantly for 30 minutes. After stirring, the solution was centrifuged at 4000 rpm at 25°C for 5 minutes. After centrifugation, the protein-rich supernatant was collected, and its pH was adjusted to isoelectric pH levels using 1M HCl. This induced protein precipitation, which was followed by a second centrifugation at 5000 rpm for 5 minutes. The precipitate was rinsed with deionized water three times, neutralized, and spray-dried at 150°C. The protein powder produced was collected, weighed, and stored.

Analytical Methods

Protein content was determined using the Bradford assay with bovine serum albumin (BSA) as the standard (Bradford, 1976). This method was selected due to its sensitivity, rapid execution, and minimal interference from non-protein components in plant extracts. Absorbance was measured at 595 nm using a UV-Vis spectrophotometer, and results were expressed as percentage protein on a dry basis. The percentage yield (% Yield) was calculated as:

$$\%Yield_{db}=(\text{Dry Weight of Raw Material})/(\text{Dry Weight of Product})\times 100$$

Statistical Analysis

Model adequacy was assessed using analysis of variance (ANOVA), with significance accepted at $p<0.05$. Key indicators included coefficient of determination (R^2), adjusted R^2, predicted R^2, lack-of-fit tests, and Adequate Precision values, which indicated the model's signal-to-noise ratio. A difference of less than 0.2 between adjusted and predicted R^2 values was considered acceptable, ensuring that the model predictions aligned closely with the observed data.

RESULTS AND DISCUSSION

Optimization of Protein Extraction Parameters

For cowpea, Run 3 had the highest protein content (54.41%), and Run 9 had the highest percent (%) yield (16.13%), as seen in Table 1. Table 2 displays the Quadratic (% protein) and reduced Quadratic (% yield) model ANOVA and fit statistics results for % protein and % yield at a $p<0.05$ significance level. For the % yield response, a reduced quadratic model was chosen as it provided the best model and diagnostic criteria. The solid-liquid ratio (A), pH basic (B), the interaction of pH Basic and Acidic (BC), and pH acidic^2 (C^2) all had a significant effect on the percentage of protein. On the other hand, pH acidic (C) and C^2 had a significant effect on the % yield. Since the lack of fit F-value is not significant ($p<0.05$), both response models fit well. Furthermore, the difference between the predicted and adjusted R^2 is less than 0.2, indicating reasonable agreement between model predictions and experimental data. Adequate precision values were greater than four (4) in both responses, indicating desirable signal-to-noise ratios.

Among the fifty-one (51) solutions generated, one (1) was selected, with a yield % of 10.42 and a protein content of 44.41%. The optimum extraction parameters were 1:14.49 solid-liquid ratio, pH basic of 10, and pH acid of 3.42, with a desirability of 0.617 (Table 3). Protein content was estimated using the Bradford assay, and yield was computed on a dry basis.

Table 1. Generated a design for the protein extraction optimization of cowpea

Block	Run	A: Solid: liquid ratio	B: pH Basic	C: pH Acidic	% Protein	%Yield
Day 1	1	17.5	9	4.5	37.9606	12.8993
Day 1	2	17.5	9	4.5	30.5692	14.7062
Day 1	3	25	10	3	54.4092	5.18695
Day 1	4	10	10	6	32.1737	5.25945
Day 1	5	17.5	9	4.5	31.9502	14.0738

Day 1	6	25	8	6	47.2649	2.13679
Day 1	7	10	8	3	42.3849	6.29806
Day 2	8	25	10	6	39.2517	1.26838
Day 2	9	17.5	9	4.5	27.0757	16.4917
Day 2	10	10	8	6	31.7826	6.98183
Day 2	11	10	10	3	50.3224	8.00203
Day 2	12	25	8	3	46.3288	7.81357
Day 2	13	17.5	9	4.5	32.965	12.959
Day 2	14	17.5	9	4.5	33.8934	14.0714
Day 3	15	25	9	4.5	32.0849	15.5788
Day 3	16	17.5	8	4.5	30.7141	14.2505
Day 3	17	17.5	9	4.5	29.4687	16.1275
Day 3	18	17.5	9	4.5	33.0836	14.3754
Day 3	19	17.5	10	4.5	31.6282	13.9992
Day 3	20	17.5	9	3	47.533	2.7314
Day 3	21	17.5	9	6	35.7718	2.64469
Day 3	22	17.5	9	4.5	30.9102	15.7606
Day 3	23	10	9	4.5	27.7396	15.0312

Table 2. Quadratic (% Protein) and reduced quadratic (% Yield) model ANOVA and fit statistics for the % protein and % yield response of cowpea protein extraction

Source	% Protein	% Yield
Model	< 0.0001[a]	< 0.0001[a]
A-Solid:liquid ratio	0.0011[a]	0.0789[b]
B-pH Basic	0.2696[b]	0.4719[b]
C-pH Acidic	< 0.0001[a]	0.0354[a]
AB	0.2732[b]	-
AC	0.0674[b]	-
BC	0.0071[a]	-
A^2	0.5975[b]	-
B^2	0.7844[b]	-
C^2	< 0.0001[a]	< 0.0001[a]
Lack of Fit	0.9870[b]	0.2179[b]
Standard Deviation	2.53	1.62
Mean	36.40	10.38
C.V. %	6.96	15.57
R^2	0.9399	0.9250
Adjusted R^2	0.8908	0.9063
Predicted R^2	0.8372	0.8247
Adequate Precision	15.0230	14.3607

Table 3. Predicted solutions given by the software for the cowpea protein extraction

Number	Solid:liquid ratio	pH Basic	pH Acidic	% Protein	Yield %	Desirability
1	**14.490**	**10.000**	**3.419**	**44.427**	**10.411**	**0.617**
2	14.478	10.000	3.419	44.426	10.412	0.617

3	14.553	10.000	3.421	44.406	10.423	0.617
4	14.424	10.000	3.418	44.434	10.408	0.617
5	14.673	10.000	3.422	44.423	10.414	0.617

From the biochemical perspective, the high protein extraction at pH 10 can be attributed to the increased solubility of plant storage proteins in alkaline conditions. At higher pH, protein molecules carry a net negative charge, resulting in electrostatic repulsion that enhances solubilization (Yoshida & Prudencio, 2020). Conversely, the precipitation step at an acidic pH near the isoelectric point (pH 3.42 in this study) reduces solubility by neutralizing net charges, facilitating protein aggregation and recovery (Mwasaru et al., 1999). This two-step pH shift is widely recognized as an effective strategy for legume protein isolation and is consistent with findings in chickpea and mung bean extraction studies (Nedumaran et al., 2015).

For the pigeon pea, *Run 6* had the highest protein content (32.1961%), and *Run 17* had the highest yield (8.8173%) (Table 4). Table 5 shows the ANOVA and fit statistics model for the percentage protein and yield response at the $p<0.05$ significance level. The reduced quadratic model was selected for both responses due to superior diagnostic criteria. Significant effects on the % protein included solid-liquid ratio (A), the interaction of the solid-liquid ratio and pH acid (AC), and pH acid^2 (C^2). For % yield, pH basic (B), pH acidic (C), and pH acidic^2 (C^2) were significant. The lack of fit was not significant ($p>0.05$), indicating model suitability.

Among the generated solutions, the highest desirability (0.781) was achieved with a yield of 8.451% and protein content of 28.619%, using parameters of 1:22.45 solid-liquid ratio, pH basic of 10, and pH acidic of 4.04 (Table 6).

The lower extraction efficiency in pigeon pea compared to cowpea could be due to differences in seed coat composition, protein structure, and endogenous anti-nutritional factors. Pigeon pea contains higher levels of tannins and phytates, which can bind to proteins and limit solubility (Mizubuti et al., 2000). Additionally, the absence of a defatting step in this study likely reduced overall protein purity and recovery, as residual lipids can hinder protein precipitation (Onyango, 2022). Defatting has been shown to improve protein recovery in legumes (Russin et al., 2011), suggesting a potential area for process enhancement in future work. The summary of optimized parameters used with reference to the data generated by the software is shown in Table 7. % Protein and %Yield were computed similarly to the optimization of cowpea.

Table 4. Generated a design for the protein extraction optimization of pigeon pea

Block	Run	A: Solid: liquid ratio	B: pH Basic	C: pH Acidic	% Protein	Yield %
Day 1	1	17.5	9	4.5	22.5612	8.48032
Day 1	2	17.5	9	4.5	27.9628	7.35656
Day 1	3	25	10	3	27.3653	7.17666
Day 1	4	10	10	6	23.9589	5.30114
Day 1	5	17.5	9	4.5	26.4543	8.12573
Day 1	6	25	8	6	32.1961	2.09655
Day 1	7	10	8	3	26.5592	6.49408
Day 2	8	25	10	6	31.241	5.80132
Day 2	9	17.5	9	4.5	25.4108	7.17147
Day 2	10	10	8	6	22.0958	5.1872
Day 2	11	10	10	3	29.4598	7.96851
Day 2	12	25	8	3	28.6086	6.66699
Day 2	13	17.5	9	4.5	27.4675	8.28091

Day 2	14	17.5	9	4.5	25.5199	7.74705
Day 3	15	25	9	4.5	30.1369	8.41654
Day 3	16	17.5	8	4.5	26.8648	6.44668
Day 3	17	17.5	9	4.5	28.5224	8.8173
Day 3	18	17.5	9	4.5	27.9073	7.2081
Day 3	19	17.5	10	4.5	30.5704	7.97866
Day 3	20	17.5	9	3	30.6258	5.95544
Day 3	21	17.5	9	6	29.7585	5.66068
Day 3	22	17.5	9	4.5	28.979	6.73972
Day 3	23	10	9	4.5	25.7389	8.62664

Table 5. Reduced quadratic model ANOVA and fit statistics for the % protein and % yield response of pigeon pea protein extraction

Source	% Protein	% Yield
Model	0.0001[a]	< 0.0001[a]
A-Solid: liquid ratio	0.0002[a]	0.2104[b]
B-pH Basic	0.1768[b]	0.0129[a]
C-pH Acidic	0.4582[b]	0.0013[a]
AC	0.0005[a]	-
C^2	0.0119[a]	< 0.0001[a]
Lack of Fit	0.9098[b]	0.4267[b]
Standard Deviation	1.40	0.8288
Mean	27.65	6.94
C.V. %	5.06	11.94
R^2	0.7836	0.7704
Adjusted R^2	0.7114	0.7130
Predicted R^2	0.4104	0.4994
Adequate Precision	12.0683	10.9660

Values with a ([a]) indicate significant model terms at 0.05 level
b ([b]) indicates no significance

Table 6. Predicted solutions given by the software for the pigeon pea protein extraction

Solution Number	Solid: liquid ratio	pH Basic	pH Acidic	% Protein	Yield %	Desirability
39	13.301	10.000	3.000	29.486	7.766	0.786
40	14.121	10.000	3.000	29.486	7.729	0.783
41	**22.449**	**10.000**	**4.044**	**28.619**	**8.451**	**0.781**
42	25.000	9.547	5.059	30.177	7.219	0.781
43	14.850	10.000	3.000	29.486	7.695	0.781

Table 7. Optimized parameters for the production of cowpea and pigeon pea powder concentrate

Protein powder	pH basic	Solid: Liquid Ratio	pH acidic
Cowpea	10	1:15	4
Pigeon Pea	10	1:22	4

Proximate analysis of protein concentrates

Table 8 presents the proximate composition of cowpea and pigeon pea protein concentrates. No significant differences ($p>0.05$) were observed in moisture, fat, or ash content, but protein and carbohydrate contents differed significantly ($p<0.05$). The protein content of cowpea and pigeon pea is 72.6% and 63.8% respectively, classifying them as protein concentrates (Codex Standard 175-1989).

These values are lower than those reported by Mune Mune et al. (2008) for cowpea (84.0%) and Pazmiño et al. (2018) for pigeon pea (76.41%). The difference is likely due to variations in processing methods, particularly the omission of defatting and dehulling in the present study. The high protein content in these legumes underscores their potential as alternative protein sources not only for regions experiencing a scarcity of meat protein but also for populations that adhere to Halal dietary practices.

In the context of food formulation, protein concentrates from cowpea and pigeon pea have potential in bakery products, meat analogs, and nutritional supplements. Their integration into food products could improve nutritional profiles while respecting cultural and religious dietary requirements. Their amino acid profile, emulsifying capacity, and water-binding properties (reported in previous literature) could support functional roles similar to soy and pea proteins in industrial applications (Dinali et al., 2025; Syed et al., 2022). Further exploration of their functional properties and bioavailability could enhance their use in food technology and nutrition, particularly in Halal markets.

Table 8. Proximate analysis of cowpea and pigeon pea concentrate

Protein concentrate	Moisture	Protein	Fat	Ash	Carbohydrates
Cowpea	7.81	72.6	5.92	4.81	8.82
Pigeon Pea	8.51	63.8	5.59	4.57	17.5

CONCLUSION

The research study successfully achieved its objectives by developing processes for producing plant proteins from local sources, focusing on underutilized local legumes such as cowpea and pigeon pea. Pre-treatment, extraction, and recovery methods were established and optimized using Response Surface Methodology with Central Composite Design (RSM-CCD) to enhance efficiency and output. The statistical results confirmed the adequacy and robustness of the models, with high R^2 values and non-significant lack-of-fit tests, validating the reliability of the optimization approach.

From a theoretical perspective, this study expands the body of knowledge on legume protein extraction methods by demonstrating the applicability of RSM-CCD in optimizing multivariable parameters for underutilized legumes. It provides empirical evidence that pH-controlled extraction combined with carefully selected solid-liquid ratios can significantly improve protein yield and content. These findings contribute to plant biochemistry and food process engineering literature by reinforcing the role of alkaline solubilization-acid precipitation as an effective extraction strategy, particularly when adapted to the physicochemical characteristics of specific legumes.

From a practical standpoint, the optimized process offers a scalable, cost-effective approach to producing plant protein concentrates from locally grown cowpea and pigeon pea. The improved protein yields (72.6 for cowpea and 63.8% for pigeon pea) demonstrate that these crops can serve as viable alternatives to imported soy protein, thereby reducing dependency on foreign supply chains. This can directly support the Philippine food industry in developing sustainable, culturally inclusive (Halal/vegetarian) food products, while also addressing protein-energy malnutrition in vulnerable populations. The integration of these protein ingredients into functional foods, mead analogs, and fortified products could help promote healthier diets and diversify the protein sources available to consumers.

Furthermore, the use of underutilized legumes aligns with sustainable food systems goals by promoting biodiversity, supporting smallholder farmers, and reducing the environmental foodprint associated with high-resource animal protein production. The approach is adaptable and could be extended to other regionally abundant but commercially neglected legumes, enhancing global plant protein availability.

Taken together, this study demonstrates that combining modern optimization tools with traditional plant protein extraction methods can yield technically robust, economically viable, and socially relevant food ingredients. Future research should focus on incorporating pre-processing steps such as defatting and dehulling to further enhance protein recovery and functional properties, as well as investigating sensory characteristics and consumer acceptability of formulated products containing these protein concentrates.

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