Dry Fractionation of Pulses Using Air Classification for Protein-Enriched Fractions and Development of Low Moisture Extruded Functional Foods

A THESIS SUBMITTED TO

THE UNIVERSITY OF TRANS-DISCIPLINARY HEALTH SCIENCES AND TECHNOLOGY

FOR THE AWARD OF THE DEGREE OF

M. SC. BY RESEARCH (FOOD SCIENCE TECHNOLOGY AND INNOVATION)

BY
MANOJ. S

UNDER THE GUIDANCE OF

DR. GURMEET SINGH,

THE UNIVERSITY OF TRANS-DISCIPLINARY HEALTH SCIENCES AND TECHNOLOGY, 74/2, JARAKABANDE KAVAL, POST-ATTUR, YELAHANKA, BANGALORE - 560064

THE UNIVERSITY OF TRANS-DISCIPLINARY HEALTH SCIENCES AND TECHNOLOGY

Private University Established in Karnataka by ACT 35 of 2013

BENGALURU - 560064

DECLARATION BY THE CANDIDATE

I declare that this thesis entitled **"Dry Fractionation of Pulses Using Air Classification for Protein-Enriched Fractions and Development of Low Moisture Extruded Functional Foods"** submitted for the award of Master of Science by Research to THE UNIVERSITY OF TRANS-DISCIPLINARY HEALTH SCIENCES AND TECHNOLOGY, Bengaluru, is my original work, conducted under the supervision of Dr. Gurmeet Singh (Guide). I also wish to inform that no part of the research has been submitted for a degree or examination at any other University. The references, help and material obtained from other sources have been duly acknowledged

I hereby confirm the originality of the work and that there is no plagiarism in any part of the dissertation.

Place: Bengaluru

Signature of the Candidate

Date:31/03/2026

Name of candidate: Manoj. S

Reg. No.: 2024MSCR01

THE UNIVERSITY OF TRANS-DISCIPLINARY HEALTH SCIENCES AND TECHNOLOGY

Private University Established in Karnataka by ACT 35 of 2013

BENGALURU - 560064

CERTIFICATE

This is to certify that the work incorporated in this thesis "**Dry Fractionation of Pulses Using Air Classification for Protein-Enriched Fractions and Development of Low Moisture Extruded Functional Foods**" submitted by **Manoj. S** was carried out under my supervision. No part of this thesis has been submitted for a degree or examination at any university. References, help and material obtained from other sources have been duly acknowledged. I hereby confirm the originality of the work and that there is no plagiarism in any part of the dissertation.

Research Supervisor

Dr. Gurmeet Singh,

Professor and Dean, Research and Outreach,

TDU, Bangalore.

REPORT OF PLAGIARISM

food science

ORIGINALITY REPORT

5%	3%	4%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.mdpi.com Internet Source	1%
2	Yang, Qiuhuizi. "Functionalities of mildly extracted mung bean proteins: From mung bean seeds to a highly functional plant-Based protein ingredient", Wageningen University and Research, 2023 Publication	<1%
3	pmc.ncbi.nlm.nih.gov Internet Source	<1%
4	Otoo, Evans Nii Okanta. "Developing New Formulations of Ready-to-Use Therapeutic Food (RUTF).", Delaware State University Publication	<1%
5	Rashmi Singh, Vijay Singh Sharanagat. "Physico-functional and structural characterization of ultrasonic-assisted chemically modified elephant foot yam starch", International Journal of Biological Macromolecules, 2020 Publication	<1%
6	Xing, Qinhui. "Dry Fractionation and Bioprocessing for Novel Legume Ingredients", Wageningen University and Research, 2021 Publication	<1%
7	www.tdx.cat Internet Source	<1%

TABLE OF CONTENTS

1. Chapter 1: Introduction 1

1.1. Background and justification 1

1.2. Air classification technology and innovation 5

1.3. Problem statement 7

1.4. Research objective and hypothesis 8

2. Chapter 2: Literature review 9

2.1. Introduction 9

2.2. Pulse protein composition and nutritional properties 9

2.2.1. General pulse protein characteristics 9

2.2.2. Vigna mungo (Black gram) nutritional profile 9

2.2.3. Vigna radiata (Green gram) nutritional profile 10

2.3. Traditional pulse protein extraction technology 10

2.3.1. Wet fraction methods 10

2.3.2. Alternative wet processing methods 11

2.3.3. Hybrid processing approaches 12

2.4. Dry fractionation and air classification technology 12

2.4.1. Principles and mechanism of dry fractionation 12

2.4.2. Air classification technology 13

2.4.3. Process parameters and optimization 14

2.5. Functional properties of pulse proteins 14

2.5.1. Water and oil absorption properties 14

2.5.2. Foaming properties 15

2.6. Extrusion processing of plant proteins 16

2.6.1. Low moisture extrusion technology 16

2.6.2. Protein behaviour during Extrusion 16

2.6.3. Formulation consideration 17

2.7. Product development applications 18

2.8. Research gaps and forthcoming directions 20

3. Chapter 3: Materials and methods 23

3.1. Raw materials 23

3.2. Dry fractionation process 24

3.3. Analytical methods 25

3.4. Extrusion processing 27

3.5. Product characterization 28

4. Chapter 4: Results and discussions 29

4.1. Raw materials characterization 29

4.2. Milling optimization 29

4.3. Air classification optimization-*Vigna mungo* (Black gram) 33

4.4. Air classification optimization-*Vigna radiata* (Green gram) 41

4.5. Extrusion technology 50

4.6. Physico-chemical analysis of air classification samples 55

4.7. Physico-chemical analysis of extrusion samples 59

4.8. Functional analysis of extrusion samples 63

5. Chapter 5: Conclusion and Reference 67

LIST OF TABLES

Table 4.1: Black gram Milling at Different RPM (Internal Classifier @ 2000 RPM, Airflow 328 CMH)

Table 4.2: Black gram Dal Milling at Different RPM (Internal Classifier @ 6000 RPM, Airflow 328 CMH)

Table 4.3: Black gram Dal Milling at 8000 Mill RPM and 6000 Internal Classifier Speed at Different Airflow Rates

Table 4.4: Fine Fraction Particle Sizes and Protein Content at Different Classifier RPM and Airflow Rates

Table 4.5 Selected the fine fractions where yield is more than 150 grams and did protein estimation.

Table 4.6: Fine Fraction Results - Optimized Processing (8000 Mill RPM, 6000 Internal Classifier)

Table 4.7: Coarse Fraction Results - Optimized Processing (8000 Mill RPM, 6000 Internal Clas Table 4.8: Pilot-Scale Black gram Processing Results (50 kg batch)

Table 4.9: Green gram Dal Milling Results (8000 Mill RPM, 8000 Internal Classifier RPM at 129,210, 286,328 CMH)

Table 4.10: Green gram Milling at Different Airflow Rates (8000 Mill RPM, 6000 Internal Classifier)

Table 4.11: Green gram Fine fractions result of selected 8000mill rpm and 6000 internal classifier RPm at air flow of 210 CMH and classified at different classifier rpm at different Air flows.

Table 4.12: Coarse fractions result of selected 8000mill rpm and 6000 internal classifier RPM at air flow of 210 CMH and classified at different classifier rpm at different air flows.

Table 4.13: Pilot-Scale Green gram Processing Results (60 kg batch)

Table 4.14: summarizes key extrusion parameters for each blend, highlighting trends in torque, melt temperature, melt pressure, expansion size, and expansion ratio.

Table 4.15: Colorimetric (L, a, b*) and Total Color Difference (ΔE) Values of Pulse Fractions

Table 4.16: Table: Functional Properties of Pulse Fractions and Flours (Water/Oil Absorption, Foaming Capacity, and Foaming Stability)

Table 4.17: Ash, Protein, and Moisture Profiles of Air-Classified Fractions and Whole-Flour Samples

4.18: Particle Size Distribution (D10, D50, D90) of Pulse Fractions and Defatted Soya Flour

4.19: *Colorimetric Parameters (L, a*, b*, ΔE) of Green Gram–Based Blend Formulations.

4.20: Functional Properties (Water and Oil Absorption) of extruded samples of Green Gram–Based Blend Formulations.

4.21: Foaming Properties of extruded samples of Defatted Soy Flour and Its Blends with Black Gram and Green Gram.

4.22: Proximate Composition of extruded samples of Defatted Soy Flour and Its Blends with Black Gram and Green Gram

4.23: Particle Size Distribution (D10, D50, D90) and Mean Particle Size of DSF–Pulse Flour Blends.

4.24: *Colorimetric Parameters (L, a*, b*, ΔE) of green gram extruded

products

Table 4.25: Bulk Density, Hydration Capacity, and Expansion Characteristics of DSF–Based Formulations

Table 4.26: Texture Profile Analysis of extruded DSF, Black Gram, and Green Gram Blends

LIST OF GRAPHS

Graph 1. Size distribution (volume weighted vs Particle diameter(µm) for black gram at different milling RPMs (2000 RPM internal classifier)

Graph 2. Size distribution (volume weighted) vs Particle diameter(µm) for Black gram at different milling RPMs (6000 RPM internal classifier)

Graph 3. Size distribution (volume weighted) of D50 vs different milling RPMs (2000 and 6000 RPM of internal classifier)

Graph 4. Size distribution (volume weighted) of D50 vs different airflow (8000 milling RPM and 6000 RPM of internal classifier)

Graph 5. Particle size distribution (volume weighted) vs different airflow (8000 milling RPM and 6000 RPM of internal classifier)

Graph 6. Particle size distribution (volume weighted) of D50 vs classifier RPM (at three different classifier RPM and airflow) of fine fractions

Graph 7. Particle size distribution (volume weighted) of D50 vs classifier RPM (at three different classifier RPM and airflow) of fine fractions

Graph 8. Particle size distribution (volume weighted) of D50 vs classifier RPM (at three different classifier RPM and airflow) of coarse fractions

Graph 9. Undersize distribution (volume weighted) vs particle diameter at 9000 classifier RPM at 286CMH of fine and coarse fractions

Graph 10. Particle size distribution (volume weighted) vs particle diameter at 9000 classifier RPM at 286CMH of whole flour, fine and coarse fractions

Graph 11. Protein content in percentage of whole flour, fine fraction and coarse fraction.

Graph 12. Particle size distribution (volume weighted) vs particle diameter (8000 milling RPM and 8000 internal classifier RPM) at 328, 286, 210, 129 CMH of whole flour

Graph 13. Particle size distribution (volume weighted) vs particle diameter (8000 milling RPM and 6000 internal classifier RPM) at 328, 286, 210, 129 CMH of whole flour

Graph 14. Particle size distribution (volume weighted) of D50 vs Classifier RPM (8000 milling RPM and 6000 internal classifier RPM) at 328, 286, 210CMH of fine fractions

Graph 15. Protein content vs particle diameter (8000 milling RPM and 6000 internal classifier RPM) at 328, 286, 210 CMH of fine fractions

Graph 16. Particle size distribution (volume weighted) of D50 vs Classifier RPM (8000 milling RPM and 6000 internal classifier RPM) at 328, 286, 210CMH of coarse fractions.

Graph 17. Protein content vs particle diameter (8000 milling RPM and 6000 internal classifier RPM) at 328, 286, 210 CMH of coarse fractions

Graph 18. Particle size distribution(volume weighted) vs particle diameter (8000 milling RPM and 6000 internal classifier RPM 210CMH) at 9000 classifier RPM and 286 CMH of whole flour, fine and coarse fractions.

Graph 19. Undersize distribution (volume weighted) vs particle diameter at 9000 classifier RPM at 286CMH of fine and coarse fractions

Graph 20. Protein content vs samples (whole flour, fine and coarse fractions)

LIST OF FIGURES

Figure 1. Black gram whole flour

Figure 2. Black gram fine fractions

Figure 3. Black gram coarse fractions

Figure 4. Green gram whole flour

Figure 5. Green gram fine fractions

Figure 6. Green gram coarse fractions

Figure 7. Feeder, water dosing and sensors positioning

Figure 8. Extrusion process profile, showing time-resolved pressure, temperature, and speed Black gram fractions process profile.

Figure 9. Extrusion process profile, showing time-resolved pressure, temperature, and speed green gram fractions process profile

Figure 10. Low moisture extruded products of blends

ABBREVIATIONS

g - Gram

Kg - Kilogram

RPM - Rotation per minute

CMH - Cubic meter per hour

(µm) - Micro meter

Kg/hr - Kilogram per hour

Wt. - Weight

DSF - Defatted soya flour

URD - Black gram dal

Green gram - Green gram dal

mm - Millimetre

D10: Particle size below which 10% of the distribution lies

D50: Median particle size (50th percentile)

D90: Particle size below which 90% of the distribution lies

ABSTRACT

The growing demand for sustainable and clean-label plant proteins has increased interest in dry fractionation as an alternative to conventional wet extraction. This study optimized an integrated dry fractionation process combining high-speed impact milling and air classification to produce clean-label, chemical-free protein-enriched fractions from black gram (*Vigna mungo*) and green gram (*Vigna radiata*) under water-free conditions at temperatures below 40 °C. The effects of milling and air-classification parameters on particle size distribution and protein enrichment were systematically evaluated, revealing that mill speed primarily influenced coarse particle size represented by D90, while airflow significantly affected D10, D50, and D90, with internal classifier wheel speed ranging from 2,000 to 8,000 RPM showing minimal independent impact. Optimized conditions enabled recovery of fine fractions with sub-20 µm D90 and effective protein–starch separation, increasing protein content from 24.81% to 45.79–46.00% in black gram, corresponding to an enrichment of 84.6% and from 24.60% to 49.16% in green gram, corresponding to an enrichment of 87%. The fine fractions exhibited small median particle sizes, with D50 values ranging from 9.59 to 10.35 micrometres, high foaming capacity approximately 90%, and enhanced water absorption capacity ranging from 3.37 to 3.71 grams per grams. Protein-enriched fractions were incorporated into low-moisture extruded products using a twin-screw extruder at 22% feed moisture, 280 RPM screw speed, and a temperature profile of zones varies from 50–125°C. Increasing levels of pulse protein fractions substitution resulted in a reduction in torque from 28.6 Nm to values between 20 and 22.9 Nm, as well as a decrease in melt pressure from 135 bar to between 77 and 90 bar, indicating changes in melt rheology. The expansion ratio decreased from 2 to 1.4, while bulk density increased, producing denser yet structurally stable extrudates. A marked reduction in hardness from 308 N to values between 60 and 75 N demonstrated improved textural acceptability. Overall, the integration of dry fractionation and extrusion successfully produced clean-label, protein-dense functional foods without chemical processing. This research establishes a scalable and environmentally sustainable approach for developing indigenous plant-protein ingredients and high-protein extruded snacks, supporting the growing demand for nutritionally enhanced, plant-based foods.

Keywords:

Dry fractionation, Air classification, Protein enrichment, Pulses, Black gram, green gram, Milling optimization, Particle size distribution, Functional properties, Extrusion, Low-moisture extrudates, Protein concentrates, Clean-label ingredients, Plant-based foods, Sustainable processing, Nutritional enhancement

CHAPTER 1: INTRODUCTION

1.1. Background and Justification

1.1.1. Global Protein Demand and Sustainability Challenges

The global food system faces unprecedented challenges as the world population approaches 8 billion people and is projected to reach 9.7 billion by 2050. This demographic growth, coupled with rising incomes in developing nations, has created an exponential increase in protein demand worldwide. Current protein demand for the global population of 7.3 billion inhabitants is approximately 202 million tonnes globally, with projections indicating that global demand for animal-derived protein will double by 2050 (Henchion et al., 2017). Within this expanding market, plant-based proteins represent a particularly dynamic segment due to growing consumer awareness of sustainability and health benefits (Alcorta et al., 2021; Boukid, 2021).

The traditional reliance on animal agriculture to meet growing protein demands presents significant environmental and sustainability concerns. Animal agriculture contributes substantially to greenhouse gas emissions, with livestock production accounting for approximately 18% of global greenhouse gas emissions according to FAO estimates, though some studies suggest the impact may be as high as 51% (Steinfeld et al., 2006; Goodland & Anhang, 2009). The environmental footprint of animal protein production is particularly evident when examining resource consumption: livestock production utilizes 70% of agricultural land while providing only 20% of global food energy, and generates over 70% of food-related greenhouse gas emissions (Steinfeld et al., 2006; Gerber et al., 2013).

Pulses, including peas, lentils, chickpeas, and beans, have emerged as critical components in addressing global protein security while maintaining environmental sustainability (Boye et al., 2010; Alcorta et al., 2021). These crops offer multiple advantages: they can fix atmospheric nitrogen, reducing dependence on synthetic fertilizers; they require less water than animal proteins; and they provide complete nutritional profiles with high protein content ranging from 20-30% in whole form. However, realizing the full potential of pulses requires advanced processing technologies to overcome inherent limitations in protein concentration and functional properties.

1.1.2. Limitations of Current Pulse Processing Methods

Traditional pulse processing methods, particularly wet fractionation, have dominated commercial protein extraction for decades. Wet fractionation typically involves alkaline extraction followed by isoelectric precipitation, producing protein isolates with high purity (>85% protein content). However, this approach presents several significant limitations that constrain its scalability and sustainability (Rivera et al., 2024; Boukid, 2021).

1.1.2.1. Resource Intensity and Environmental Impact

Wet fractionation is extremely resource-intensive, requiring substantial quantities of water, chemicals, and energy. Research demonstrates that producing plant protein isolates through wet methods consumes significant resources and energy, with energy requirements primarily driven by extensive drying processes needed to remove water after protein precipitation (Schutyser & van der Goot, 2011; Pelgrom et al., 2015). Studies indicate that wet fractionation achieves substantially lower energy efficiency compared to alternative methods, contributing to the overall carbon footprint of protein production and challenging the sustainability credentials of plant-based alternatives.

1.1.2.2. Chemical Usage and Clean Label Concerns

Modern consumers increasingly demand clean label products—ingredients that are natural, minimally processed, and free from synthetic additives (Boukid, 2021). Wet fractionation inherently conflicts with clean label principles due to its reliance on harsh chemicals including sodium hydroxide, hydrochloric acid, and organic solvents. These chemicals can leave residues in final products and require extensive processing to achieve acceptable levels. Furthermore, the extreme pH conditions (often >11 or <3) used in wet fractionation can denature proteins, reducing their nutritional value and functional properties (Rivera et al., 2024).

1.1.2.3. Protein Functionality Challenges

The harsh conditions employed in wet fractionation—including extreme pH levels, high temperatures, and chemical treatments—can significantly impact protein structure and functionality. These processing conditions often lead to protein denaturation, reducing properties such as solubility, emulsification capacity, and gelation strength (De Angelis et al., 2024). While wet fractionation produces high protein purity, the resulting isolates may have compromised functional properties compared to native proteins, limiting their applications in food product development.

1.1.3. Advantages of Dry Fractionation Technologies

Dry fractionation represents a paradigm shift in plant protein processing, offering solutions to many limitations of wet fractionation while maintaining product quality and functionality. This approach relies on mechanical separation techniques that exploit differences in particle size, density, and electrical properties to achieve protein enrichment without water or chemicals (Schutyser & van der Goot, 2011; Pelgrom et al., 2015).

1.1.3.1. Environmental Sustainability

Dry fractionation offers dramatic environmental advantages over conventional wet methods. Comparative studies indicate that dry fractionation reduces environmental impact substantially for most plant protein sources by eliminating water usage, chemical consumption, and associated waste streams (Schutyser et al., 2015). Energy requirements are substantially lower, with dry fractionation achieving significantly improved energy efficiency compared to wet fractionation methods. The environmental benefits extend beyond direct processing impacts by eliminating the need for wastewater treatment, chemical disposal, and associated infrastructure.

1.1.3.2. Clean Label Compatibility

Dry fractionation aligns perfectly with clean label requirements and consumer preferences for natural, minimally processed foods. The process involves only mechanical operations—milling and air classification—without chemicals, solvents, or extreme processing conditions (De Angelis et al., 2024). This approach produces protein concentrates that qualify for organic certification and do not require complex chemical descriptions in ingredient listings. The native state of proteins is preserved throughout processing, maintaining their original nutritional value and functional properties.

1.1.3.3. Protein Quality and Functionality

Dry fractionation preserves the native structure of proteins, resulting in superior functional properties compared to chemically extracted proteins. Research demonstrates that dry fractionated proteins exhibit enhanced foaming capacity, emulsification properties, and gelation strength (De Angelis et al., 2024; Schutyser & van der Goot, 2011). These improved functional characteristics expand application possibilities in food product development, enabling the creation of innovative plant-based foods with superior texture and sensory properties.

The preservation of protein nativity also maintains nutritional quality. Amino acid profiles remain intact, and bioactive compounds associated with proteins are retained. This nutritional preservation is particularly important for essential amino acids that can be sensitive to chemical and thermal processing (Zhang et al., 2024).

1.1.3.4. Economic Advantages

The economic benefits of dry fractionation are substantial and multifaceted. Capital investment requirements are significantly lower due to simplified equipment needs and the absence of chemical handling infrastructure. Operating costs are reduced through elimination of chemical purchases, waste disposal fees, and water treatment expenses. Energy costs are dramatically lower due to the absence of extensive drying operations (Schutyser et al., 2015).

1.1.3.5. Scalability and Processing Flexibility

Dry fractionation offers superior scalability compared to wet methods. The process is inherently modular, allowing for incremental capacity expansion without major infrastructure changes. Equipment is commercially available at various scales, from laboratory to industrial production levels. The absence of complex chemical handling and waste treatment systems simplifies scaling and reduces regulatory compliance requirements.

Processing flexibility is another key advantage. Dry fractionation can be easily adapted to different pulse varieties and compositions, enabling processors to respond to seasonal availability and market demands. The process is also compatible with hybrid approaches, where dry fractionation serves as a pre-treatment step before selective wet processing, combining the advantages of both methods (Schutyser & van der Goot, 2011).

1.1.4 Selection of Black gram and Green gram for Research

This study focuses on Black gram (black gram, *Vigna mungo*) and Green gram (green gram, *Vigna radiata*).

1.1.4.1. Nutritional Excellence and Protein Quality

Green gram (Green gram, *Phaseolus aureus*) and black gram (Black gram, *Phaseolus mungo*) were selected as the primary substrates for dry fractionation through air classifier technology due to their good protein content and distinct physicochemical properties. According to the Indian Food Composition Tables, both pulses exhibit high protein concentrations of 23.88 ± 0.61 g/100g and 23.06 ± 0.59 g/100g respectively (National Institute of Nutrition, 2017). The contrasting dietary fiber compositions between the two pulses—with Green gram containing higher insoluble fiber (6.13 ± 0.20 g/100g) compared to Black gram (3.23 ± 0.05 g/100g), while Black gram exhibits significantly higher soluble fiber content (4.35 ± 0.15 g/100g versus 1.62 ± 0.19 g/100g in Green gram)—offers an opportunity to investigate how different fiber matrices influence separation efficiency during air classification (National Institute of Nutrition, 2017). Additionally, both pulses are widely cultivated and consumed across India, ensuring consistent raw material availability and practical relevance for developing scalable protein enrichment processes that could benefit the regional food industry and nutritional security.

The nutritional value of these selected pulses extends beyond basic protein content to encompass amino acid profiles that justify their use in advanced fractionation studies. Black gram demonstrates exceptional nutritional characteristics with protein content ranging from 24.5% to 34.41% depending on variety, with research indicating that black gram proteins contain all essential amino acids in appreciable quantities, where the percentage of essential amino acids being higher than the non-essential group (Modgil et al., 2019; Sharma et al., 2020). Green gram contains 20.97-31.32% protein with a well-balanced amino acid composition, where essential amino acids constitute approximately 43% of total amino acids in mung bean protein (Li et al., 2018). Mung bean proteins are particularly rich in glutamic acid and arginine (Li et al., 2018). This combination of good protein content and contrasting fibre profile makes the two pulses good substrates for investigating air classification efficiency and developing optimized protein enrichment protocols.

1.1.4.2. Morphological Structure and Processing Suitability

Studies show that black gram seeds have favorable physical properties for processing, including appropriate particle size distribution after milling (Bakane et al., 2024). The relatively low-fat content minimizes powder cohesiveness issues that can compromise air classification efficiency. Research has demonstrated that low-fat pulses experience better separation efficiency due to improved powder flow characteristics.

Green gram flour also offers excellent powder flow characteristics. It also has minimal anti-nutritional factors compared to other pulses. Physical properties studies indicate that green gram has favorable characteristics for mechanical processing, including appropriate density and particle size distribution (Bakane et al., 2024). The seed structure allows for effective protein liberation during milling while maintaining protein functionality (Li et al., 2018).

1.1.4.3. Functional Properties and Product Development Potential

Both pulses exhibit exceptional functional properties essential for product development applications. Research on black gram proteins demonstrates superior functional characteristics, including good water absorption capacity and emulsification properties, making them suitable for both low and high moisture extrusion applications (Modgil et al., 2019). The proteins maintain their functional characteristics during processing, supporting texture development in extruded products.

Green gram proteins offer excellent gelation properties and thermal stability. Research shows that mung bean protein isolates exhibit superior foaming capacity and emulsion stability compared to many other plant proteins (Li et al., 2018). These properties are particularly valuable for high moisture extrusion applications where protein functionality directly impacts final product texture and consumer acceptance.

1.1.4.4. Market Potential

The growing global demand for clean label, sustainable protein ingredients create substantial market potential for products derived from these pulses. Consumer acceptance studies indicate high preference for familiar pulse varieties over novel protein sources, supporting the commercial viability of Black gram and Green gram protein ingredients.

1.1.4.5. Comparative Processing Advantages

This comparative approach allows for the development of processing protocols that can be adapted to different pulse varieties, enhancing the commercial applicability of the research outcomes. The different protein body sizes and starch granule characteristics between the two pulses provide valuable data for optimizing air classification parameters across diverse feedstocks (Pelgrom et al., 2015).

1.1.4.6. Research Strategy and Complementary Analysis

The inclusion of both Black gram and Green gram enables comprehensive evaluation of air classification technology effectiveness. Different processing characteristics provide opportunities to optimize milling parameters, air classification conditions, and product development protocols for diverse pulse varieties. This approach ensures that research outcomes contribute broadly applicable knowledge rather than pulse-specific findings, enhancing the commercial impact and scientific contribution of the work.

1.2. Air Classification Technology and Innovation

1.2.1. Principles and Mechanisms of Air Classification

Air classification represents the core separation technology in dry fractionation systems, exploiting fundamental differences in particle size and density to achieve protein enrichment. The technology operates on aerodynamic principles, where particles are subjected to controlled airflow patterns that selectively transport lighter, smaller particles (primarily protein bodies) while allowing heavier, larger particles (primarily starch granules and fibre) to settle (Pelgrom et al., 2015).

The theoretical foundation of air classification lies in understanding the morphological structure of pulse seeds. Pulses exhibit a characteristic cellular organization where protein bodies (1-3 µm diameter) are embedded within a matrix containing starch granules (approximately 20 µm diameter) and surrounded by fibre-rich cell walls (Rivera et al., 2024). This size differential provides the physical basis for separation, as proper milling can liberate protein bodies into particles smaller than starch granules, enabling aerodynamic separation.

Modern air classifiers employ centrifugal force combined with controlled airflow to achieve high-resolution separation. The classifier wheel creates a rotating air pattern that generates size-dependent particle trajectories (Wang et al., 2015). Fine particles follow the airstream and are collected as the protein-rich fraction, while coarse particles are centrifugally separated and collected as the starch-rich fraction. Process optimization involves balancing classifier wheel speed, air flow rate, and feed rate to maximize separation efficiency and protein enrichment.

1.2.2. Processing Parameters and Optimization

Successful air classification requires careful optimization of the multiple interdependent parameters mentioned above – feed rate, classifier wheel speed, air flow rate and the milling wheel speed. Classifier wheel speed represents a critical control variable, affecting the centrifugal force field and the cut point between fine and coarse fractions. Research has demonstrated that wheel speeds can significantly influence protein recovery and purity, with optimal conditions varying by pulse type and milling characteristics (Pelgrom et al., 2015; Wang et al., 2015).

Air flow rate serves as the primary separation driving force, determining the aerodynamic drag on particles and their classification trajectory. Flow rates must be balanced to provide sufficient particle suspension while avoiding excessive turbulence that can cause particle agglomeration or misclassification. Studies indicate that optimal flow rates depend on material characteristics and desired separation efficiency (Schutyser et al., 2015).

Feed rate optimization ensures optimal residence time for particle separation while maintaining system throughput. Excessive feed rates can overload the classifier, reduce separation efficiency and cause particle agglomeration. Conversely, very low feed rates may result in economic inefficiency despite achieving high separation quality. Research indicates that feed rates must be optimized based on classifier design and material characteristics (Pelgrom et al., 2015).

Temperature and humidity control represent additional process considerations. Moisture content affects particle flow characteristics and can cause agglomeration, reducing separation efficiency. Optimal moisture levels typically range from 8-12% for most pulse varieties. Temperature affects air density and flow characteristics, requiring consideration in process design and control (Schutyser et al., 2015).

1.2.3. Integration with Product Development

The integration of air classification technology with downstream product development creates opportunities for innovative food applications. Protein-enriched fractions from air classification can serve as functional ingredients in various food systems, offering improved nutritional profiles and enhanced functionality compared to whole pulse flours (Zhang et al., 2024).

Low moisture extrusion applications benefit significantly from air classified proteins due to their concentrated protein content and preserved functionality. The higher protein concentration enables the development of high-protein snacks, cereals, and convenience foods with improved nutritional profiles. The native protein structure supports texture development during extrusion, contributing to desirable sensory properties (Rivera et al., 2024).

1.3. Problem Statement

Despite the clear advantages of dry fractionation and air classification technology, several critical knowledge gaps and technical challenges limit the widespread adoption and optimization of these processes for pulse protein production. These limitations constrain the realization of sustainable, economical plant protein production at the scale required to address global protein security (Boukid, 2021).

1.3.1. Process Optimization Challenges

Current air classification processes lack comprehensive optimization protocols that account for the diverse characteristics of different pulse varieties. While research has demonstrated the feasibility of protein enrichment through air classification, systematic studies examining the interactive effects of process parameters across multiple pulse types remain limited (Rivera et al., 2024). This knowledge gap results in suboptimal processing conditions that may compromise protein recovery, purity, or functional properties.

The relationship between pre-processing conditions (particularly milling parameters) and air classification efficiency is not fully understood. Milling significantly affects particle size distribution, protein liberation, and subsequent separation efficiency. However, optimization of milling parameters specifically for air classification applications requires additional research to identify conditions that maximize protein recovery while minimizing starch damage and maintaining protein functionality (Pelgrom et al., 2015).

Scale-up challenges present additional obstacles to commercial implementation. Most research has been conducted at laboratory or pilot scale, with limited investigation of full-scale processing considerations. Industrial air classification involves different equipment designs, process dynamics, and control requirements that may significantly affect separation performance. The translation of laboratory optimization results to commercial scale requires validation and potentially re-optimization of process parameters (Schutyser et al., 2015).

1.3.2. Product Development Limitations

While air classification can produce protein-enriched fractions, the development of these fractions into finished food products remains challenging. Limited research has examined the functional properties of air classified proteins in specific food applications, particularly in extrusion processing (Zhang et al., 2024). Understanding how air classification affects protein functionality in different food systems is essential for successful product development.

The integration of air classified proteins into low moisture extrusion processes requires investigation of protein-starch interactions, processing parameter optimization, and product quality assessment. Current knowledge of how air classification affects protein behavior during extrusion is limited, constraining the development of high-quality extruded products (Rivera et al., 2024).

Nutritional optimization represents another development challenge. While air classification can increase protein concentration, the effects on amino acid profiles, vitamin content, and mineral bioavailability require systematic investigation. Understanding these nutritional changes is essential for developing products that meet consumer expectations and regulatory requirements (Boukid, 2021).

1.4. Research Objectives and Hypotheses

This research addresses the identified knowledge gaps through a comprehensive investigation of pulse dry fractionation using air classification technology, combined with the development and evaluation of extruded products from protein-enriched fractions.

1.4.1. Primary Objective

To develop and optimize a dry fractionation process using air classification for producing protein-enriched fractions from Black gram and Green gram, and to evaluate the potential of these fractions in low extrusion application for creating nutritious, functional food products.

1.4.2. Specific Objectives

Objective 1: Optimize Air Classification Parameters for Maximum Protein Enrichment

This objective focuses on systematic optimization of air classification processing parameters to achieve maximum protein recovery and purity from Black gram and Green gram. The investigation will examine the interactive effects of mill speed, classifier wheel speed, air flow rate, feed rate, and raw material characteristics on separation efficiency. Response surface methodology will be employed to develop predictive models for process optimization and to identify optimal operating conditions for both pulse varieties.

Objective 2: To develop low moisture extruded products utilizing air-classified protein fractions from pulses and to comprehensively characterize their physicochemical and functional properties

The research will optimize formulation and extrusion parameters to produce nutritionally enhanced, protein-rich snacks with desirable texture, sensory quality, and storage stability. Detailed characterization of the air-classified protein fractions will encompass assessments of protein content, particle size and key functional attributes including solubility, water and oil absorption, pasting properties, and foaming capacity. These findings will establish quality specifications for the protein fractions and guide the selection of optimal processing conditions for advanced product applications.

CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

The growing global demand for sustainable protein sources has intensified research into plant-based alternatives from animal proteins. Pulses, representing a critical component of this transition, offer exceptional nutritional profiles combined with environmental sustainability advantages. However, the challenge lies in efficiently extracting and processing pulse proteins while maintaining their functional properties and commercial viability. This literature review examines the current state of knowledge in pulse protein processing technologies, with particular emphasis on dry fractionation methods, air classification techniques, and product development applications.

The review is structured to provide comprehensive coverage of the key research areas relevant to this thesis. Beginning with an overview of pulse protein composition and properties, the review progresses through traditional and emerging processing technologies, focusing on the advantages and limitations of various extraction methods. Special attention is given to dry fractionation and air classification technologies, which represent the core focus of this research. The review also examines functional properties of fractionated proteins, extrusion processing applications, and current research gaps that justify the present investigation.

2.2. Pulse Protein Composition and Nutritional Properties

2.2.1. General Pulse Protein Characteristics

Pulses represent an exceptional source of plant proteins, containing approximately 20-35% protein content depending on variety and growing conditions (Boye et al., 2010; Singh et al., 2017). The protein composition of pulses is characterized by a distinctive fractionation pattern consisting primarily of two major protein classes: albumins and globulins. Globulins constitute the dominant storage protein fraction, representing 60-80% of total seed protein, while albumins typically comprise 10-20% of the total protein content (Singh et al., 2017).

Research has demonstrated that pulse proteins provide well-balanced essential amino acid profiles when consumed in combination with cereals. They contain high amounts of lysine, leucine, aspartic acid, glutamic acid, and arginine, making them particularly valuable for addressing protein quality concerns in plant-based diets (Boye et al., 2010). However, like most legume proteins, pulses are typically deficient in sulfur-containing amino acids, particularly methionine and cysteine, which represents a nutritional limitation that can be addressed through complementary protein combinations.

2.2.2. *Vigna mungo* (Black Gram) Nutritional Profile

Black gram (*Vigna mungo*) demonstrates exceptional nutritional characteristics that make it an ideal candidate for protein fractionation research. Comprehensive compositional analysis reveals protein content ranging from 24.5% to 34.41% depending on cultivar and growing conditions (Modgil et al., 2019). This protein concentration significantly exceeds that of many other pulse varieties, providing a strong foundation for achieving high protein yields through fractionation processes.

The structural organization of Black gram seeds exhibits characteristics favourable for mechanical fractionation. Research indicates uniform starch granule distribution with distinct protein body organization, facilitating effective separation through size-based classification methods (Singh et al., 2017). The relatively low-fat content (1.64% in raw form) minimizes powder cohesiveness issues that can compromise air classification efficiency, making black gram particularly suitable for dry fractionation applications.

2.2.3. *Vigna radiata* (Green Gram) Nutritional Profile

Green gram (*Vigna radiata*) complements Black gram with its own distinctive nutritional advantages and processing characteristics. Protein content analysis reveals concentrations ranging from 20.97% to 31.32% depending on variety and processing conditions (Li et al., 2018). While generally lower than Black gram protein concentrations, green gram proteins exhibit superior digestibility and functional properties that enhance their value for food applications.

The protein structure of mung beans demonstrates favorable characteristics for both functional properties and processing efficiency. Storage proteins comprise approximately 85% of total proteins, with globulins representing 60% and albumins 25% of total protein content (Li et al., 2018). This protein distribution contributes to excellent solubility characteristics and functional properties, including superior foaming capacity and emulsion stability compared to many other plant proteins.

Research indicates that mung bean proteins maintain their structural integrity and functional properties better during mechanical processing compared to chemical extraction methods, making them particularly suitable for dry fractionation applications (Li et al., 2018). The seed morphology exhibits appropriate size differentials between protein bodies and starch granules, facilitating effective air classification separation.

2.3. Traditional Pulse Protein Extraction Technologies

2.3.1. Wet Fractionation Methods

Wet fractionation has dominated commercial pulse protein extraction for several decades, employing aqueous-based systems to separate proteins from other seed components. The conventional approach involves alkaline extraction followed by isoelectric precipitation, producing protein isolates with high purity typically exceeding 80-90% protein content (Rivera et al., 2024). This process has been extensively studied and optimized for various pulse varieties, establishing benchmarks for protein purity and yield that alternative methods must address.

The alkaline solubilization process typically operates at pH 8-11 using sodium hydroxide, exploiting the pH-dependent solubility characteristics of pulse proteins. Research demonstrates that protein solubility increases dramatically under alkaline conditions, reaching maximum extraction efficiency at pH 9-10 for most pulse varieties (Singh et al., 2017). The subsequent isoelectric precipitation at pH 4-5 causes protein aggregation and precipitation, enabling separation from soluble carbohydrates and other components.

Comprehensive studies have demonstrated that wet fractionation can achieve protein isolates with 84-94% protein content from various pulses, including lentils, mung beans, and yellow peas, with protein yields ranging from 54-68% (Rivera et al., 2024). However, these impressive purity levels come with significant resource requirements and processing challenges that limit scalability and sustainability.

The resource intensity of wet fractionation represents its most significant limitation. Detailed life cycle analyses reveal that producing 1 kg of protein isolate requires substantial inputs: over 80 kg of water, significant quantities of chemicals including sodium hydroxide and hydrochloric acid, and extensive energy requirements for drying operations (Schutyser et al., 2015). This resource consumption translates to high environmental impact and operational costs that challenge the economic viability of plant protein production.

Process-induced modifications represent another critical concern with wet fractionation. The extreme pH conditions (often >11 or <3) combined with thermal treatments can cause significant protein denaturation, reducing functional properties and nutritional quality. Research indicates that alkaline treatment can cause amino acid modifications, including racemization and destruction of sensitive amino acids such as lysine and cysteine (Rivera et al., 2024).

2.3.2. Alternative Wet Processing Methods

Beyond conventional alkaline extraction, several alternative wet processing methods have been developed to address the limitations of traditional approaches. Ultrafiltration represents one such alternative, employing membrane separation to concentrate proteins without extreme pH conditions. Research demonstrates that ultrafiltration can achieve protein concentrates with 75% protein content while maintaining better functional properties compared to isoelectric precipitation (Rivera et al., 2024).

Salt extraction or micellization methods exploit the different solubility characteristics of pulse protein fractions. This approach selectively dissolves globulin proteins in salt solutions (0.2-0.8 M sodium chloride) at neutral pH, followed by protein recovery through dialysis or dilution precipitation. Studies indicate that micellization can produce protein concentrates with 67-75% protein content while preserving native protein structure and functionality better than alkaline extraction methods.

Enzyme-assisted extraction has emerged as a promising approach for improving protein yield and quality while reducing harsh chemical treatments. Research demonstrates that pre-treatment with cell wall-degrading enzymes, including pectinase, cellulase, and xylanase, can increase protein extraction efficiency by 13-21% compared to conventional alkaline extraction alone (Rivera et al., 2024). The enzymatic approach operates under milder conditions, potentially preserving protein functionality while improving extraction efficiency.

Despite these technological advances, all wet fractionation methods share fundamental limitations including high water consumption, energy-intensive drying requirements, and the need for chemical inputs. These constraints have motivated research into alternative processing approaches that can address sustainability concerns while maintaining product quality.

2.3.3. Hybrid Processing Approaches

The recognition of individual limitations in both wet and dry processing methods has led to the development of hybrid approaches that combine multiple technologies to optimize both sustainability and product quality. These integrated systems typically employ dry fractionation as a pre-concentration step followed by selective wet processing of enriched fractions, potentially achieving the benefits of both approaches while minimizing their individual limitations.

Research demonstrates that hybrid processing can achieve protein isolates with 80-85% purity while reducing water consumption by 60-70% compared to conventional wet fractionation (Schutyser et al., 2015). The approach involves initial dry fractionation to produce protein concentrates of 50-60% purity, followed by wet processing of only the protein-enriched fraction. This strategy significantly reduces the volume of material requiring wet processing, correspondingly reducing resource requirements.

The economic implications of hybrid processing appear favorable, with studies indicating improved protein delivery efficiency of 29.1 g protein per MJ compared to 14.6 g protein per MJ for conventional wet fractionation (Schutyser et al., 2015). This improvement reflects both the energy efficiency of the initial dry fractionation step and the reduced processing volume in the subsequent wet fractionation stage.

However, hybrid approaches introduce additional complexity in process control and equipment requirements. The optimization of both dry and wet processing parameters requires sophisticated understanding of protein behaviour across multiple unit operations. Research indicates that the success of hybrid approaches depends critically on the selection of appropriate intermediate protein concentrations and the optimization of wet processing parameters for pre-concentrated feedstocks.

2.4. Dry Fractionation and Air Classification Technology

2.4.1. Principles and Mechanisms of Dry Fractionation

Dry fractionation represents a paradigm shift in pulse protein processing, relying entirely on mechanical separation techniques that exploit physical differences between cellular components. The process capitalizes on the natural organization of pulse seeds, where smaller protein bodies (1-3 µm diameter) are embedded within matrices containing larger starch granules (20-40 µm diameter) surrounded by fibre-rich cell walls (Pelgrom et al., 2015). This size differential provides the physical foundation for separation through controlled milling and air classification operations.

The milling process serves as the critical first step in dry fractionation, disrupting cellular structures to liberate protein bodies from the starch matrix. Research demonstrates that proper milling intensity, seed moisture content, and chemical composition significantly influence the degree of protein liberation and subsequent separation efficiency (Pelgrom et al., 2015). Impact classifier milling has proven particularly effective, producing fine flours with particle sizes below 45 µm while minimizing starch damage, which is essential for effective air classification.

Studies examining various pulse varieties reveal that milling effectiveness varies significantly among species based on cellular organization and mechanical properties. Faba beans, yellow peas, and red lentils subjected to impact classifier milling produce fine flours with minimal starch damage (1.3-2.4%), facilitating effective protein separation (Pelgrom et al., 2015). The optimization of milling parameters represents a critical factor in maximizing separation efficiency and protein recovery.

The theoretical maximum protein content achievable through dry fractionation is determined by the intrinsic protein content of individual protein bodies, which typically ranges from 70-88% depending on pulse variety (Pelgrom et al., 2015). This theoretical limit explains why dry fractionation typically achieves protein concentrations of 40-65% compared to the 80-90% possible with wet methods, but at significantly lower resource and energy requirements.

2.4.2. Air Classification Technology

Air classification serves as the core separation technology in dry fractionation systems, employing aerodynamic principles to achieve size-based particle separation. The technology operates on the principle that particles of different sizes and densities respond differently to controlled airflow patterns, enabling selective transport of protein-rich fine particles while allowing starch-rich coarse particles to settle (Wang et al., 2015).

Modern air classifiers employ centrifugal force combined with controlled airflow to achieve high-resolution separation. The classifier wheel creates a rotating air pattern that generates size-dependent particle trajectories, with fine particles following the airstream to the protein-rich fraction while coarse particles are centrifugally separated to the starch-rich fraction. Process optimization requires careful balancing of classifier wheel speed, air flow rate, and feed rate to maximize separation efficiency (Wang et al., 2015).

Research examining air classification of various pulse varieties demonstrates protein concentrations in fine fractions ranging from 49-70% depending on pulse type and processing conditions (Pelgrom et al., 2015). Legumes generally achieve higher protein purity than cereal grains due to their higher native protein concentration and larger, more uniform starch granules. The starch granule size largely determines separation potential, with optimal results obtained when particle size distributions between protein and starch fractions are maximally distinct.

The cut point in air classification represents a critical process parameter, defining the boundary between fine and coarse fractions. Research indicates that this parameter must be optimized for each pulse variety based on the characteristic size distributions of cellular components. Optimal cut points typically range from 10-25 μm depending on pulse morphology and milling conditions (Pelgrom et al., 2015).

2.4.3. Process Parameters and Optimization

Successful air classification requires systematic optimization of multiple interdependent parameters that collectively determine separation efficiency and product quality. Classifier wheel speed represents the primary control variable, affecting the centrifugal force field and the boundary between fine and coarse fractions. Research demonstrates that wheel speeds ranging from 2,000-6,000 RPM significantly influence protein recovery and purity, with optimal conditions varying by pulse type and milling characteristics (Wang et al., 2015).

Air flow rate serves as the driving force for particle separation, determining the aerodynamic drag on particles and their classification trajectory. Studies indicate that flow rates must be precisely balanced to provide sufficient particle suspension while avoiding excessive turbulence that causes particle agglomeration or misclassification. Typical industrial classifiers operate at flow rates of 50-200 m^3/hour per kilogram of processed material, requiring optimization based on material characteristics and desired separation efficiency.

Feed rate optimization ensures appropriate residence time for particle separation while maintaining economic throughput. Research indicates that excessive feed rates overload the classifier, reducing separation efficiency and causing particle agglomeration, while very low feed rates may be economically inefficient despite achieving high separation quality (Pelgrom et al., 2015). Optimal feed rates typically range from 50-100 kg/hour per meter of classifier diameter, providing balance between efficiency and throughput.

Environmental conditions, particularly temperature and humidity, significantly affect air classification performance. Moisture content influences particle flow characteristics and can cause agglomeration, reducing separation efficiency. Research indicates that optimal moisture levels typically range from 8-12% for most pulse varieties, requiring careful control of storage and processing conditions (Schutyser et al., 2015).

2.5. Functional Properties of Pulse Proteins

2.5.1. Water and Oil Absorption Properties

Water and oil absorption capacities represent critical functional properties that influence texture, mouthfeel, and processing behaviour in food applications. These properties reflect the protein's ability to bind and retain water or oil through various molecular interactions, including hydrogen bonding, electrostatic interactions, and hydrophobic associations. Research demonstrates that pulse proteins exhibit excellent water absorption capacities, typically ranging from 150-400% depending on protein source and processing conditions (Karaca et al., 2011).

Black gram proteins demonstrate particularly superior water absorption capacity, with studies indicating values of 150-250% of protein weight under standard testing conditions (Modgil et al., 2019). This exceptional water-binding capacity makes black gram proteins particularly suitable for applications requiring moisture retention, including bakery products, extruded snacks, and meat analog formulations. The high-water absorption correlates with the protein's structural characteristics and the presence of hydrophilic amino acids.

Oil absorption capacity varies significantly among pulse varieties, reflecting differences in protein structure and hydrophobic characteristics. Research indicates that pulse proteins typically achieve oil absorption capacities of 100-200% of protein weight, with variations depending on protein source and processing method (Karaca et al., 2011). This property is particularly important for applications in fried foods, salad dressings, and other oil-containing products where fat binding contributes to texture and stability.

The relationship between protein structure and absorption properties has been extensively studied, revealing that denaturation generally increases water absorption while potentially affecting oil absorption differently depending on the extent and nature of structural changes. Dry fractionation, by preserving native protein structure, typically maintains balanced water and oil absorption properties that are optimal for most food applications.

2.5.2. Foaming Properties

Foaming properties determine the suitability of pulse proteins for applications requiring aeration and volume enhancement, including baked goods, confectionery products, and whipped formulations. Foaming capacity reflects the protein's ability to rapidly migrate to air-water interfaces, unfold to accommodate conformational changes, and form stable films around air bubbles (Karaca et al., 2011). Foam stability indicates the protein's ability to maintain foam structure over time through elastic film formation.

Research demonstrates that pulse proteins exhibit excellent foaming properties, with significant variations among varieties and protein fractions. Albumin fractions consistently demonstrate superior foaming performance compared to globulin fractions, reflecting their higher solubility and greater structural flexibility. Studies indicate that lentil and horse gram albumins achieve foaming capacities of 76.7% and 79% respectively, substantially higher than corresponding globulin fractions (Karaca et al., 2011).

The relationship between protein solubility and foaming performance has been clearly established through comparative studies. Research indicates a positive correlation between foaming properties and both surface charge (zeta potential magnitude) and protein solubility across various pulse varieties (Karaca et al., 2011). Higher surface charges contribute to enhanced foaming by weakening hydrophobic interactions, increasing protein flexibility, and enabling rapid interface spreading.

Processing conditions significantly affect foaming properties, with pH representing a critical control parameter. Studies demonstrate that pulse proteins exhibit maximum foaming activity under acidic and alkaline conditions where solubility is high, while showing minimum foaming near the isoelectric point where solubility is lowest. This pH-dependent behavior must be considered in formulation development for foam-based applications.

2.6. Extrusion Processing of Plant Proteins

2.6.1. Low Moisture Extrusion Technology

Low moisture extrusion (LME) represents a well-established technology for producing textured vegetable proteins (TVP) with feed moisture contents typically ranging from 10-40%. This process produces expanded, dry products that require rehydration before consumption but offer excellent storage stability and versatile application possibilities. Research demonstrates that LME can effectively process various plant proteins to create products with meat-like textures suitable for numerous food applications (Osen et al., 2014).

The mechanism of low moisture extrusion involves protein denaturation, alignment, and cross-linking under conditions of high temperature (140-180°C), mechanical shear, and controlled moisture. The sudden pressure release at the die exit causes expansion and structure formation, creating the characteristic fibrous texture associated with TVP products. Studies indicate that protein content, moisture level, and processing parameters critically influence final product texture and quality (Osen et al., 2014).

Process parameter optimization for low moisture extrusion requires careful balance of temperature, screw speed, feed rate, and moisture content. Research demonstrates that barrel temperature represents the most critical parameter for protein texturization, with optimal ranges varying by protein type and desired product characteristics. Temperatures below 90°C hinder expansion and layer formation, while excessive temperatures can cause protein degradation and reduced functionality (Osen et al., 2014).

The application of pulse proteins in low moisture extrusion has shown promising results, with studies demonstrating successful production of textured products from various pulse varieties. Air-classified pulse proteins offer particular advantages due to their concentrated protein content and preserved native structure, enabling effective texturization while maintaining nutritional quality. Research indicates that pulse protein TVP can achieve protein contents of 50-70%, significantly higher than conventional cereal-based products.

2.6.2. Protein Behaviour During Extrusion

The molecular transformations occurring during extrusion processing are complex and critically influence final product quality. Research demonstrates that proteins undergo sequential denaturation, unfolding, realignment, and cross-linking during extrusion, with the extent and nature of these changes depending on processing conditions and protein characteristics (Dekkers et al., 2018). Understanding these molecular mechanisms is essential for optimizing processing parameters and achieving desired product properties.

Protein denaturation during extrusion occurs primarily through thermal and mechanical stress, causing disruption of native protein structure and exposure of previously buried regions. Studies indicate that the degree of denaturation correlates with processing temperature and residence time, with implications for both functional properties and final product characteristics. Controlled denaturation can enhance protein-protein interactions and facilitate structure formation, while excessive denaturation may impair functionality (Dekkers et al., 2018).

The formation of new protein networks during extrusion involves various molecular interactions including hydrogen bonds, disulfide bonds, and hydrophobic associations. Research demonstrates that the balance between different bond types critically influences final product texture and quality. Studies examining protein solubility in different extraction media reveal that extruded products are primarily stabilized by non-covalent interactions, with hydrogen bonds and hydrophobic interactions predominating over disulfide bonds (Liu et al., 2023).

The alignment of proteins during extrusion creates the anisotropic structure characteristic of meat-like products. Research indicates that shear forces generated during extrusion cause protein molecules to align in the direction of flow, creating directional structure that persists through cooling and solidification. This alignment is responsible for the fibrous texture and directional strength properties that distinguish extruded meat analogs from isotropic protein products (Dekkers et al., 2018).

2.6.3. Formulation Considerations

Successful extrusion processing requires careful formulation design that considers not only protein content but also the inclusion of other ingredients that influence processing behaviour and final product quality. Research demonstrates that the addition of polysaccharides, fibers, and other functional ingredients can significantly enhance extrusion performance and product characteristics (Liu et al., 2023). Understanding ingredient interactions and their effects on processing is essential for developing optimal formulations.

Polysaccharide additions have shown particular promise for enhancing extrusion performance, with studies demonstrating that specific types and concentrations can improve protein network formation and product texture. Research indicates that sodium alginate at 4% addition level significantly enhances fibrous structure formation, while xanthan gum at 2% optimally improves thermal stability and protein alignment (Liu et al., 2023). These additions work by modifying protein-water interactions and enhancing the stability of protein networks during processing.

Moisture management represents a critical aspect of formulation design, particularly for high moisture extrusion applications. Studies demonstrate that different ingredients exhibit varying water-binding capacities and influence moisture distribution within the protein matrix during processing. Research indicates that maltodextrin addition at 2% level creates the highest percentage of free water, while sodium alginate enhances bound water retention, both contributing to improved processing performance (Liu et al., 2023).

The pH of extrusion formulations significantly influences protein behaviour and final product quality. Research demonstrates that pH affects protein solubility, intermolecular interactions, and processing behaviour, with optimal pH ranges varying by protein type and desired product characteristics. Studies indicate that neutral pH typically provides optimal processing performance for most pulse proteins, while acidic or alkaline conditions can impair protein functionality and product quality.

2.7. Product Development Applications

2.7.1. Meat Analog Development

The development of plant-based meat analogs represents one of the most commercially significant applications for fractionated pulse proteins. Research demonstrates that pulse proteins, particularly when concentrated through dry fractionation, offer excellent potential for creating products that closely mimic the texture, appearance, and nutritional profile of animal meats (Zhang et al., 2024). The combination of high protein content, favorable amino acid profiles, and excellent functional properties makes fractionated pulse proteins ideal candidates for meat analog applications.

Current commercial meat analog products utilize various plant protein sources, with soy proteins dominating the market due to their established processing infrastructure and functional characteristics. However, research indicates that pulse proteins offer several advantages including superior nutritional profiles, absence of common allergens, and excellent processing characteristics for both low and high moisture extrusion applications (Zhang et al., 2024). The development of pulse-based meat analogs represents a significant market opportunity with substantial growth potential.

The sensory characteristics of pulse protein meat analogs have been extensively studied, with research demonstrating that properly formulated products can achieve consumer acceptance levels comparable to conventional meat products. Studies indicate that texture represents the most critical sensory attribute, with successful products achieving fibrous, meat-like structures through optimized extrusion processing (Zhang et al., 2024). Color, flavor, and aroma development require careful formulation and processing optimization but can be successfully achieved through ingredient selection and processing parameter control.

Nutritional optimization of pulse protein meat analogs offers advantages over both conventional meat products and other plant-based alternatives. Research demonstrates that pulse-based products can provide complete amino acid profiles when properly formulated, while offering superior fiber content, lower saturated fat levels, and beneficial micronutrient profiles including substantial iron content from pulses like black gram (Zhang et al., 2024).

2.7.2. Bakery and Snack Applications

Pulse protein concentrates from dry fractionation offer excellent potential for enhancing the nutritional profile of bakery products and snack foods. Research demonstrates that air-classified pulse proteins can be successfully incorporated into bread, cookies, crackers, and various extruded snack products while maintaining acceptable sensory characteristics and improving nutritional value (Boye et al., 2010). The preserved functional properties of dry fractionated proteins contribute to successful product development across diverse applications.

Bread applications represent a particularly promising area for pulse protein incorporation, with studies demonstrating that protein concentrates can replace portions of wheat flour while enhancing protein content and amino acid balance. Research indicates that pulse protein additions of 10-20% can significantly improve bread protein quality while maintaining acceptable loaf volume and texture characteristics (Boye et al., 2010). The water absorption and dough handling properties of pulse proteins contribute to successful bread applications.

Extruded snack applications offer excellent opportunities for pulse protein utilization, with research demonstrating successful development of high-protein snacks with 20-30% protein content. Studies indicate that air-classified pulse proteins perform excellently in extrusion applications, creating products with desirable expansion, texture, and flavor characteristics (Boye et al., 2010). The combination of high protein content and excellent processing characteristics makes pulse proteins ideal for premium snack product development.

Cookie and cracker applications have shown particular success with pulse protein incorporation, with research demonstrating that protein concentrates can enhance nutritional value while contributing to desirable texture characteristics. Studies indicate that pulse proteins can improve the protein quality and mineral content of baked goods while providing functional benefits including enhanced water absorption and structure formation (Boye et al., 2010).

2.7.3. Beverage and Dairy Alternative Applications

The development of protein-enriched beverages represents a rapidly growing market segment where pulse proteins offer significant advantages. Research demonstrates that air-classified pulse proteins exhibit superior solubility characteristics compared to chemically extracted isolates, making them particularly suitable for beverage applications where clarity and stability are critical (Pelgrom et al., 2015). The preserved native structure and enhanced solubility of dry fractionated proteins contribute to successful beverage formulation.

Plant-based milk alternatives represent a significant application opportunity for pulse proteins, with research demonstrating successful development of milk analogs with excellent nutritional profiles and acceptable sensory characteristics. Studies indicate that pulse proteins can provide the protein content and amino acid quality necessary for nutritionally complete milk alternatives while contributing to desirable texture and mouthfeel characteristics (Zhang et al., 2024).

Protein shakes and smoothie applications offer excellent potential for pulse protein utilization, with research demonstrating that concentrated pulse proteins can provide high-quality protein in convenient, ready-to-drink formats. Studies indicate that the superior solubility and neutral flavor characteristics of air-classified pulse proteins make them particularly suitable for these applications (Pelgrom et al., 2015).

Yogurt and fermented product alternatives represent an emerging application area where pulse proteins show promise for creating dairy-free products with excellent nutritional and sensory characteristics. Research indicates that the functional properties of pulse proteins, including gelation and water-binding capacity, contribute to successful fermented product development with textures comparable to conventional dairy products.

2.8. Research Gaps and Future Directions

2.8.1. Process Optimization Challenges

Despite significant advances in dry fractionation technology, several critical knowledge gaps remain that limit the widespread adoption and optimization of these processes for pulse protein production. Current air classification processes lack comprehensive optimization protocols that account for the diverse characteristics of different pulse varieties, resulting in suboptimal processing conditions that may compromise protein recovery, purity, or functional properties (Rivera et al., 2024). This limitation represents a significant barrier to commercial implementation and scaling of dry fractionation technology.

The relationship between pre-processing conditions and air classification efficiency requires additional research to establish optimal protocols. Milling parameters significantly affect particle size distribution, protein liberation, and subsequent separation efficiency, but systematic studies examining these relationships across multiple pulse varieties remain limited (Pelgrom et al., 2015). Understanding these relationships is essential for maximizing protein recovery while maintaining protein functionality and minimizing processing costs.

Scale-up challenges represent another significant research gap, with most studies conducted at laboratory or pilot scale with limited investigation of full-scale processing considerations. Industrial air classification involves different equipment designs, process dynamics, and control requirements that may significantly affect separation performance

(Rivera et al., 2024). The translation of laboratory optimization results to commercial scale requires validation and potentially re-optimization of process parameters.

Quality standardization represents an additional challenge, with the absence of established protocols for assessing air-classified protein quality limiting industry adoption. Unlike wet fractionated proteins with established purity and functionality standards, air-classified proteins lack comprehensive quality specifications that complicate product development and commercial transactions (Pelgrom et al., 2015).

2.8.2. Product Development Limitations

While dry fractionation can produce protein-enriched fractions, the development of these fractions into finished food products presents ongoing challenges that require additional research. Limited studies have examined the functional properties of air-classified proteins in specific food applications, particularly in extrusion processing where protein behavior may differ significantly from conventional protein sources (Zhang et al., 2024). Understanding these application-specific characteristics is essential for successful product development.

The integration of air-classified proteins into both low moisture extrusion processes requires systematic investigation of protein-starch interactions, processing parameter optimization, and product quality assessment. Current knowledge of how air classification affects protein behavior during extrusion remains limited, constraining the development of high-quality extruded products (Rivera et al., 2024). This research gap represents a significant opportunity for advancing product development capabilities.

Nutritional optimization represents another area requiring additional research, particularly regarding the effects of processing on amino acid profiles, vitamin content, and mineral bioavailability. While air classification can increase protein concentration, the impacts on overall nutritional quality require systematic investigation to ensure that products meet consumer expectations and regulatory requirements (Zhang et al., 2024).

Sensory optimization represents a critical but under-researched area, with limited studies examining flavour development, texture optimization, and consumer acceptance of products derived from air-classified proteins. Understanding these sensory characteristics and developing optimization strategies is essential for successful commercial product development and market acceptance.

2.8.3. Sustainability Assessment

While dry fractionation offers apparent sustainability advantages over conventional wet processing, comprehensive life cycle assessments comparing different processing approaches remain limited. Research is needed to quantify the environmental benefits of dry fractionation across multiple impact categories including energy consumption, water usage, greenhouse gas emissions, and waste generation (Schutyser et al., 2015). Such assessments are essential for validating sustainability claims and supporting investment decisions.

Economic analysis represents another critical research need, with limited studies examining the full economic implications of dry fractionation compared to conventional processing methods. Research should consider not only direct processing costs but also infrastructure requirements, product quality premiums, and market positioning advantages to provide comprehensive economic evaluation (Rivera et al., 2024).

The integration of dry fractionation into existing food processing infrastructure requires investigation to understand implementation barriers and opportunities. Research examining retrofit possibilities, equipment compatibility, and operational integration could facilitate industry adoption and scaling of dry fractionation technology.

2.8.4. Technology Innovation Opportunities

Advanced separation technologies represent significant opportunities for improving the protein purity achievable through dry fractionation. Research into electrostatic separation, density-based separation, and other emerging technologies could enhance separation efficiency while maintaining the sustainability advantages of dry processing (Wang et al., 2015). These technological advances could potentially achieve protein purities approaching those of wet fractionation while preserving the economic and environmental benefits of dry processing.

Process monitoring and control technologies offer opportunities for optimizing air classification performance through real-time feedback and dynamic parameter adjustment. Research into in-line particle size analysis, protein content monitoring, and automated control systems could improve both separation efficiency and product consistency compared to conventional open-loop control approaches.

Novel formulation approaches for extrusion processing represent another area of opportunity, with research into functional ingredient additions, pH optimization, and processing aid utilization potentially enhancing product quality and expanding application possibilities. Understanding ingredient interactions and their effects on processing could enable development of superior products with enhanced nutritional and sensory characteristics (Liu et al., 2023).

CHAPTER 3: MATERIALS AND METHODS

3.1. Raw Materials

3.1.1. Pulse Selection and Sourcing

The raw materials for this study comprised two primary pulse varieties selected based on their protein content, commercial availability, and significance in Indian dietary patterns. Green gram (*Vigna radiata* L. Wilczek) and Black gram (*Vigna mungo* L. Hepper) were procured from established wholesale suppliers in the local market of Bengaluru, Karnataka, India. The selection criteria included uniform grain size, consistent color, absence of visible damage or foreign matter, and moisture content below 12% to ensure optimal processing characteristics. Both pulse varieties were sourced from a single supplier to maintain consistency in cultivar and post-harvest handling practices. The pulse samples were cleaned manually to remove any damaged grains, foreign seeds, and debris before processing.

Defatted soy flour was purchased from a commercial food ingredient supplier in the local market. The defatted soy flour served as a comparative protein source and was selected based on its established use in protein fractionation studies and its distinct particle size characteristics compared to pulse samples. The soy flour was procured in sealed packaging and verified for protein content (minimum 50%) and fat content (maximum 1.5%) as per supplier specifications.

3.1.2. Equipment and Instrumentation

The experimental work was conducted using specialized equipment for air classification, milling operations, and analytical characterization. The primary processing equipment included:

DP® ACM-5 Air Classifier Mill: served as the integrated milling and classification unit, combining size reduction with simultaneous air classification in a single operation.

DP® AEROVA 120 Air: Classifier was employed for independent air classification studies to optimize separation parameters.

Kjeldahl apparatus: Protein content determination was performed following standard AOAC methods for nitrogen content analysis

Radwag MA 50 R Moisture Analyzer: Moisture content analysis was conducted providing rapid and accurate moisture determination through thermogravimetric principles.

Analytical weighing balance: Sample weighing operations utilized a precision with appropriate sensitivity for both bulk and analytical samples.

Brabender Twin Lab F-20/40 Extruder: was employed for extrusion studies of fractionated materials.

Konica Minolta CR-400 Chroma Meter: Colour analysis was performed for objective colour parameter measurement in the CIE L*a*b* colour space.

Eppendorf Centrifuge 5430 G: for sample preparation and separation studies

Muffle furnace: for ash content determination and high-temperature treatments

High shear homogenizer (Ultra-Turrax T 25, IKA Co., Germany): for foaming capacity

Particle size analyser: for granulometric characterization of air classified protein fractions

Rheometer (MCR 302e, Anton Paar GmbH, Graz, Austria): for flow property measurements

Texture analyser (Ametek Brookfield Inc, USA): for mechanical property evaluation

Hot Air Tray Dryer: for controlled drying operation of extruded protein fractions.

METHODS:

3.2. Dry Fractionation Process

3.2.1. Pre-treatment and Milling

3.2.1.1. Equipment Specifications

Milling operations were conducted using the DP® ACM-5 Air Classifier Mill (DP pulveriser, India), which combines impact milling with integrated air classification in a single unit. The ACM-5 features a horizontal rotor design with adjustable classifier wheel speed (500-8000 rpm) and integrated air flow control system.

3.2.1.2. Milling Parameter Optimization

Systematic optimization of milling parameters was conducted to achieve optimal particle size distribution while minimizing starch damage and protein denaturation. The key parameters investigated included:

- Rotor speed: 1000-9000 rpm in 1000 rpm increments
- Classifier wheel speed: 1000-8000 rpm in 100 rpm increments
- Feed rate: 5-15 kg/h
- Air flow rate: 80-320 CMH

Each parameter combination was evaluated in triplicate with sample collection after steady-state operation (minimum 15 minutes). Particle size distribution was analyzed using laser diffraction particle size analyzer, and samples were collected for compositional analysis.

3.2.2. Air Classification Setup

3.2.2.1. Classifier System Configuration

Independent air classification studies were conducted using the DP® AEROVA 120 Air Classifier (DP pulveriser, India) to optimize separation parameters independently of milling effects. The AEROVA 120 features a horizontal classifier wheel design with adjustable cut point control and is equipped with:

- Variable frequency drive for classifier wheel (1000-9500 rpm)
- Precision air flow control system (80-320 CMH)
- Feeding system with gravimetric control
- Integrated cyclone separation system
- Fine and coarse fraction collection vessels

3.2.2.2. Classification Parameter Investigation

The independent variables and their ranges were:

- Classifier wheel speed: 1000-9500 rpm
- Air flow rate: 80-320 CMH
- Feed rate: 10-15 kg/h

The dependent variables (responses) evaluated included:

- Protein enrichment ratio: Protein content of fine fraction/protein content of feed material
- Protein recovery: (Protein mass in fine fraction/total protein mass) × 100
- Separation efficiency: Based on Tromp curve analysis

3.2.1.3. Particle Size Analysis Protocol

Particle size distribution analysis was performed using a laser diffraction particle size analyzer following ISO 13320 guidelines. Dry method was used and the key parameters measured included:

- D10: Particle size below which 10% of the distribution lies
- D50: Median particle size (50th percentile)
- D90: Particle size below which 90% of the distribution lies

3.3. Analytical Methods

3.3.1. Compositional Analysis

3.3.1.1. Protein Content Determination

Kjeldahl Method Protocol

Protein content determination was conducted using the Kjeldahl method (AOAC 990.03) with automated Kjeldahl apparatus. The detailed protocol included:

1. Sample Preparation: Accurately weigh 0.3 ± 0.1 g of ground sample into digestion tubes
2. Digestion: Add 10 mL concentrated H_2SO_4 and 5 g Kjeldahl catalyst tablets (K_2SO_4:$CuSO_4$:Se = 10:1:0.1). Heat at 420°C for 90-120 minutes until clear solution is obtained
3. Distillation: Add 75 mL distilled water and 50 mL 40% NaOH solution. Steam distil into 25 mL 4% boric acid solution containing mixed indicator
4. Titration: Titrate with standardized 0.1 N HCl until colour change endpoint
5. Calculation: $\%N = (V_1 - V_2) \times N \times 1.4007 / \text{Sample weight}$
   $\%\text{Protein} = \%N \times \text{conversion factor}$ (6.25 for pulses)

Quality control measures included analysis of certified reference material (pea protein) with each batch and duplicate analysis for 10% of samples.

3.3.1.2. Ash Content Analysis

Gravimetric Method (AOAC 923.03)

Ash content determination was performed using muffle furnace incineration:

1. Crucible Preparation: Pre-heat porcelain crucibles at 525°C for 1 hour, cool in desiccator, and record tare weight
2. Sample Preparation: Accurately weigh 5g of ground sample into crucibles
3. Pre-charring: Heat crucibles on hot plate until sample stops smoking (avoid ignition)
4. Ashing: Transfer to muffle furnace and heat at 525°C for 5-8 hours until white/gray ash is obtained
5. Cooling and Weighing: Cool crucibles in desiccator for 1 hour and weigh immediately
6. Calculation: Ash content (%) = ((Weight of crucible + ash) - (Weight of empty crucible))/(Sample weight) × 100

Quality control included duplicate analysis for 20% of samples and blank determinations.

3.3.2. Functional Properties Assessment

3.3.2.1 Water and Oil Absorption Capacity

Water Absorption Capacity (WAC): Following modified AACC Method 56-30, 1 g sample was dispersed in 10 mL distilled water, vortexed for 1 minute, allowed to stand for 30 minutes with intermittent stirring, then centrifuged at 3000 × g for 25 minutes. The supernatant was drained and pellets weighed.

WAC (g/g) = (Weight of sediment - Sample weight)/Sample weight

Oil Absorption Capacity (OAC): Using refined sunflower oil instead of water following the same protocol as WAC.

3.3.2.2. Foaming Properties

Foaming Properties: 1% (w/v) protein suspension (100 mL) was whipped using high-speed homogenizer at 9000 rpm for 5 minutes. Foam capacity and stability were measured at 0, 10, 20, 30, 60 minutes.

Foam Capacity (%) = (Volume after whipping - Initial volume)/Initial volume × 100
Foam Stability (%) = (Foam volume at time t/Initial foam volume) × 100

3.4. Extrusion Processing

3.4.1. Low Moisture Extrusion

3.4.1.1. Extruder Configuration

Low moisture extrusion trials were conducted using a twin-screw extruder equipped with co-rotating intermeshing screws. The extruder configuration included:

- Screw Configuration: Two-element shear configuration with L/D ratio of 40/20
- Temperature Control: Six independently controlled heating zones (Z1-Z6)
- Feed System: Screw feeder
- Die Configuration: Circular die with 2mm diameter
- Cutting System: Variable speed rotary knife system
- Monitoring Systems: Real-time torque, temperature, and pressure monitoring

3.4.1.2. Process Parameters and Conditions

Feed preparation:
Sample blends were prepared with different ratios of defatted soy flour (DSF), Black gram flour, and Green gram flour. Feed moisture content was adjusted to 22% (wet basis) using distilled water and mixed thoroughly to ensure uniform distribution.

Operating Conditions:

- Screw Speed: 280 rpm
- Throughput: 4.5 kg/h
- Cutting Speed: 250 rpm
- Temperature Profile:
    - Zone 1 (Feed): 50°C
    - Zone 2: 90°C

- Zone 3: 110°C
- Zones 4-6 (Die): 125°C

Process Control:
Steady-state operation was maintained for a minimum of 10 minutes before sample collection. Process parameters including torque, melt temperature, and melt pressure were continuously monitored and recorded at 30-second intervals. Extrudate samples were collected and immediately dried using a tray dryer at 60°C for 8-12 hours to achieve final moisture content below 10%.

3.5. Product Characterization

3.5.1. Physical Properties Measurement

3.5.1.1. Bulk density

Following the method of Ojokoh et al. (2015), ground extruded samples were gently filled into a pre-weighed 50 mL graduated cylinder and tapped repeatedly on a laboratory bench until no further volume reduction occurred. The settled volume was recorded and sample weight determined by difference.

Bulk Density (g/mL) = Weight of sample (g) / Settled volume (mL)

3.5.1.2. Hydration

Water Absorption Capacity (WAC): Following the method of Singh et al. (2007) with modifications, 10 g of extruded sample was immersed in 100 mL distilled water at room temperature (25 ± 2°C) for 5 minutes. The hydrated sample was then strained through a fine mesh strainer (0.5 mm) for 2 minutes to remove excess water and immediately weighed.

WAC (g/g) = (Weight of hydrated sample - Initial sample weight) / Initial sample weight

3.5.1.3. Color Analysis

Objective color measurement was performed using Konica Minolta CR-400 Chroma Meter with standard illuminant D65 and 2° observer angle. Color parameters measured included:

- L^*: Lightness (0 = black, 100 = white)
- a^*: Green-red chromaticity (-60 = green, +60 = red)
- b^*: Blue-yellow chromaticity (-60 = blue, +60 = yellow)
- ΔE^*: Total color difference calculated as $\sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$

Calibration was performed using manufacturer-supplied white calibration plate before each measurement session.

3.5.1.4. Textural Analysis

Texture profile analysis was conducted using a Texture Analyzer equipped with appropriate probes for different product forms:

Parameters measured: Hardness, cohesiveness, springiness index, chewiness, and resilience.

CHAPTER 4: RESULTS AND DISCUSSIONS

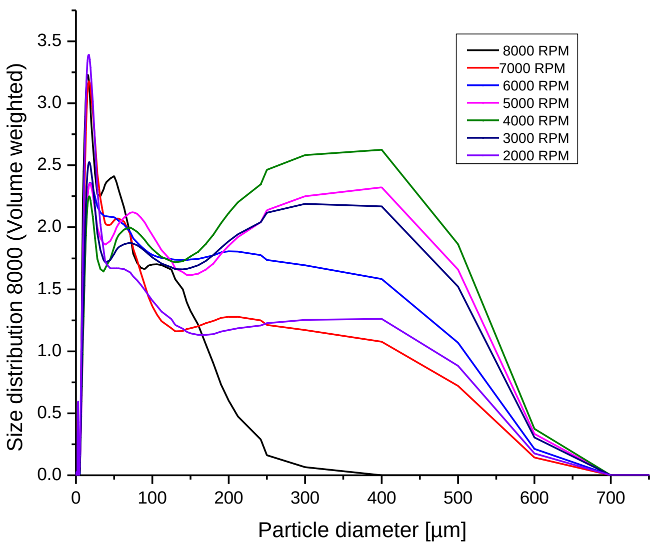
4.1. Raw Material Characterization

The proximate composition analysis of raw materials established baseline characteristics essential for understanding fractionation behaviour and process optimization. Black gram (*Vigna mungo*) exhibited a protein content of 23.06 ± 0.59%, total dietary fiber of 11.93 ± 0.26% (comprising 7.58 ± 0.13% insoluble and 4.35 ± 0.15% soluble fiber), fat content of 1.69 ± 0.12%, and ash content of 3.17 ± 0.02%. The moisture content was maintained at 9.16 ± 0.35%, which falls within the optimal range for dry processing applications. Green gram (*Vigna radiata*) demonstrated comparable protein content at 23.88 ± 0.61%, with slightly lower total dietary fiber at 9.37 ± 0.38% (7.75 ± 0.39% insoluble, 1.62 ± 0.19% soluble), fat content of 1.35 ± 0.20%, and ash content of 3.04 ± 0.03%. The moisture content was 9.77 ± 0.67%, suitable for mechanical processing operations. These compositional characteristics align with published values for Indian pulse varieties and confirm the suitability of both materials for protein enrichment through dry fractionation (Pelgrom et al., 2013; Schutyser & van der Goot, 2011).

4.2. Milling Optimization

4.2.1. Effect of Mill RPM on Particle Size Distribution - Black gram

Initial milling experiments with Black gram demonstrated the critical relationship between mill rotor speed and particle size reduction efficiency. When operating with a constant internal classifier speed of 2,000 RPM and airflow of 328 CMH, increasing mill RPM from 1,000 to 8,000 RPM resulted in systematic changes in particle size distribution.



Graph 1. Size distribution (volume weighted) vs Particle diameter(µm) for black gram at different milling RPMs (2000 RPM internal classifier)

Table 4.1: Black gram Milling at Different RPM (Internal Classifier @ 2000 RPM, Airflow 328 CMH)

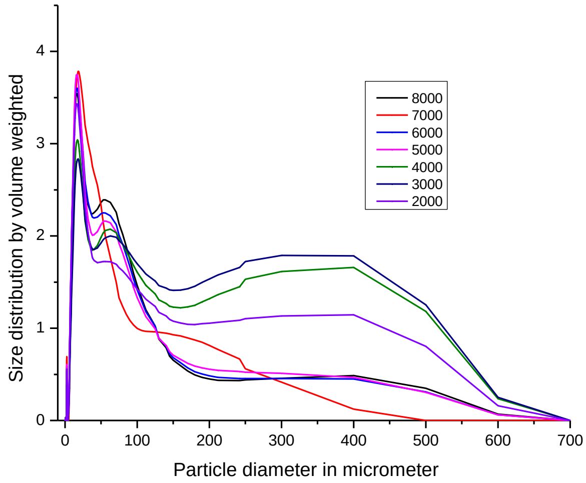
Mill RPM	Black gram whole flour	Input wt.(kg)	Output wt. (kg)	D50 particle size (µm)	D10 particle size (µm)	D90 particle size (µm)	Mean size (µm)
1000	Black gram whole	1	0.404	0	0	0	0
2000	Black gram whole	1	0.768	27.76	8.86	250.93	86.09
3000	Black gram whole	1	0.864	54.44	10.73	326.30	124.39
4000	Black gram whole	1	0.944	67.22	11.35	347.7	139.36
5000	Black gram whole	1	0.914	59.69	11.60	334.80	129.21
6000	Black gram whole	1	0.932	47.9	10.5	285.7	108.68
7000	Black gram whole	1	1.012	31.38	9.53	230.76	83.04
8000	Black gram whole	1	0.898	26.48	9.02	140.4	47.2

At 1,000 RPM, material recovery was limited to 40.4%, indicating insufficient mechanical energy for effective size reduction. Optimal particle size reduction was achieved at 4,000 mill RPM, producing a median particle size (D_{50}) of 67.22 µm with 94.4% material recovery, representing the best balance between size reduction efficiency and throughput.

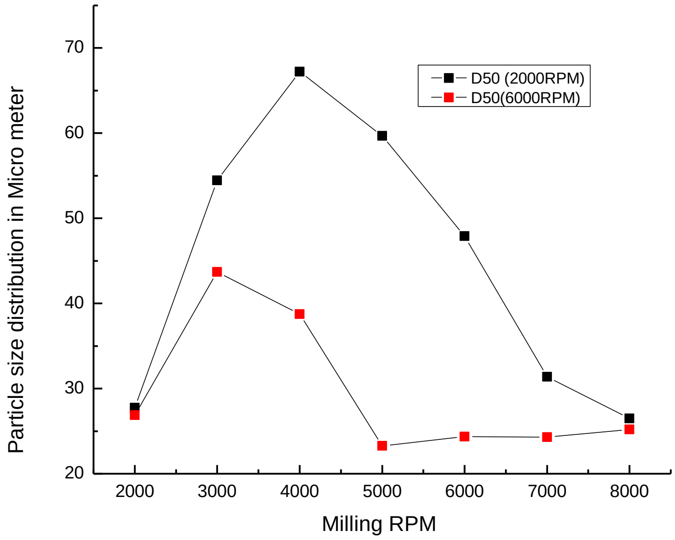
When the internal classifier speed was increased to 6,000 RPM while maintaining the same airflow conditions, different trends emerged:

Table 4.2: Black gram Milling at Different RPM (Internal Classifier @ 6000 RPM, Airflow 328 CMH)

Mill RPM	Black gram whole flour	Input wt.(kg)	Output wt. (kg)	D50 particle size (µm)	D 10 particle size (µm)	D 90 particle size (µm)
1000	Black gram whole	1	0 .342	0	0	0
2000	Black gram whole	1	0.710	26.89	8.82	234.98
3000	Black gram whole	1	0.866	43.71	10.47	301.66
4000	Black gram whole	1	0.924	38.75	10.06	291.91
5000	Black gram whole	1	0.924	23.27	8.56	118.6
6000	Black gram whole	1	0.928	24.35	8.71	113.0
7000	Black gram whole	1	0.988	24.29	9.1	112.14
8000	Black gram whole	1	0.924	25.19	8.86	113.2



Graph 2. Size distribution (volume weighted) vs Particle diameter(µm) for Black gram at different milling RPMs (6000 RPM internal classifier)



Graph 3. Size distribution (volume weighted) of D50 vs different milling RPMs (2000 and 6000 RPM of internal classifier)

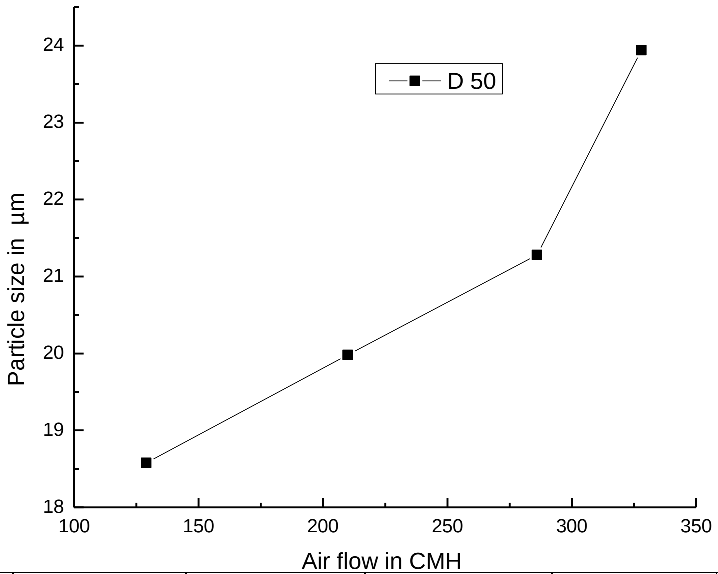
Mill speeds of 5,000-8,000 RPM consistently produced finer particle distributions, with D_{50} values ranging from 23.27 to 25.19 µm, demonstrating improved classification efficiency at higher internal classifier speeds. This finding supports the principle that increased centrifugal forces enhance particle separation by overcoming inter-particle adhesion forces common in pulse flours (Bishop & Cogan, 2022).

4.2.2. Optimization of Integrated Milling-Classification Parameters

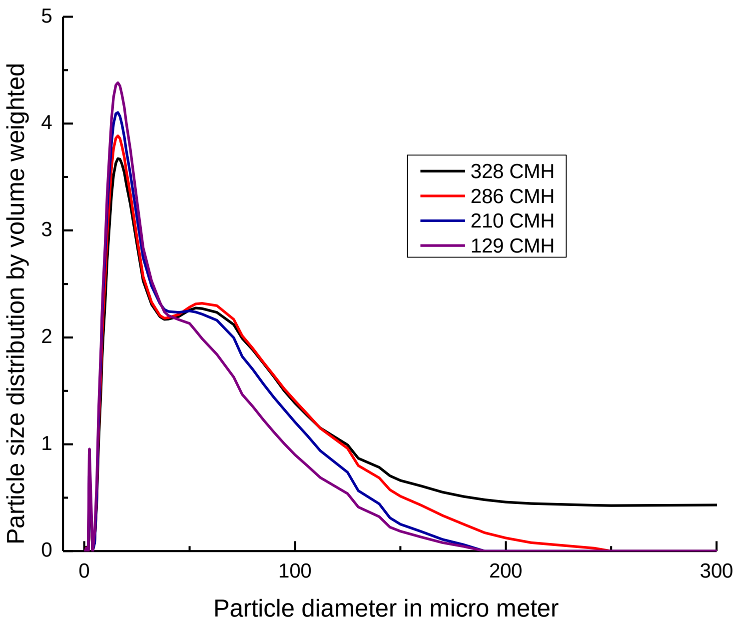
Based on preliminary experiments, the integrated approach using 8,000 mill RPM combined with 6,000 internal classifier RPM was selected for systematic optimization. Varying the airflow from 328 to 82 CMH revealed that lower airflow rates consistently produced finer particles:

Table 4.3: Black gram Milling at 8000 Mill RPM and 6000 Internal Classifier Speed at Different Airflow Rates

Sample	Air flow (CMH)	Input wt. (kg)	Output wt. (kg)	D 50 (µm)	D 10 (µm)	D 90 (µm)
Black gram	328	1	1.014	23.94	8.77	110.89
Black gram	286	1	0.947	21.28	8.33	81.02
Black gram	210	1	0.906	19.98	8.12	71.86
Black gram	129	1	0.818	18.58	7.58	63.70
Black gram	100	1	0	0	0	0
Black gram	82	1	0	0	0	0



Graph 4. Size distribution (volume weighted) of D50 vs different airflow (8000 milling RPM and 6000 RPM of internal classifier)



Graph 5. Particle size distribution (volume weighted) vs different airflow (8000 milling RPM and 6000 RPM of internal classifier)

The finest distribution (D_{50} = 18.58 µm) was achieved at 129 CMH. This relationship demonstrates the inverse correlation between airflow velocity and particle fineness, where reduced air velocity allows for more selective particle entrainment and improved separation of fine, protein-rich particles (Barakat et al., 2020).

4.3. Air Classification Optimization - Black gram

4.3.1. Single Parameter Effects

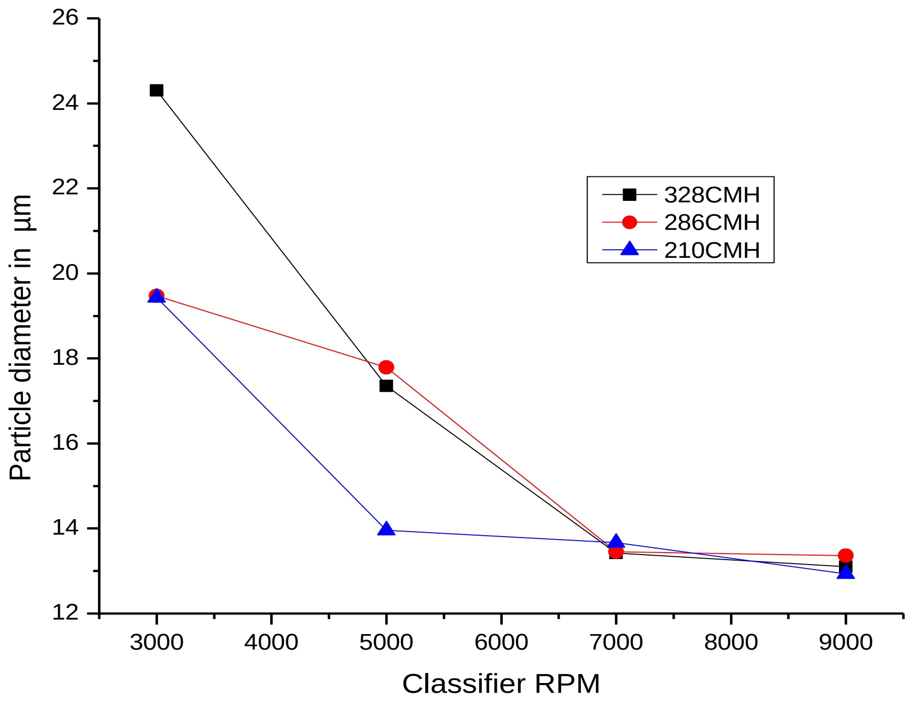
4.3.1.1. Fine Fraction Analysis

Systematic investigation of classifier wheel speed effects revealed strong correlations between rotational speed and both particle size selectivity and protein enrichment efficiency. The material milled at optimal conditions was subjected to air classification at various classifier RPMs and airflow rates. Initial milling experiments were performed by varying the milling RPM while maintaining the internal classifier RPM at a constant value of either 2,000 or 6,000, in order to assess the effect of milling speed on particle size reduction. Notably, milling at an operating condition of 4,000 mill RPM in combination with an internal classifier RPM of 2,000 yielded material with a median particle size (d_{50}) of 67 µm. This milled material was subsequently subjected to systematic classification trials, wherein different classifier RPMs and airflow configurations were evaluated to identify the optimal conditions for achieving the targeted particle size distributions.

Table 4.4: Fine Fraction Particle Sizes at Different Classifier RPM and Airflow Rates

Classifier RPM	Air flow in CMH	Input Wt. in Kg	Output Wt. in kg of fine Fraction	D 50 (µm)	D 10 (µm)	D 90 (µm)

9000	328	1	0.045	13.1	6.42	16.17
7000	328	1	0.146	13.42	6.63	16.37
5000	328	1	0.208	17.35	7.91	20.72
3000	328	1	0.193	24.3	9.14	32.26
9000	286	1	0.082	13.36	6.18	36.03
7000	286	1	0.06	13.45	6.36	35.22
5000	286	1	0.208	17.79	8.06	39.18
3000	286	1	0.236	19.47	8.23	50.5
9000	210	1	0.012	12.93	6.09	32.99
7000	210	1	0.044	13.67	6.28	38.42
5000	210	1	0.034	13.96	6.84	28.98
3000	210	1	0.161	19.43	8.25	48.64



Graph 6. Particle size distribution (volume weighted) of D50 vs classifier RPM (at three different classifier RPM and airflow) of fine fractions.

Table 4.5 Selected the fine fractions where yield is more than 150 grams and did protein estimation.

Classifier	Air	Input	Output	D 50	D 10	D 90	Fine	Coarse

RPM	flow	Wt. in Kg	Wt. in kg Fine Fraction	(µm)	(µm)	(µm)	Protein content in %	protein content in %
5000	328	1	0.208	17.35	7.91	20.72	20.43	24.93
3000	328	1	0.193	24.3	9.14	32.26	23.61	25.88
5000	286	1	0.208	17.79	8.06	39.18	21.52	27.01
3000	286	1	0.236	19.47	8.23	50.5	23.11	26.19
7000	328	1	0.146	13.42	6.63	16.37	26.47	24.06
3000	210	1	0.161	19.43	8.25	48.64	25.46	25.39

Milling conditions of 4,000 mill RPM combined with an internal classifier speed of 2,000 RPM did not yield the desired median particle size (d_{50}) of 20 µm, and the protein content of the fine fractions remained limited, ranging only from 24% to 28%. Furthermore, classification of the material produced under these parameters did not result in effective particle separation. To address these limitations, the milling parameters were intensified to 8,000 mill RPM and 6,000 internal classifier RPM in order to achieve a finer particle size distribution more suitable for downstream processing. The resulting optimized milled material was then subjected to systematic classification trials; wherein various classifier RPMs and three distinct airflow rates were employed to investigate their effects on particle size fractionation and protein enrichment.

4.3.2. Optimized Processing Results

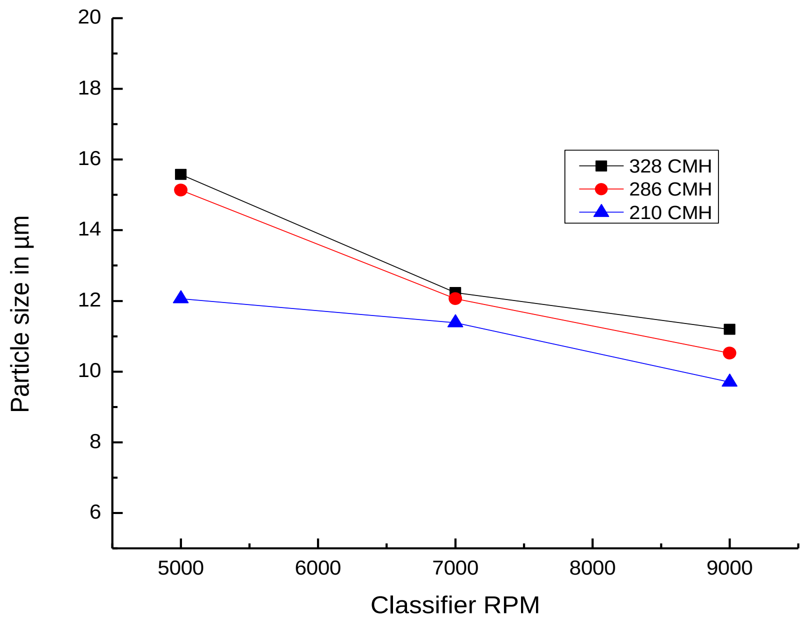
For subsequent experiments, milling was carried out at 8,000 RPM with an internal classifier speed of 6,000 RPM, and an initial airflow setting of 210 CMH. This milled flour was then classified at four distinct classifier RPMs, each tested across three separate airflow rates to systematically evaluate the impact of these parameters on particle size distribution. The resulting whole flour exhibited a d_{10} of 7.58 µm, a d_{50} of 20.06 µm, and a d_{90} of 77.58 µm, reflecting the particle size range obtained under these optimized milling and classification conditions.

Following parameter intensification to 8,000 mill RPM and 6,000 internal classifier RPM, superior fractionation results were achieved:

Classifier RPM	Air flow	Input Weight in Kg	Output Weight in kg Fine Fraction	D 10 (µm)	D 50 (µm)	D 90 (µm)	Protein content in %
9000	328	1	0.19	5.16	11.19	22.46	31.70

7000	328	1	0.586	5.96	12.23	24.50	27.62
5000	328	1	0.590	6.75	15.53	32.66	21.96
9000	286	1	0.16	5.09	10.52	23.05	36.73
7000	286	1	0.28	4.99	12.06	26.86	27.18
5000	286	1	0.688	7.06	15.13	30.62	23.64
9000	210	1	0.07	4.49	9.70	19.43	36.33
7000	210	1	0.116	5.50	11.38	25.42	34.15
5000	210	1	0.193	6.00	12.06	24.33	24.86

Table 4.6: Fine Fraction Results - Optimized Processing (8000 Mill RPM, 6000 Internal Classifier)

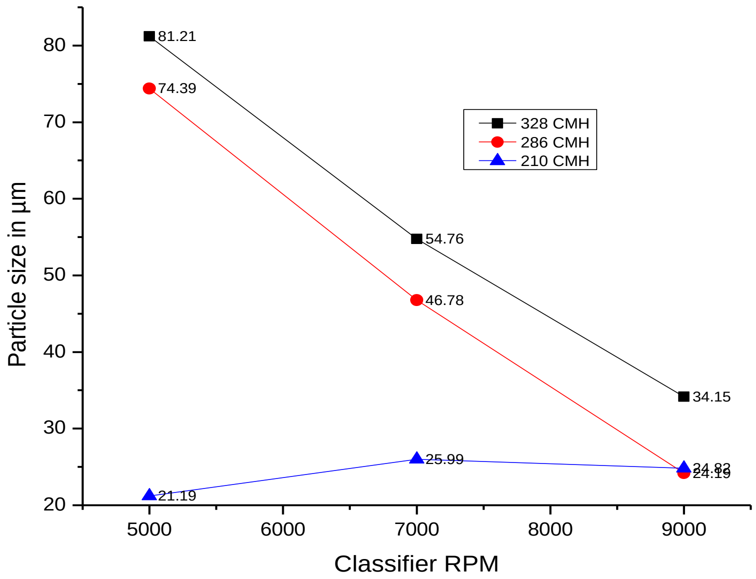


Graph 7. Particle size distribution (volume weighted) of D50 vs classifier RPM(at three different classifier RPM and airflow) of fine fractions.

Table 4.7: Coarse Fraction Results - Optimized Processing (8000 Mill RPM, 6000 Internal Classifier).

Classifier RPM	Air flow	Input Wt. in Kg	Output Wt. in kg Coarse Fraction	D 10 (µm)	D 50 (µm)	D 90 (µm)	Protein content in %
9000	328	1	0.63	11.51	34.15	170.70	22.85
7000	328	1	0.506	16.19	54.76	190.35	24.09

5000	328	1	0.351	36.50	81.21	238.78	26.71
9000	286	1	0.78	10.28	24.19	126.11	20.50
7000	286	1	0.654	14.38	46.78	101.98	25.13
5000	286	1	0.350	32.81	74.39	232.32	26.88
9000	210	1	0.75	10.60	24.82	105.75	20.87
7000	210	1	0.69	11.11	25.99	104.36	23.35.
5000	210	1	0.433	11.99	21.19	119.42	26.10



Graph 8. Particle size distribution (volume weighted) of D50 vs classifier RPM (at three different classifier RPM and airflow) of coarse fractions.

At the highest classifier speed (9,000 RPM), fine fractions exhibited the smallest median particle size (D_{50} = 9.70 µm) and achieved protein content of 36.33% at 210 CMH airflow, representing a significant improvement over initial processing condition.

The classification experiments demonstrated that increasing the classifier RPM led to a reduction in particle size and enhanced protein enrichment in the fine fractions. At the highest classifier speed of 9,000 RPM combined with the lowest airflow of 210 CMH, the finest particles with a d_{50} as low as 4.49 µm and the highest protein content of 36.33% were obtained. Conversely, lower classifier speeds and higher airflow resulted in coarser fine fractions with protein content dropping below 25%. The coarse fractions showed larger particle sizes, with d_{50} values ranging from 21.19 to 81.21 µm, and consistently lower protein contents, typically between 20% and 27%, indicating enrichment in starch and fiber. Yields of fine fractions decreased with higher classifier RPM, while coarse fraction yields increased, confirming effective separation by centrifugal and airflow forces. These results highlight that optimizing classifier RPM and airflow is critical to achieving targeted particle size distributions and protein enrichment, supporting the efficacy of dry fractionation for producing specialized pulse protein and fiber ingredients.

Following these results, we proceeded to classify material milled at 8,000 RPM with an internal classifier speed also set at 8,000 RPM, conducting trials at three different airflow rates to further evaluate the impact on particle size distribution and protein enrichment.

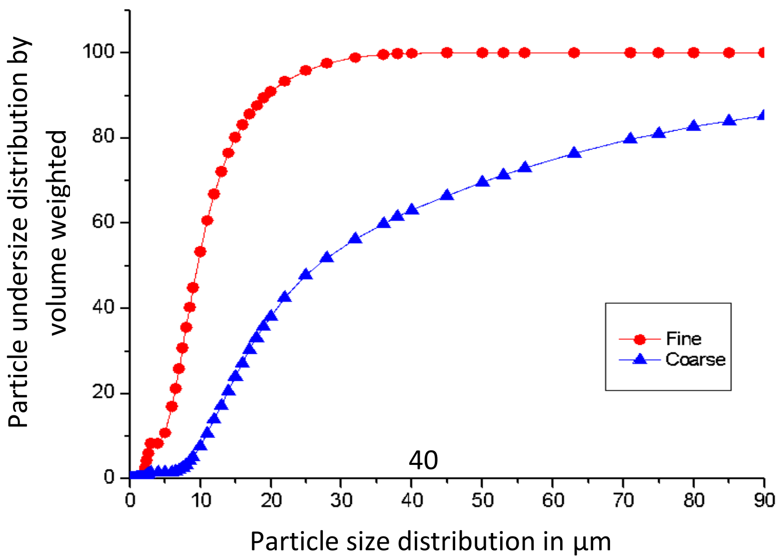
4.3.3. Pilot-Scale Processing Validation - Black gram

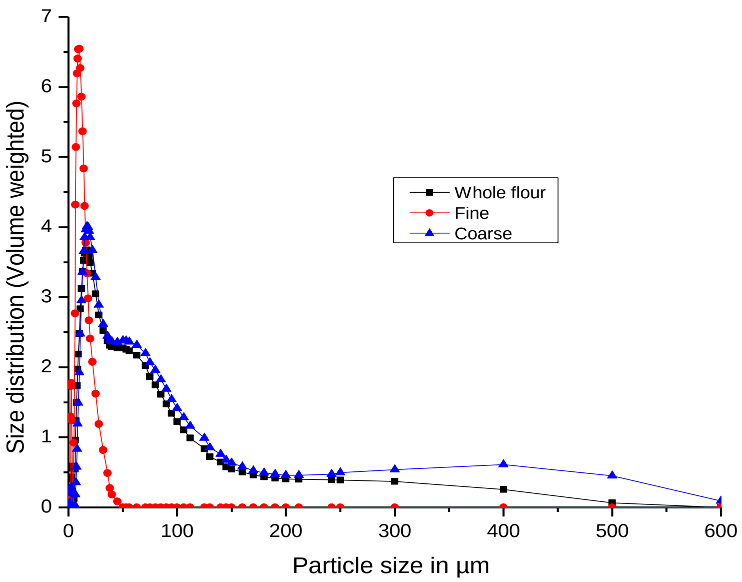
Pilot-scale processing validation was conducted with 50 kg of Black gram under optimized conditions (8,000 mill RPM, 8,000 internal classifier RPM, 328 CMH initial airflow, followed by classification at 9,000 RPM with 286 CMH airflow):

Table 4.8: Pilot-Scale Black gram Processing Results (50 kg batch)

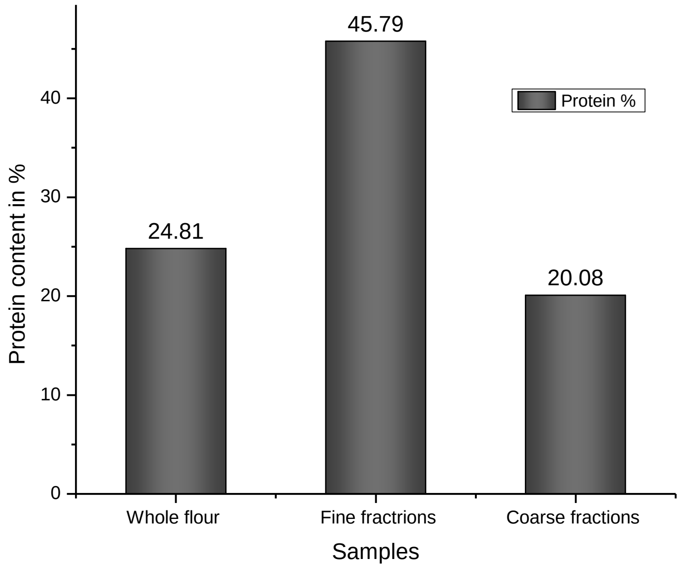
Samples	Input Weight in Kg	Output weight of fractions in kg	D 10 (µm)	D 50 (µm)	D 90 (µm)	Protein content in %
WHOLE flour	50	47.35	8.32	22.54	92.64	24.81
Fine fractions	47	5.62	4.65	9.59	19.36	45.79
Coarse fractions	47	41.8	10.74	26.55	122.27	20.08
DUST	50	4	2.66	7.54	17.42	43.37

Graph 9. Undersize distribution (volume weighted) vs particle diameter at 9000 classifier RPM at 286CMH of fine and coarse fractions.





Graph 10. Particle size distribution (volume weighted) vs particle diameter at 9000 classifier RPM at 286CMH of whole flour, fine and coarse fractions.



Graph 11. Protein content in percentage of whole flour, fine fraction and coarse fraction.

The optimized Black gram fractionation process, employing 8,000 mill RPM with 8,000 internal classifier RPM at 328CMH airflow and subsequent classification at 9,000 RPM with 286CMH airflow, efficiently processed 50kg of raw material. This setup achieved substantial results: an 85.7% reduction in particle size (from initial 67µm to final 9.59µm D_{50}), 84.6% protein enrichment in fine fractions (45.79% versus 24.81% in whole flour), and 94.7% material recovery efficiency. The process yielded 5.62kg of high-protein fine fractions (11.9% yield) and 4.00kg of high-protein dust (43.37% protein content), with 19.3% of processed material reaching protein concentrations exceeding 43%, all while maintaining uniform particle size distributions and effective separation of coarse, starch-rich fractions. This optimization demonstrates that industrial-scale, chemical-free dry fractionation can deliver significant value addition, transforming basic pulse materials into premium high-protein ingredients suitable for diverse applications in food and nutraceutical industries.

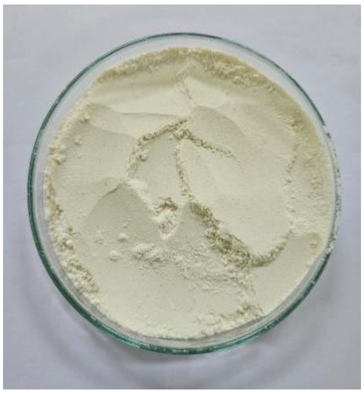


Figure 1. Black gram whole flour


Figure 2. Black gram coarse fractions


Figure 3. Black gram fine fractions

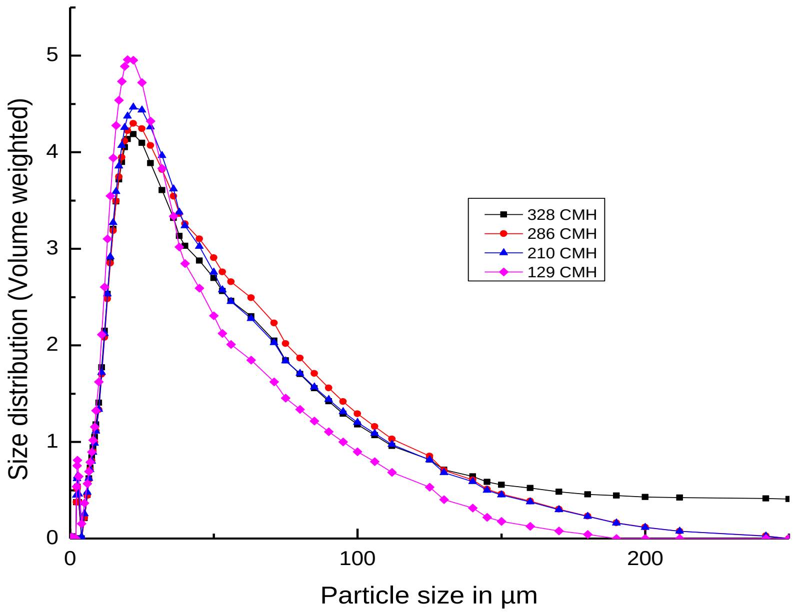
4.4. Air classification optimization- green gram

4.4.1. Initial Processing Trials

We investigated the effect of airflow variation on particle size reduction by milling Green gram at a constant 8,000 RPM for both the mill and internal classifier. Each 1 kg batch was subjected to a range of decreasing airflow settings (from 328 CMH down to 82 CMH), with the goal of systematically evaluating how lower airflow rates influence flour yield and size classification.

Table 4.9: Green gram Milling Results (8000 Mill RPM, 8000 Internal Classifier RPM at 129,210, 286,328 CMH)

Sample	Air flow (CMH)	Input wt. (kg)	Output wt. (kg)	D 10 µm	D 50 µm	D 90 µm
Green gram	328	1	.920	19.99	26.52	92.23
Green gram	286	1	.856	10.13	26.32	77.12
Green gram	210	1	.659	9.78	25.46	74.83
Green gram	129	1	.550	8.49	22.05	62.55
Green gram	100	1	0	0	0	0
Green gram	82	1	0	0	0	0

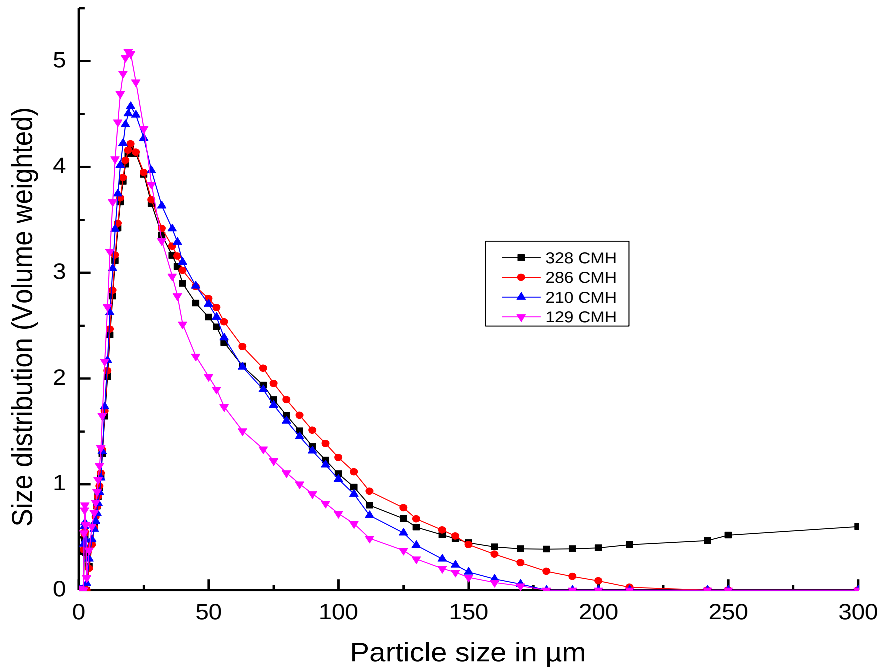


Graph 12. Particle size distribution (volume weighted) vs particle diameter (8000 milling RPM and 8000 internal classifier RPM) at 328, 286, 210, 129 CMH of whole flour

The results show that as airflow decreased from 328 to 129 CMH, there was a steady reduction in both output weight and particle sizes (D_{10} and D_{50}), with the smallest median particle size (D_{50} = 22.05 µm) achieved at the lowest measured airflow (129 CMH). These findings indicate that reduced airflow enhances fine particle separation, but also results in lower flour yields due to more selective recovery of smaller particles. Attempts to further optimize by switching to 8,000 mill RPM and 6,000 internal classifier RPM, as previously done for Black gram, reflected the ongoing challenge of consistently achieving a d_{50} value near 20 µm for Green gram. This underscores the pulse-specific variability in response to air classification and highlights the need for process adjustments beyond simple parameter replication to achieve targeted size specifications in different pulse flours.

Table 4.10: Green gram Milling at Different Airflow Rates (8000 Mill RPM, 6000 Internal Classifier)

Sample	Air flow (CMH)	Input wt. (kg)	Output wt. (kg)	D 10 µm	D 50 µm	D 90 µm
Green gram	328	1	.918	9.55	26.58	80.05
Green gram	286	1	.840	8.83	24.03	68.44
Green gram	210	1	.661	6.77	20.48	56.42
Green gram	129	1	.585	5.57	22.03	87.63
Green gram	100	1	0	0	0	0
Green gram	82	1	0	0	0	0



Graph 13. Particle size distribution (volume weighted) vs particle diameter (8000 milling RPM and 6000 internal classifier RPM) at 328, 286, 210, 129 CMH of whole flour

When Green gram was milled at 8,000 mill RPM and 6,000 internal classifier RPM across various airflow settings, the median particle size (D_{50}) of the resulting fractions demonstrated a clear dependence on airflow rate. At the highest airflow (328 CMH), the D_{50} was 26.58 µm, while lower airflow rates steadily reduced the D_{50}: 24.03 µm at 286 CMH, 20.48 µm at 210 CMH, and 22.03 µm at 129 CMH. The smallest median particle size obtained in this series of experiments approached the critical 20 µm threshold (at 210 CMH), which has been identified in previous research as essential for maximizing protein enrichment during air classification of pulse flours. This trend confirms that decreasing airflow promotes finer particle selection, driving D_{50} values toward the targeted specification needed for effective protein separation. As demonstrated, obtaining a D_{50} near 20 µm is a crucial benchmark for boosting protein content in the fine fractions, supporting literature recommendations for optimal pulse fractionation (Rivera, 2022; Epic Powder Machinery, 2024).

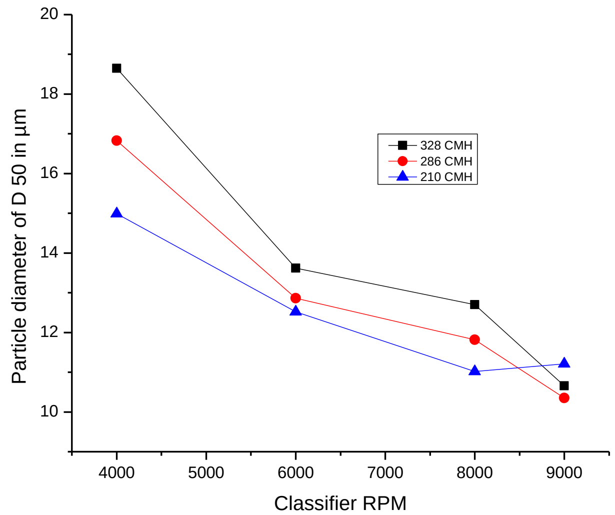
The smallest median particle size (D_{50} = 20.48 µm) was achieved at 210 CMH airflow, which approached the critical 20 µm threshold essential for maximizing protein enrichment during air classification.

4.4.3. Optimized Green gram Classification Results

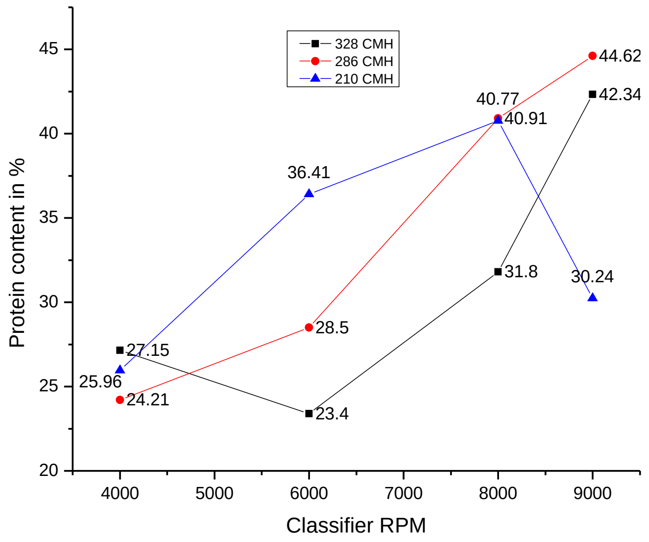
Using material milled at 8,000 RPM with 6,000 internal classifiers at 210 CMH airflow, systematic classification trials were conducted:

Table 4.11: Green gram Fine fractions result of selected 8000mill rpm and 6000 internal classifier RPM at air flow of 210 CMH and classified at different classifier rpm at different Air flows.

Classifier RPM	Air flow in CMH	Input Weight in Kg	Output Weight in kg of fine Fraction	D 10 µm	D 50 µm	D 90 µm	Protein Content %
9000	328	1	0.230	4.13	10.66	24.72	42.34
8000	328	1	0.184	4.25	12.70	27.56	31.80
6000	328	1	0.320	4.31	13.62	27.63	23.40
4000	328	1	0.446	9.23	18.65	38.47	27.15
9000	286	1	0.104	3.44	10.35	25.04	44.62
8000	286	1	0.082	4.13	11.82	26.19	40.91
6000	286	1	0.286	5.24	12.86	26.87	28.50
4000	286	1	0.310	8.49	16.83	34.61	24.21
9000	210	1	0.230	4.22	11.21	33.77	30.24
8000	210	1	0.164	3.89	11.02	24.36	40.77
6000	210	1	0.068	4.71	12.52	27.68	36.41
4000	210	1	0.196	6.73	14.99	36.72	25.96



Graph 14. Particle size distribution (volume weighted) of D50 vs Classifier RPM (8000 milling RPM and 6000 internal classifier RPM) at 328, 286, 210CMH of fine fractions



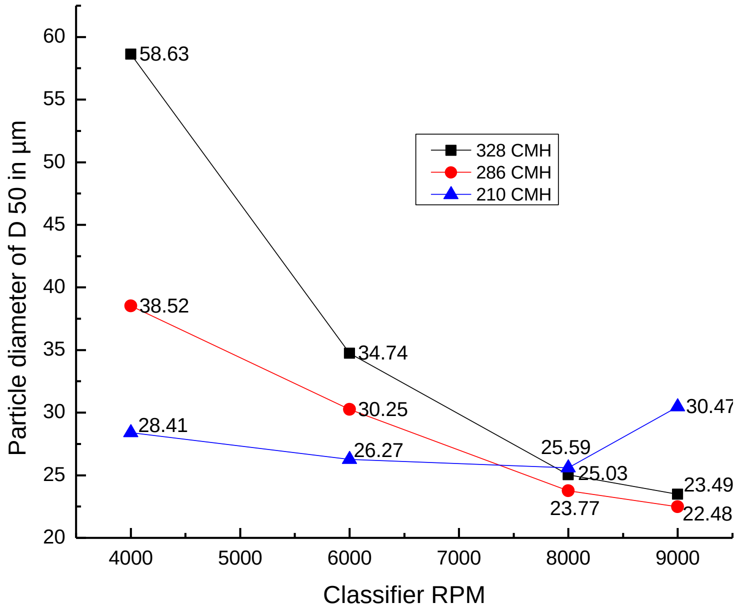
Graph 15. Protein content vs particle diameter (8000 milling RPM and 6000 internal classifier RPM) at 328, 286, 210 CMH of fine fractions

Green gram demonstrated superior fractionation performance, with the highest protein concentration (44.62%) achieved at 9,000 RPM classifier speed with 286 CMH airflow, representing an 87.0% protein enrichment over whole flour (23.85%).

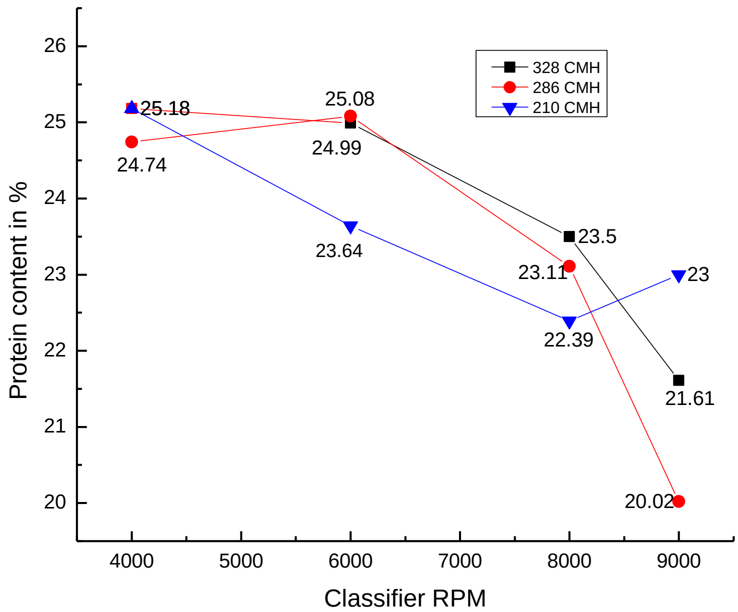
Table 4.12: Coarse fractions result of selected 8000mill rpm and 6000 internal classifier RPM at air flow of 210 CMH and classified at different classifier rpm at different air flows.

Classifier RPM	Air flow in CMH	Input Weight in Kg	Output Weight in kg of Coarse Fraction	D 10 µm	D 50 µm	D 90 µm	Protein Content %
9000	328	1	0.754	12.28	23.49	73.93	21.61
8000	328	1	0.770	12.96	25.03	71.43	23.50
6000	328	1	0.610	15.94	34.74	124.34	24.99
4000	328	1	0.650	22.41	58.63	177.80	25.18
9000	286	1	0.986	11.8	22.48	63.59	20.02
8000	286	1	0.796	12.72	23.77	68.20	23.11
6000	286	1	0.672	14.79	30.25	107.85	25.08
4000	286	1	0.784	19.48	38.52	356.38	24.74

9000	210	1	0.840	12.84	30.47	133.32	23.00
8000	210	1	0.654	12.64	25.59	104.10	22.39
6000	210	1	0.690	12.90	26.27	92.83	23.64
4000	210	1	0.811	13.22	28.41	91.10	25.18



Graph 16. Particle size distribution (volume weighted) of D50 vs Classifier RPM (8000 milling RPM and 6000 internal classifier RPM) at 328, 286, 210CMH of coarse fractions.



Graph 17. Protein content vs particle diameter (8000 milling RPM and 6000 internal classifier RPM) at 328, 286, 210 CMH of coarse fractions

The data in Table 4.12 presents the fine and coarse fractionation results when Green gram was milled at 8,000 mill RPM and 6,000 internal classifier RPM with an airflow of 210 CMH, then classified at various classifier RPMs and airflow conditions.

For the fine fractions, lower classifier RPMs (4,000 RPM) consistently resulted in coarser particles, with D_{50} values ranging from 15.99 to 22.65 µm and relatively lower protein contents (25.96% to 27.15%). Increasing the classifier RPM to 6,000, 8,000, and 9,000 RPM significantly reduced the median particle size of fine fractions, with D_{50} ranging approximately from 10.35 to 14.21 µm, and protein content improving markedly, reaching the highest recorded protein concentration of 44.62% at 9,000 RPM and 286 CMH airflow. These trends demonstrate that higher classification speeds enhance separation efficiency, enabling finer particle enrichment and better protein concentration in the fine fraction.

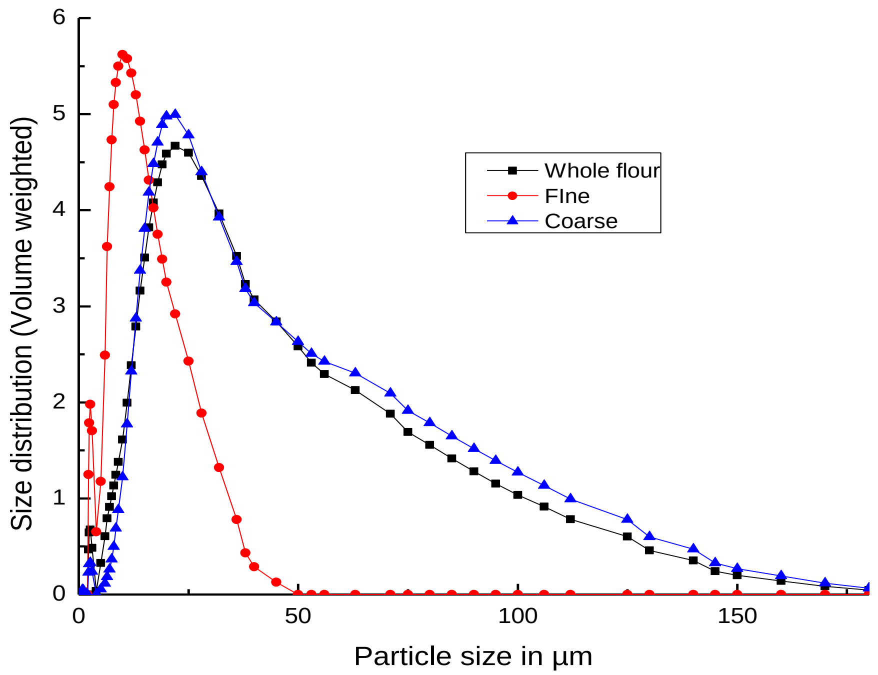
Conversely, the coarse fractions showed much larger particle sizes (D_{50} up to 58.63 µm) and consistently lower protein contents across all conditions, confirming effective segregation of starch- and fiber-rich particles from the protein-rich fines. The protein content in the coarse fraction mostly remained between approximately 20% to 25%, highlighting a clear compositional difference from the fine fractions.

Based on these findings, the highest protein-enriched fine fraction was produced at a classifier speed of 9,000 RPM and airflow of 286 CMH, which provided an optimal balance between particle size reduction and protein concentration. Leveraging this optimized milling and classification protocol, a pilot-scale milling of 60 kg of green gram was conducted to generate sufficient protein-enriched material for extrusion processing.

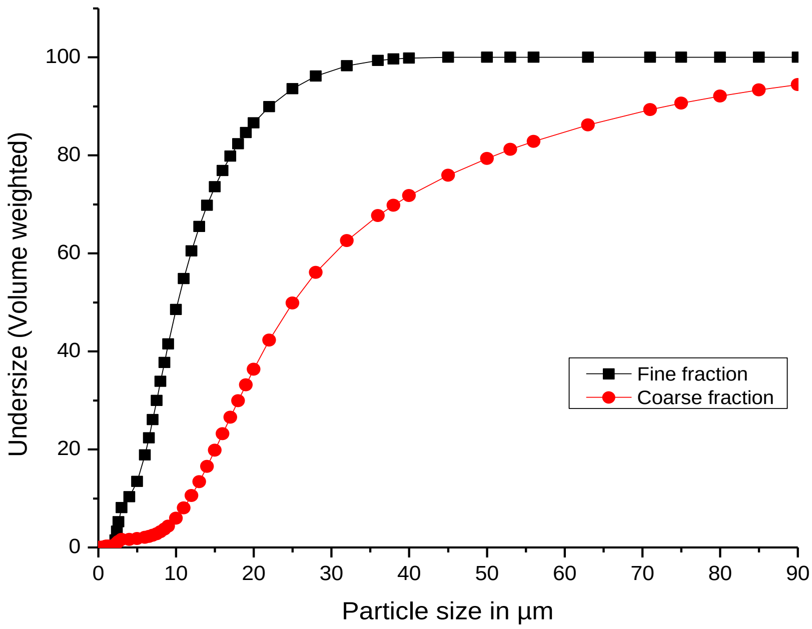
4.4.4. Pilot-Scale Green gram Processing

Table 4.13: Pilot-Scale Green gram Processing Results (60 kg batch)

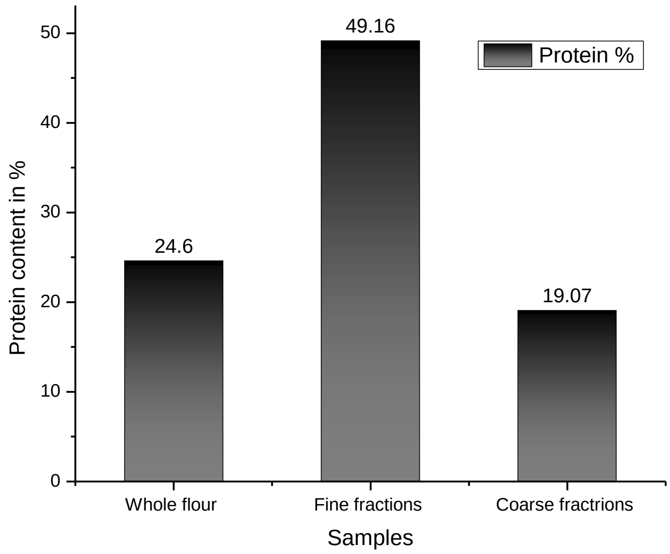
Samples	Input Weight in Kg	Output weight of fractions in kg	D 10 µm	D 50 µm	D 90 µm	Protein content
Whole flour	60	53	6.77	20.48	56.42	24.60
Fine fractions	53	5.38	3.44	10.35	25.04	49.16
Coarse fractions	53	46.73	11.80	22.48	63.59	19.07
Dust	60	3.94	2.91	9.58	24.10	45.11



Graph 18. Particle size distribution (volume weighted) vs particle diameter (8000 milling RPM and 6000 internal classifier RPM 210CMH) at 9000 classifier RPM and 286 CMH of whole flour, fine and coarse fractions.



Graph 19. Undersize distribution (volume weighted) vs particle diameter at 9000 classifier RPM at 286CMH of fine and coarse fractions.



Graph 20. Protein content vs samples (whole flour, fine and coarse fractions).

In this experiment, 60 kg of Green gram was milled at 8,000 mill RPM with a 6,000 RPM internal classifier and an airflow of 210 CMH, producing 53 kg of whole flour. The whole flour exhibited a median particle size (D_{50}) of 21.58 µm and a protein content of 24.60%. Fractionation yielded 5.38 kg of fine fractions with significantly smaller median particle size (D_{50} = 10.22 µm) and a substantially higher protein content of 49.16%, demonstrating effective enrichment. The coarse fractions, comprising 46.73 kg, had a larger median particle size of 22.05 µm and lower protein content of 19.07%, confirming successful separation of starch-rich material. Additionally, 3.94 kg of dust was collected, characterized by very fine particles (D_{50} = 9.58 µm) with a high protein concentration of 45.11%. These results indicate that the milling and classification parameters employed efficiently reduced particle size and enhanced protein concentration in fine fractions and dust, supporting the process's suitability for producing protein-enriched Green gram fractions for further

applications.

Fig. 4 Green gram whole flour Fig. 5 Green gram coarse fraction Fig. 6 Green gram fine fractions

4.5. Extrusion technology

The low-moisture extrusion experiment was designed to investigate the behavior of pulse-based blends, primarily defatted soya flour (DSF) with Black gram and Green gram , at different blend ratios while maintaining constant protein content and extrusion parameters. Air classification was used to obtain fine fractions, subsequently blended at varying ratios to systematically analyze process responses such as torque, melt temperature, melt pressure, and expansion characteristics. By keeping process variables—moisture (22%), screw RPM (280), temperature setpoints (50/90/110/125°C), screw configuration, throughput, and cutting speed—constant, the experiment isolates the effect of blend composition on product functionality and extrusion performance.

4.5.1. Calibration of blended powder for volumetric feeder.

For calibration of the volumetric feeder filled the hopper of the feeder with the blend which we prepared previously. Feeder started on local mode (manually) at highest rpm for passing the powder up to the bottom of the screw of the feeder. The feeder pushed to the remote mode. Then the feeder calibrated at rpm of 2, 5, 10 and 15 for 1 min for each rpm, powder which passed out of the feeder outlet was weighed at each rpm. Plotted the graph of material in gram vs rpm of the feeder as follows.

4.5.2. Calibration of water for dosing

For calibration of the dosing pump filled the tube with water completely. Then the pump calibrated at rpm of 15, 110, 200 rpm for 1 min for each rpm, water which passed out from the pump outlet was weighed at each rpm. Plotted the graph of water in gram vs rpm of the feeder as follows.

4.5.3. Feeder, water dosing and sensors positioning

Figure (7) shows the positioning of the accessories required for the extrusion process. The feeder is positioned at the 1st zone. Next to the feeder water dosing pump was positioned in the 2nd zone. A pressure transducer and a thermocouple equipped at the 4^{th} zone, one more pressure transducer and a more thermocouple equipped in the die section.

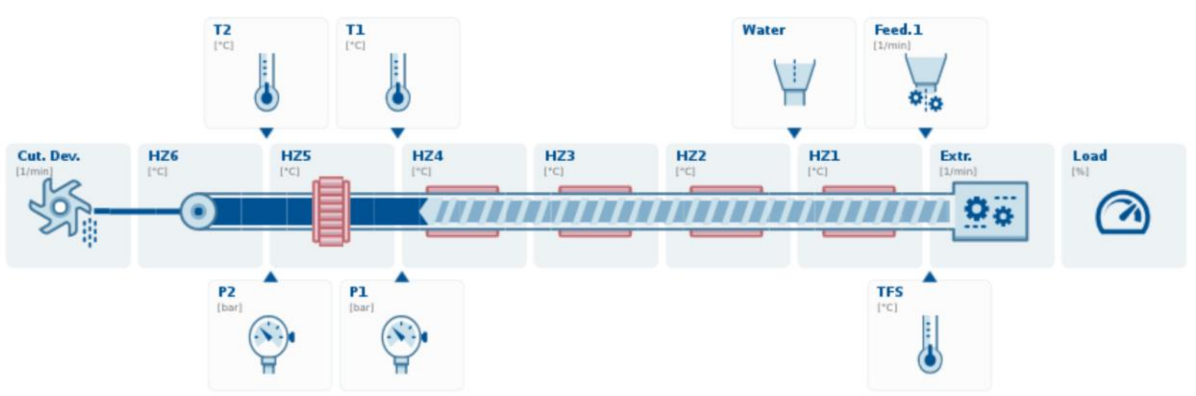


Figure 7: Feeder, water dosing and sensors positioning

4.5.4. Temperature profile in extruder barrel:

The Brabender twin lab F-20/40 barrel is equipped with four heating zones (HZ1, HZ2, HZ,3 and HZ4). HZ1 is used only for conveying the blend powder and water, so the temperature of HZ1 was kept at 50°C. After HZ1 temperature increased up to 90°C, 110°C and 125°C for HZ2, HZ3 and HZ4 respectively so as it can form gel.

Table 4.14. summarizes key extrusion parameters for each blend, highlighting trends in torque, melt temperature, melt pressure, expansion size, and expansion ratio.

Sample Name	Blend Protein %	Torque	Melt Temp	Melt Pressure	Expansion Size	Expansion Ratio
DSF 100%	50	28.6	145	135	6.1	2.03
DSF 80: black gram 20	49	25.8	144.5	132	5.9	1.97
DSF60: black gram 40	48	25.3	142	124	5.5	1.83
DSF 40: black gram 60	47	24.2	143	122	5.2	1.73
DSF 20 : black gram 80	46	22.2	144	99	4.7	1.57
black gram 100%	45	20	143.5	90	4.4	1.47
DSF 80: green gram 20	49.8	22.1	139.7	124	5.7	1.9
DSF60: green gram 40	49.4	21.4	140	129	5.2	1.73

DSF 40: green gram 60	49	23	137.5	104	4.8	1.6
DSF20 : green gram 80	49.2	22.5	135	84	4.5	1.5
green gram 100%	49.1	22.9	134	77	4.2	1.4

As seen in Table 4.14, increasing the proportion of pulse flour significantly decreased torque—from 28.6 Nm (DSF 100%) to 20 Nm (Black gram 100%) and 22.9 Nm (Green gram 100%)—while melt temperature remained relatively steady. Melt pressure also declined sharply with higher pulse content (135 bar for DSF vs. 77–90 bar for pure pulses), indicating changes in flow resistance and hydration during extrusion. Correspondingly, both expansion size and ratio decreased with increasing pulses, implying lower gas retention and expansion capacity in Black gram and green gram-rich blends.

Figures 8 and 9 visualize real-time changes in pressure, temperature, and extrusion speed for representative blends. The pressure curves (green and blue lines) reveal distinct stages in the process, with pulse-rich blends displaying lower peak pressures and greater fluctuations. Such real-time tracking illustrates the immediate impact of blend composition on the physical stresses within the extruder and supports the observed numerical trends.

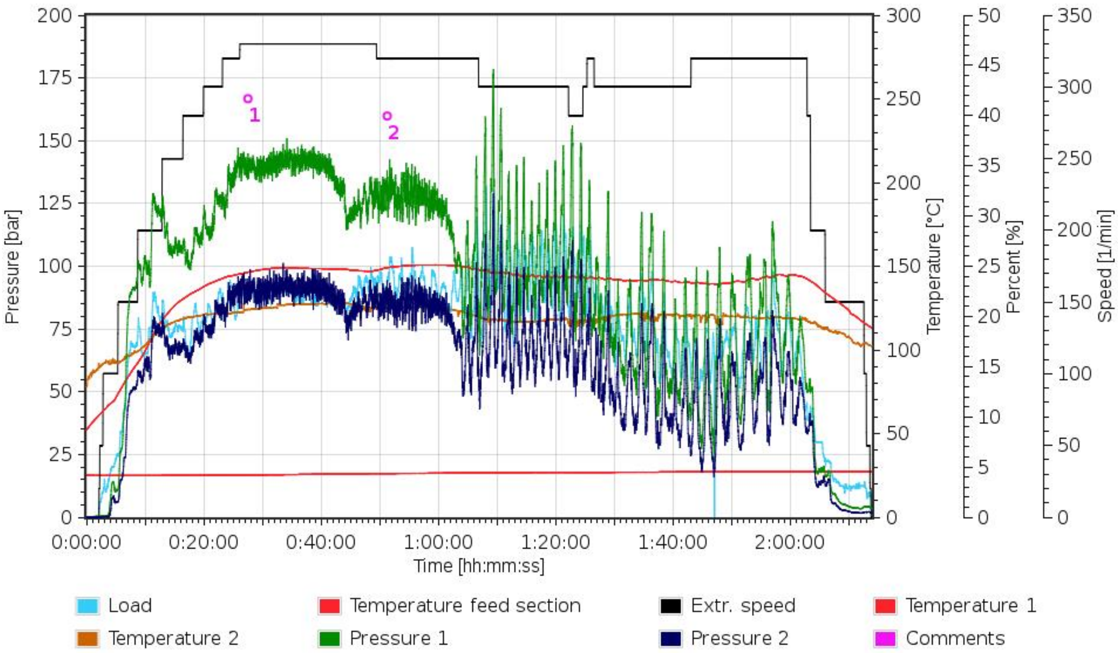


Figure 8: Graphical representation of RPM, pressure and temperature profile for extrusion process profile of black gram fractions.

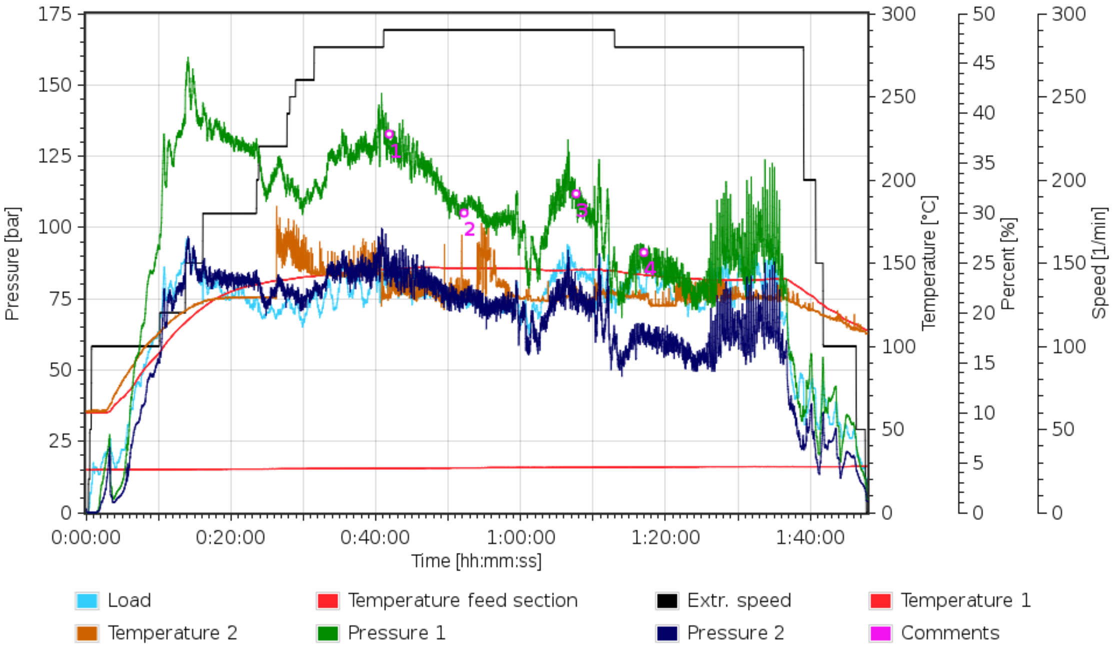


Figure 9: Graphical representation of RPM, pressure and temperature profile of alternative blend green gram fractions process profile

Overall, the results demonstrate that increasing the proportion of pulse flours (Black gram or green gram) in DSF-based extruded blends results in lower torque, melt pressure, and expansion properties, likely due to compositional and structural changes that affect melt viscosity, gas cell formation, and product texturization.

Figure 10. Extruded product photos

a) 100% Green gram flour

b) 80% green gram and 20% DSF

c) 60% green gram and 40% DSF

d) 40% green gram and 60% DSF e) 20% green gram and 80% DSF

f) 100% DSF g) 80% DSF and 20% black gram

h) 60% DSF and 40% black gram

i) 40% DSF and 60% black gram   j) 20% DSF and 80% black gram   k) 100% black gram

4.6. Physio-chemical analysis

Table 4.15. Colorimetric (L, a, b*) and Total Color Difference (ΔE) Values of Pulse Fractions

Samples	L	a	b	ΔE
Black gram Whole Flour	94.02 ±0.05	4.65 ±0.03	5.96 ±0.06	5.34 ±0.03
Black gram fine fraction	95.94 ±0.08	4.5 ±0.1	2.84 ±0.05	5.64 ±0.05
Black gram Coarse fraction	93.42 ±0.27	4.55 ±0.02	6.08 ±0.03	5.3 ±0.02
Green gram whole flour	93.94 ±0.08	3.15 ±0.03	16.92 ±0.10	13.17 ±0.04
Green gram fine fractions	95.24 ±0.04	3.3 ±0.02	10.66 ±0.05	8.16 ±0.06
Green gram Coarse fractions	93.88 ±0.06	2.89 ±0.03	16.82 ±0.04	13.24 ±0.05
Defatted soya flour	42.67 ±0.58	10.68 ±0.02	19.09 ±0.02	54.87 ±0.05

The chromometer analysis colour space revealed significant colour variations between air classifier fractions and defatted soya flour, with lightness (L*) values ranging from 42.06 for DSF to 95.95 for Black gram fine fractions, demonstrating the effectiveness of colour measurement in characterizing flour quality and composition differences. Black gram fractions consistently exhibited higher L* values (93.61-95.95) and lower b* values (2.83-6.05) compared to Green gram fractions, which showed greater yellowness with b* values ranging from 10.66-16.96, while DSF displayed distinctly different colour characteristics with significantly lower L* values and higher a* and b* values, reflecting its processed nature and different protein matrix. The total colour difference (ΔE) values effectively distinguished between samples, with pulse fractions showing values of 5.30-13.25 while DSF exhibited a substantially higher value of 54.81, indicating its visual distinctiveness and potential impact on product appearance when used in food formulations.

4.6.2. Water absorption

Table 4.16.: Functional Properties of Pulse Fractions and Flours (Water/Oil Absorption, Foaming Capacity, and Foaming Stability)

Samples	Water absorption in gm	Oil absorption in gm	Foaming capacity %	Foaming stability %
Black gram Whole Flour	2.43 ±0.05	1.36 ±0.06	75	68.57
Black gram fine fraction	3.71 ±0.027	1.76 ±0.05	90	92.15
Black gram Coarse fraction	2.64 ±0.05	1.34 ±0.01	35	77.78
Green gram whole flour	1.28 ±0.02	1.35 ±0.04	60	66.25
Green gram fine fractions	0.92 ±0.004	1.8 ±0.01	90	89.47
Green gram Coarse fractions	1.31 ±0.005	1.25 ±0.01	40	75.00
DSF	3.37 ±0.02	1.64 ±0.03	30	84.62

The water absorption capacity results demonstrate significant variability among the different air classifier fractions and samples tested, with Black gram fractions exhibiting substantially higher water absorption capabilities compared to Green gram fractions. Among the Black gram samples, the fine fraction demonstrated the highest water absorption capacity at 3.6859 g per gram of sample, followed by the coarse fraction at 2.6904 g/g and whole flour at 2.4194 g/g, indicating that air classification successfully concentrated hydrophilic components in the fine fraction. In contrast, Green gram samples showed considerably lower water absorption capacities, with an unusual pattern where the coarse fraction absorbed 1.3049 g/g, which was higher than both the whole flour at 1.2502 g/g and the fine fraction at 0.912 g/g, suggesting different protein structural characteristics compared to Black gram. Defatted soya flour demonstrated high water absorption capacity at 3.3724 g/g, ranking second only to Black gram fine fraction, reflecting its high protein concentration and processed nature. The substantial difference between Black gram and Green gram varieties, with Black gram samples showing 2.5 to 4 times higher water absorption than corresponding Green gram fractions, indicates fundamental differences in protein structure and composition between these legume species, which has important implications for functional food applications where water absorption capacity directly influences texture, mouthfeel, and processing characteristics in food formulations.

4.6.3. Oil Holding capacity

The oil absorption capacity analysis showed more uniform values across samples, with fine fractions achieving the highest capacities at 1.7796 g/g for Black gram and 1.7929 g/g for green gram, compared to their respective coarse fractions at 1.351 g/g and 1.248 g/g. Whole flour samples exhibited intermediate values of 1.4 g/g (black gram) and 1.3449 g/g (green gram). Unlike water absorption where dramatic differences were observed between pulse varieties, oil absorption remained relatively consistent, suggesting uniform hydrophobic protein characteristics across fractions, making all samples suitable for applications requiring fat binding in food systems.

4.6.4. Foaming capacity

The foaming capacity analysis showed that fine fractions achieved the highest values at 90.0% for both Black gram and Green gram varieties, while defatted soya flour exhibited the lowest capacity at 30.0%. Foam stability measurements after 30 minutes revealed that Black gram fine fraction maintained the best stability at 92.11%, whereas Green gram whole flour showed the poorest stability at 66.25%. These results demonstrate that air classification effectively concentrates proteins with superior surface-active properties in fine fractions, making them particularly suitable for applications requiring stable foam formation, while the processing conditions of DSF may have compromised its foaming functionality despite high protein content.

4.6.5. Ash content

Table 4.17. Ash, Protein, and Moisture Profiles of Air-Classified Fractions and Whole-Flour Samples

Samples	Ash content in gm	Protein content	Moisture content %
Black gram Whole Flour	4.85 ±0.05	24.91 ±0.17	11.5 ±0.10
Black gram fine fraction	4.66 ±0.4	45.86 ±0.13	12.35 ±0.04
Black gram Coarse fraction	4.87 ±0.02	20.68 ±0.53	12.15 ±0.02
Green gram whole flour	4.77 ±0.06	24.46 ±0.31	11.08 ±0.03
Green gram fine fractions	4.55 ±0.06	49.53 ±0.51	12.24 ±0.03
Green gram Coarse fractions	4.84 ±0.04	19.04 ±0.07	12.09 ±0.09
Defatted soya flour	4.63 ±0.03	50.17 ±0.42	11.53 ±0.35

The ash content analysis revealed distinct fractionation patterns, with fine fractions consistently demonstrating the highest mineral concentrations across both pulse varieties, where Black gram fine fraction achieved 7.19% ash content and Green gram fine fractions reached 7.97%, compared to significantly lower values in their respective coarse fractions of 3.70% and 4.01%. This pronounced difference between fine and coarse fractions indicates that the air classification process effectively concentrated mineral-rich components along with proteins in the smaller particles, as evidenced by the nearly two-fold increase in ash content from coarse to fine fractions in both Black gram and Green gram samples. The whole flour samples exhibited intermediate ash values of 4.51% for Black gram and 4.48% for green gram, representing the baseline mineral content before fractionation, while defatted soya flour demonstrated high ash content at 7.18%, comparable to the fine fractions. The elevated mineral content in fine fractions suggests successful concentration of essential minerals such as potassium, phosphorus, magnesium, calcium, and iron that are naturally bound to protein bodies in legumes, making these fractions particularly valuable for nutritional fortification applications. The consistent pattern observed across both pulse varieties confirms that air classification not only enhances protein content but simultaneously enriches the mineral profile, positioning fine fractions as nutrient-dense ingredients suitable for developing functional foods with improved micronutrient density.

4.6.6. Protein content

The protein content analysis demonstrated effective concentration through air classification, with fine fractions achieving 49.16% (green gram) and 45.79% (black gram) protein compared to coarse fractions containing only 19.07% and 20.08% respectively. This represents approximately 2.5-fold protein enrichment from coarse to fine fractions, while whole flours showed intermediate values of 24.60-24.81%. The results confirm successful separation of protein-rich particles from starch components, producing fine fractions with protein levels comparable to commercial protein concentrates suitable for nutritional fortification applications

The moisture content of air-classified fractions from Black gram, green gram, and defatted soya flour (DSF) was found to be consistently within a narrow range of 11.17–12.34%, with a mean of 11.90% and low standard deviation, indicating uniform drying and effective sample handling. Slightly higher values in Black gram fractions (mean 12.00%) compared to green gram (11.79%) and DSF (11.90%) may reflect varietal differences in water retention, but all samples fall well within the optimal range for stability and storage according to standard flour analysis guidelines. These results confirm that air classification did not introduce significant moisture variation between fractions, preserving the quality of the raw material for further processing and analytical assessments.

Table 4.18. Particle Size Distribution (D10, D50, D90) of Pulse Fractions and Defatted Soya Flour

Samples	+D 10	D 50	D 90
Black gram whole flour	8.32 ±0.04	22.54 ±0.03	92.64 ±0.02
Black gram Fine fractions	4.65 ±0.02	9.59 ±0.05	19.36 ±0.01
Black gram Coarse fractions	10.74 ±0.01	26.55 ±0.02	122.27 ±0.09
Green gram whole flour	6.77 ±0.05	20.48 ±0.06	56.42 ±0.08

Green gram Fine fractions	3.44 ±0.2	10.35 ±0.04	25.04 ±0.06
Green gram Coarse fractions	11.80 ±0.03	22.48 ±0.02	63.59 ±0.08
Defatted soya flour	12.01 ±0.07	24.55 ±0.01	191.86 ±0.09

The particle size distribution analysis confirmed effective separation through air classification, with fine fractions achieving significantly smaller D_{50} values of 9.59 µm (black gram) and 10.35 µm (green gram) compared to coarse fractions at 26.55 µm and 22.48 µm respectively. Fine fractions also demonstrated lower D_{10} values (3.44-4.65 µm) and D_{90} values (19.36-25.04 µm), while coarse fractions contained larger particles with D_{90} values reaching 122.27 µm for Black gram and 63.59 µm for green gram. Whole flour samples exhibited intermediate particle sizes with D_{50} values of 20.48-22.54 µm, representing the original distribution before fractionation. This successful particle size separation validates the air classification process and correlates with the observed protein enrichment in fine fractions, as smaller protein-rich particles were effectively concentrated while larger starch-containing particles predominated in coarse fractions.

4.7. Analysis of extrusion samples

Table 4.19. ***Colorimetric Parameters (L, a***, **b*, ΔE) of extruded samples of Green Gram–Based Blend Formulations.**

Sample	L	a	b	E
MNG fine	95.57 ± 0.73	3.28 ± 1.07	13.33 ±0.71	9.1 ±0.71
DSF	98.43 ± 0.57	4.57 ± 0.01	2.84 ±0.19	5.76 ±0.10
DSF40: green gram60	93.02 ± 0.13	4.05 ± 0.04	10.91 ±0.31	8.15 ±0.06
DSF60: green gram40	91.58 ± 0.12	4.63 ± 0.12	11.14 ±0.44	8.78 ±0.40
DSF80: green gram20	89.99 ± 0.17	5.22 ± 0.06	11.38 ±0.43	9.79 ±0.38
DSF20: green gram80	94.96 ± 0.49	3.51 ± 0.01	11.09 ±0.21	7.94 ±0.20
DSF40: black gram60	93.03 ± 0.12	5.15 ±0.04	5.7 ±0.13	5.8 ±0.08
DSF60: black gram40	92.08 ± 0.21	5.24 ±0.03	0.36 ±0.55	6.48 ±0.22
DSF80: black gram20	90.01 ± 0.10	5.51 ±0.05	9.17 ±0.26	8.51 ±0.16
DSF20: black gram80	94.78 ± 0.31	4.66 ±0.04	4.39 ±0.50	5.24 ±0.05
Black gram	95.95 ± 0.21	4.5 ±0.07	2.82 ±0.12	5.63 ±0.11

Values are expressed as mean ± standard deviation (n = 3). L = lightness, a* = red/green coordinate, b* = yellow/blue coordinate, and ΔE = total color difference.

The colour analysis of the extruded samples showed clear variations across blends of DSF, MNG fine, and UPF, reflecting changes in raw material composition and their influence on Maillard browning. Lightness (L*) values ranged from 89.99 to 98.43, with DSF exhibiting the highest brightness (98.43 ± 0.57), whereas increasing incorporation of MNG or UPF gradually reduced L* due to their darker intrinsic pigment profile and higher protein content, which promote browning during extrusion. The redness values (a*) varied between 3.28 and 5.51, with higher a* observed in DSF–UPF blends (e.g., 5.51 ± 0.05 in DSF80:UPF20), indicating increased non-enzymatic browning and melanoidin formation. Yellowness (b*) values were markedly higher in MNG-rich samples, reaching 13.33 ± 0.71 in MNG fine, attributable to natural carotenoid pigments and their enhanced visibility in lighter matrices. The total colour difference (ΔE) ranged from 5.24 to 13.33, with maximum ΔE observed in MNG fine, indicating significant colour deviation from DSF caused by compositional differences such as protein, starch, and mineral content. Overall, increased substitution of DSF with MNG or UPF resulted in darker, redder, and more intense yellow hues, consistent with the higher protein content and pigment load of pulse-based fractions, which intensify browning reactions during extrusion.

Table 4.20. Functional Properties (Water and Oil Absorption) of extruded samples of Green Gram–Based Blend Formulations.

Samples	Water absorption in gm	Oil absorption in gm
DSF	3.37 ± 0.021	1.64 ± 0.03
DSF 80 : Black gram 20	2.50 ± 0.032	1.47 ± 0.04
DSF 60 : Black gram 40	3.03 ± 0.044	1.43 ± 0.03
DSF 40 : Black gram 60	3.75 ± 0.032	1.63 ± 0.02
DSF 20 : Black gram 80	3.89 ± 0.045	1.59 ± 0.03
Black gram 100%	3.71 ± 0.027	1.76 ± 0.05
DSF 80 : Green gram 20	2.30 ± 0.054	1.69 ± 0.08
DSF 60 : Green gram 40	2.04 ± 0.034	1.54 ± 0.06
DSF 40 : Green gram 60	1.69 ± 0.029	2.04 ± 0.05
DSF 20 : Green gram 80	1.12 ± 0.031	2.15 ± 0.06
Green gram 100%	0.92 ± 0.004	1.8 ± 0.01

The functional properties of the blends revealed distinct patterns in water absorption capacity (WAC) and oil absorption capacity (OAC) depending on the proportion of DSF, Black gram fine, and Green gram fine incorporated. WAC ranged from 0.92 to 3.89 g/g, with the highest values observed in DSF20:Black gram80 (3.89 g/g) and DSF40:Black gram60 (3.75 g/g), indicating that black gram-enriched blends possess a stronger ability to bind water due to their higher protein and soluble fibre content. Pure Black gram fine (3.71 ± 0.027 g/g) also demonstrated high WAC, whereas green gram-rich blends showed a progressive decline, with Green gram recording the lowest WAC (0.92 ± 0.004 g/g), likely due to its finer particle size and lower soluble fibre, which reduces water-binding capacity. Conversely, OAC values ranged from 1.43 to 2.15 g/g, with the highest absorption recorded in DSF20:Green gram80 (2.15 g/g) and DSF40:Green gram60 (2.04 g/g). This suggests that green gram-enriched blends exhibit a greater affinity for oil, potentially due to the presence of hydrophobic amino acids and finer particles that enhance surface–oil interactions. Black gram-rich blends maintained moderate OAC (1.47–1.76 g/g), while DSF alone exhibited balanced functionality (1.64 ± 0.03 g/g). Overall, the results indicate that Black gram fractions significantly enhance water-binding properties, whereas Green gram fractions strengthen oil-binding characteristics, offering flexibility in tailoring functional properties for extrusion and product development.

Table 4.21. Foaming Properties of extruded samples of Defatted Soy Flour and Its Blends with Black Gram and Green Gram.

Sample	Foam Stability %	Foam Capacity %
DSF	84.62	30.00
DSF 80: Black gram 20	86.12	42.00
DSF 60: Black gram 40	87.61	54.00
DSF 40: Black gram 60	89.11	66.00
DSF 20: Black gram 80	90.61	78.00
Black gram 100%	92.15	90.00
DSF 80: Green gram 20	85.39	42.00
DSF 60: Green gram 40	86.16	54.00
DSF 40: Green gram 60	86.93	66.00
DSF 20: Green gram 80	87.70	78.00
Green gram 100%	89.47	90.00

The foaming properties of the blends showed a clear improvement with increasing incorporation of Black gram and Green gram fine fractions. Foam stability increased from 84.62% in DSF to a maximum of 92.15% in Black gram fine and 89.47% in Green gram fine, indicating that pulse proteins possess superior film-forming ability compared to DSF. Foaming capacity exhibited an even more pronounced enhancement, rising steadily from 30% in DSF to 90% in both Black gram and Green gram fine fractions, with intermediate blends showing a proportional increase. Black gram-based blends displayed slightly higher stability and capacity compared to green gram-based blends at equivalent substitution levels, suggesting stronger interfacial protein interactions and improved air entrapment ability. The progressive improvement across blends reflects the higher protein concentration and functionality of the fine fractions obtained through dry fractionation, which promote rapid unfolding and adsorption of proteins at the air–water interface. Overall, these results confirm that incorporation of pulse protein fractions significantly enhances foaming behaviour, making them suitable for applications requiring aeration and improved textural attributes in extruded or functional food formulations.

Table 4.22. Proximate Composition of extruded samples of Defatted Soy Flour and Its Blends with Black Gram and Green Gram

Samples	Ash content in	Protein content in %	Moisture content %

	gm		
DSF	4.64 ± 0.02	50 ±0.45	11.95 ±0.067
DSF 80: Black gram 20	4.65 ± 0.07	49 ±0.23	10.31 ±0.08
DSF60: Black gram 40	4.66 ±0.06	48 ±0.32	10.28 ±0.045
DSF 40: Black gram 60	4.66 ±0.06	47 ±0.35	10.46 ±0.09
DSF 20: Black gram 80	4.67 ±0.04	46 ±0.6	11.56 ±0.08
Black gram 100%	4.67 ±0.034	45 ±0.23	12.34 ±0.07
DSF 80: Green gram 20	4.63 ±0.045	49.8 ±0.48	9.99 ±0.043
DSF60: Green gram 40	4.63 ±0.032	49.4 ±0.11	9.37 ±0.08
DSF 40: Green gram 60	4.62 ±0.023	49 ±0.42	11.14 ±0.032
DSF20 : Green gram 80	4.61 ±0.029	49.2 ±0.5	10.89 ±0.042
Green gram 100%	4.60 ±0.012	49.1 ±0.71	11.89 ±0.034

The proximate analysis of the blends showed consistent trends across ash, protein, and moisture content as the proportion of DSF, Black gram fine, and Green gram fine varied. Ash content exhibited a slight but progressive increase with higher Black gram incorporation, rising from 4.643 g in DSF to 4.676 g in Black gram fine, indicating the presence of higher mineral content in black gram-derived fractions. Conversely, ash content marginally decreased with increasing Green gram substitution (4.634 to 4.602 g), reflecting the comparatively lower mineral density of Green gram fine fractions. Protein content displayed a predictable dilution effect, decreasing from 50% in DSF to 45% in Black gram fine, as Black gram fine fractions inherently possess lower protein concentration than DSF. Green gram blends, however, maintained protein levels closer to DSF (49–49.8%), consistent with the relatively higher protein content of green gram fine fractions. Moisture content remained within safe limits (9.37–12.34%), with black gram-rich blends showing slightly higher moisture (up to 12.34%) due to their greater water-binding capacity, while Green gram blends exhibited lower moisture (as low as 9.37%) associated with finer particle size and reduced hydration ability. Overall, the results indicate that incorporation of Black gram and Green gram fine fractions modify mineral, protein, and moisture characteristics but maintains functional stability suitable for extrusion and storage.

Table 4.23. Particle Size Distribution (D10, D50, D90) and Mean Particle Size of DSF–Pulse Flour Blends.

Samples	D 10 µm	D 50 µm	D 90 µm	Mean size µm
DSF 100%	12.01	24.55	191.86	69.54
DSF 80: black gram 20	5.71	19.97	187.13	66.30
DSF60: black gram 40	5.18	12.71	149.6	50.08
DSF 40: black gram 60	5.11	10.25	28.05	14.58

DSF 20 : black gram 80	5.08	10.24	23.36	13.02
black gram 100%	4.65	9.59	19.36	11.23
DSF 80: 20	5.48	19.53	187.99	65.66
DSF60: green gram 40	4.765	13.60	118.01	44.65
DSF 40: green gram 60	4.16	11.14	28.53	14.88
DSF20 : green gram 80	4.27	10.97	25.17	13.80
green gram 100%	3.44	10.35	25.04	13.15

The particle size distribution analysis revealed a clear reduction in particle size with increasing incorporation of Black gram and Green gram fine fractions. DSF exhibited the largest particle sizes (D10 = 12.01 µm, D50 = 24.55 µm, D90 = 191.86 µm), reflecting its coarser structure. As the proportion of pulse fine fractions increased, particle sizes progressively decreased, with Black gram fine achieving the smallest distribution (D10 = 4.65 µm, D50 = 9.59 µm, D90 = 19.36 µm), followed closely by Green gram fine (D10 = 3.44 µm, D50 = 10.35 µm, D90 = 25.04 µm). The blends showed intermediate values, with substantial reductions observed in high-pulse blends such as DSF40:Black gram60 (D50 = 10.25 µm) and DSF40:Green gram60 (D50 = 11.14 µm). Mean particle size followed the same trend, decreasing from 69.54 µm in DSF to 11.23–13.15 µm in Black gram and Green gram fine fractions. The sharper reduction in D90 values for pulse-rich samples indicates more uniform and refined particle structures, attributable to the fine milling characteristics of dry-fractionated proteins. Overall, the data confirm that incorporating Black gram and green gram fine fractions significantly reduce particle size, enhances uniformity, and improves powder functionality, which supports better hydration, mixing behaviour, and extrusion performance.

4.8. Functional properties

Extruded products

Table 4.24. ***Colorimetric Parameters (L, a*, **b*, ΔE) of Green gram extruded products.**

Sample	L	a	b	E
MNG fine	62.19 ±0.95	6.56 ±0.46	33.81 ±0.64	43.97 ±0.63
DSF	41.06 ±1.63	10.66 ±0.54	19.07 ±0.65	57.07 ±1.06

DSF40: green gram60	55.79 ±0.71	8.2 ±0.34	24.62 ±0.59	43.84 ±0.90
DSF60: green gram40	49.16 ±0.89	9.48 ±0.24	21.24 ±0.63	50.18 ±1.10
DSF80: green gram20	47.58 ±1.1	9.44 ±0.36	20.04 ±1.1	49.99 ±1.3
DSF20: green gram80	61.39 ±0.35	7.48 ±0.05	30.41 ±0.47	42.27 ±0.15
DSF40: green gram60	54.99 ±0.94	8.37 ±0.54	21.29 ±0.12	43.47 ±0.40
DSF60: green gram40	48.78 ±1.43	8.74 ±0.54	19.93 ±0.95	49.74 ±0.66
DSF80: green grsm20	48.56 ±0.72	10.21 ±0.27	21.32 ±0.42	49.36 ±1.66
DSF20: green gram80	53.95 ±1.14	7.91 ±0.14	21.59 ±0.77	44.78 ±0.52
Green gram	62.16 ±0.77	5.93 ±0.28	19.30 ±0.69	36.49 ±0.93

The colour characteristics of the extruded products varied significantly across blends, reflecting differences in raw material composition and their influence on Maillard browning during extrusion. Lightness (L*) ranged from 41.06 in DSF to 62.19 in Green gram fine, indicating that DSF produced the darkest extrudates, while Green gram and Black gram fine fractions resulted in lighter, more visually appealing products. Increasing substitution with green gram or Black gram fine fractions consistently increased L*, with high-pulse blends such as DSF20:MNG80 (L = 61.39) and UPF (L = 62.16) showing brightness similar to pure fine fractions. Redness (a*) values decreased with higher pulse incorporation, dropping from 10.66 in DSF to as low as 5.93 in UPF, suggesting reduced browning intensity due to lower sugar–protein reaction rates in pulse-rich formulations. Yellowness (b*) values were highest in green gram-based extrudates (b = 33.81 in MNG fine), reflecting natural carotenoid pigments, and lower in black gram-rich samples (b = 19.30 in UPF). The total colour difference (ΔE) was highest for DSF (57.07), while pulse-rich blends exhibited more moderate ΔE values (36.49–44.78), indicating a closer colour resemblance to their respective fine fractions. Overall, incorporation of pulse fine fractions enhanced lightness and reduced browning intensity, producing visually lighter extrudates with more desirable colour attributes suitable for consumer-acceptable functional snack products.

4.8.2. Bulk density

Table 4.25. Bulk Density, Hydration Capacity, and Expansion Characteristics of DSF–Based Formulations

Samples	Bulk Density	Hydration capacity	Expansion ratio	Expansion size mm
DSF80: green gram20	0.30 ±0.012	14 ±0.32	1.9	5.7

DSF60: green gram40	0.32 ±0.025	15 ±0.12	1.7	5.2
DSF40: green gram60	0.34 ±0.04	19 ±0.3	1.6	4.8
DSF20: green gram80	0.40 ±0.04	16 ±0.42	1.5	4.5
Green gram 100%	0.42 ±0.05	15± 0.21	1.4	4.2
DSF	0.40 ±0.05	8±0.19	2.0	6.1
DSF 80: black gram 20	0.38 ±0.034	10 ±0.12	1.9	5.9
DSF 60: black gram 40	0.28 ±0.03	14±0.17	1.8	5.5
DSF 40: black gram 60	0.26 ±0.07	14 ±0.129	1.7	5.2
DSF 20: black gram 80	0.32 ±0.05	13 ±0.16	1.5	4.7
Black gram 100%	0.20 ±0.07	16 ±0.2	1.4	4.4

The physical properties of the extruded products showed distinct trends depending on the proportion of Green gram and Black gram fine fractions incorporated into the DSF matrix. Bulk density increased progressively with Green gram enrichment, from 0.30 g/mL in DSF:MNG 80:20 to 0.42 g/mL in Green gram 100%, indicating the formation of denser and more compact structures due to reduced starch content and lower expansion. A similar pattern was observed in Black gram blends, though with a more pronounced decrease in density at higher Black gram levels; Black gram 100% recorded the lowest bulk density (0.20 g/mL), suggesting greater puffing efficiency and lighter product structure. Hydration capacity was highest in pulse-rich blends, with DSF:MNG 40:60 (19 g/g) and Black gram 100% (16 g/g) showing superior water uptake, likely due to higher protein content and smaller particle size enhancing hydration. Expansion ratio decreased consistently with higher pulse incorporation, dropping from 2.0 in DSF to 1.4 in both green gram and Black gram 100%, reflecting the reduced starch-driven puffing behaviour of protein-dense formulations. Correspondingly, expansion size is reduced from 6.1 mm (DSF) to 4.2–4.4 mm in pure pulse extrudates. Overall, the results indicate that increasing the proportion of dry-fractionated pulse proteins produces denser, less-expanded extrudates with higher hydration capacity, demonstrating how protein–starch interactions influence structural development during extrusion. These findings support the use of pulse-protein fractions for developing compact, nutrient-dense functional extruded foods.

Table 4.26. Texture Profile Analysis of extruded DSF, Black Gram, and Green Gram Blends

Samples	Hardness Cycle 1	Resilience	Cohesiveness	Springiness Index	Chewiness
DSF 100%	308.45 ±0.21	0.55 ±0.34	0.90 ±0.4	0.81 ±0.22	4.19 ±0.14
DSF 80: black gram 20	160.81 ±0.25	0.24 ±0.03	0.70 ±0.02	0.66 ±0.07	1.01 ±0.05
DSF60: black gram 40	124.56 ±0.32	0.15 ±0.032	0.60 ±0.012	0.44 ±0.03	0.61 ±0.05
DSF 40: black gram 60	114.72 ±0.034	0.12 ±0.022	0.50 ±0.03	0.37 ±0.03	0.53 ±0.07
DSF 20 : black gram 80	43.88 ±0.043	0.04 ±0.012	0.41 ±0.04	0.30 ±0.04	0.13 ±0.08
Black gram 100%	75.62 ±0.051	0.19 ±0.015	0.44 ±0.02	0.36 ±0.04	0.23 ±0.04
DSF 80: green gram 20	286.00 ±0.019	0.49 ±0.05	0.71 ±0.03	0.75 ±0.02	3.66 ±0.03
DSF60: green gram 40	155.14 ±0.07	0.55 ±0.027	0.81 ±0.01	0.66 ±0.04	1.93 ±0.05
DSF 40: green gram 60	142.03 ±0.011	0.27 ±0.03	0.57 ±0.02	0.70 ±0.05	1.36 ±0.032
DSF20 : green gram 80	71.04 ±0.08	0.07 ±0.04	0.40 ±0.04	0.44 ±0.01	0.28 ±0.05
Green gram 100%	60.80 ±0.09	0.17 ±0.02	0.38 ±0.08	0.32 ±0.07	0.18 ±0.02

The texture profile analysis revealed a consistent decline in hardness with increasing incorporation of Black gram and Green gram fine fractions. DSF exhibited the highest hardness (308.45 N), reflecting its dense, high-fibre structure, whereas pulse-rich extrudates showed significantly softer textures, with Green gram 100% (60.80 N) and DSF20:Black gram80 (43.88 N) demonstrating the lowest hardness values. This softening effect is attributed to the higher protein content and finer particle size of the pulse fractions, which disrupt starch continuity and reduce structural rigidity during extrusion. Resilience and cohesiveness followed a similar declining trend; DSF showed the highest resilience (0.55) and cohesiveness (0.90), indicating strong internal bonding, while blends with higher pulse proportions exhibited weaker structural recovery and lower internal matrix strength. Springiness also decreased with increasing Black gram or Green gram levels, with DSF showing the highest springiness (0.81) and pulse-rich samples showing more fragile, less elastic structures. Chewiness values were highest for DSF (4.19) and gradually reduced to 0.13–0.28 in high-pulse blends, reflecting the combined effects of reduced hardness and cohesiveness. Overall, the incorporation of dry-fractionated pulse proteins significantly reduced hardness, chewiness, and structural integrity of the extrudates, producing softer, less dense textures suitable for formulations aimed at improved digestibility and consumer acceptability in high-protein functional snacks.

CHAPTER 5: CONCLUSIONS

This study demonstrated that integrated dry fractionation, combining high-speed impact milling with optimized air classification (no water usage, temperatures below 40°C), efficiently produces clean-label, chemical-free protein-enriched fractions (45-50% protein) from black gram and green gram, suitable as direct food ingredients or for further concentration/isolation. For milling, three parameters were optimized (mill RPM, classifier wheel RPM, airflow) at standard feed rate; mill RPM impacted D90 primarily, airflow strongly affected D10/D50/D90, while classifier wheel RPM (2,000-8,000) showed minimal effect when others were constant. Classifier optimization focused on wheel RPM and airflow, achieving sub-20 µm D90 fine fractions with effective protein-starch separation: Black gram reached 45.79-46% protein (84.6% enrichment over 24.81% whole flour baseline, D_{50} ≈18.58 µm at 8,000 mill and internal classifier 6,000 classifier RPM), while Green gram hit 49.16% (87% over 24.60%, D_{50} ≈20.48 µm at 8,000 mill and 8,000 internal classifier RPM, 129-210 CMH).Classification process has been done by keeping classifier RPM at 9000 with air flow of 286CMH. Across both pulses, fine fractions consistently exhibited small particle sizes (D_{50} = 9.59–10.35 µm), high foaming capacity (90%), enhanced water absorption (3.37–3.71 g/g confirming that dry fractionation effectively concentrates functional and nutritional components.

Low moisture extrusion of 100% DSF results in hard spongy expanded matrices. Addition of dry fractions of Black gram and Green gram result in reducing the hardness, springiness, cohesiveness and chewiness values as measured by texture analyser. This opens up opportunities of creating ‘meat-like’ granules. The advantage of using dry fractions is that the protein content of the blends of DSF and dry fractions remains of the order of 45 to 55%. Thus, good protein content can be obtained without the need for soy concentrates or isolates.

The extrusion trials further validated the applicability of fractionated pulse proteins in value-added foods. Increasing the proportion of black gram and green gram fine fractions in DSF blends resulted in predictable adjustments, with torque decreasing from 28.6 Nm (DSF 100%) to 20.0–22.9 Nm (black gram/green gram 100%), and melt pressure reducing from 135 bar to 77–90 bar, indicating reduced melt viscosity and altered flow behaviour. Expansion ratio correspondingly declined from 2.0 (DSF) to 1.4 (black gram/green gram 100%), while bulk density increased to 0.40–0.42 g/mL in pulse-rich samples, reflecting denser extrudate structures. Despite lower expansion, pulse-based extrudates exhibited desirable hydration (14–19 g water gain) and stable textural properties, with hardness values decreasing from 308 N (DSF) to 60–75 N in 100% pulse extrudates. Collectively, these findings demonstrate that optimized dry fractionation produces nutritionally superior protein concentrates that integrate successfully into extrusion systems, offering a sustainable pathway to develop high-protein, functional, plant-based foods from indigenous legumes. The outcomes establish a scalable model for clean-label protein ingredient production and support broader application in snacks, meat analogues, and functional food formulations.

REFERENCES

Boye, J., Zare, F., & Pletch, A. (2010). Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Research International*, 43(2), 414-431. https://doi.org/10.1016/j.foodres.2009.09.003

Dekkers, B. L., Boom, R. M., & van der Goot, A. J. (2018). Structuring processes for meat analogues. *Trends in Food Science & Technology*, 81, 25-36. https://doi.org/10.1016/j.tifs.2018.08.011

Karaca, A. C., Low, N., & Nickerson, M. (2011). Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Research International*, 44(9), 2742-2750. https://doi.org/10.1016/j.foodres.2011.06.012

Li, R., Cui, Q., Wang, G., Liu, J., Chen, S., Wang, X., Wang, M., Kang, W., & Xu, B. (2018). Mung bean proteins and peptides: Nutritional, functional and bioactive properties. *Food & Function*, 9(6), 3171-3194. https://doi.org/10.1039/c8fo00511g

Liu, Y., Zhang, X., Wang, Y., Chen, L., Liu, K., & Huang, J. (2023). High-moisture extrusion cooking on soybean-wheat protein mixtures: Effect of polysaccharide addition on protein structure and fibrous properties. *Food Hydrocolloids*, 135, 108151. https://doi.org/10.1016/j.foodhyd.2022.108151

Modgil, R., Tanwar, B., Bajwa, H. K., & Khajuria, P. (2019). Bio-chemical and functional characteristics of black gram (*Vigna mungo*) cultivars. *International Journal of Current Microbiology and Applied Sciences*, 8(4), 2149-2158. https://doi.org/10.20546/ijcmas.2019.804.251

Osen, R., Toelstede, S., Wild, F., Eisner, P., & Schweiggert-Weisz, U. (2014). High moisture extrusion cooking of pea protein isolates: Raw material characteristics, extruder responses, and texture properties. *Journal of Food Engineering*, 127, 67-74. https://doi.org/10.1016/j.jfoodeng.2013.11.023

Pelgrom, P. J., Boom, R. M., & Schutyser, M. A. (2015). Functional analysis of mildly refined fractions from yellow pea. *Food Hydrocolloids*, 44, 12-22. https://doi.org/10.1016/j.foodhyd.2014.09.001

Rivera, J., Siliveru, K., & Li, Y. (2024). A comprehensive review on pulse protein fractionation and extraction: Processes, functionality, and food applications. *Critical Reviews in Food Science and Nutrition*, 64(13), 4179-4201. https://doi.org/10.1080/10408398.2022.2139223

Schmitt, M., Schieber, A., & Carle, R. (2022). High moisture extrusion of plant proteins: Fundamentals of the extrusion process and protein ingredient considerations. *Comprehensive Reviews in Food Science and Food Safety*, 21(3), 2125-2150. https://doi.org/10.1111/1541-4337.12937

Schutyser, M. A., Pelgrom, P. J., van der Goot, A. J., & Boom, R. M. (2015). Dry fractionation for sustainable production of functional legume protein concentrates. *Trends in Food Science & Technology*, 45(2), 327-335. https://doi.org/10.1016/j.tifs.2015.04.013

Sharma, S., Singh, N., & Kaur, M. (2020). Assessment of black gram milling by-product as a potential source of protein and dietary fiber. *Food Research International*, 137, 109717. https://doi.org/10.1016/j.foodres.2020.109717

Singh, B., Singh, J. P., Kaur, A., & Singh, N. (2017). Bioactive compounds in banana and their associated health benefits – A review. *Food Chemistry*, 206, 1-11. https://doi.org/10.1016/j.foodchem.2016.03.033

Tang, C. H., Wang, X. S., & Yang, X. Q. (2014). Enzymatic hydrolysis of hemp (*Cannabis sativa* L.) protein isolate by various proteases and antioxidant properties of the resulting hydrolysates. *Food Chemistry*, 114(4), 1484-1490. https://doi.org/10.1016/j.foodchem.2008.11.049

Wang, J., de Wit, M., Schutyser, M. A., & Boom, R. M. (2015). Analysis of electrostatic powder charging for fractionation of foods. *Innovative Food Science & Emerging Technologies*, 26, 360-365. https://doi.org/10.1016/j.ifset.2014.06.011

Zhang, Z., He, S., Wang, Y., Liu, C., Xu, D., Chen, Y., Zhang, H., Agyekum, A. A., Zandile, M., & Xu, X. (2024). Recent progress in plant-based proteins: From extraction and processing to food applications. *Comprehensive Reviews in Food Science and Food Safety*, 23(4), e13356. https://doi.org/10.1111/1541-4337.13356

Alcorta, A., Porta, A., Tárrega, A., Alvarez, M. D., & Vaquero, M. P. (2021). Foods for plant-based diets: Challenges and innovations. *Foods*, 10(2), 293. https://doi.org/10.3390/foods10020293

Bakane, P. H., Shende, D., & Mehta, B. (2024). Physical properties of green gram split. *International Journal of Current Microbiology and Applied Sciences*, 6(Special Issue), 1891-1897. https://doi.org/10.20546/ijcmas.2017.606.221

Boukid, F. (2021). Plant-based meat analogues: From niche to mainstream. *European Food Research and Technology*, 247(2), 297-308. https://doi.org/10.1007/s00217-020-03630-9

Boye, J., Zare, F., & Pletch, A. (2010). Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Research International*, 43(2), 414-431. https://doi.org/10.1016/j.foodres.2009.09.003

De Angelis, D., Latrofa, V., Squeo, G., Pasqualone, A., & Summo, C. (2024). Techno-functional, rheological, and chemical properties of plant-based protein ingredients obtained with dry fractionation and wet extraction. *Food Research International*, 195, 114905. https://doi.org/10.1016/j.foodres.2024.114905

Gerber, P. J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A., & Tempio, G. (2013). *Tackling climate change through livestock: A global assessment of emissions and mitigation opportunities*. Food and Agriculture Organization of the United Nations (FAO).

Goodland, R., & Anhang, J. (2009). Livestock and climate change: What if the key actors in climate change are... cows, pigs, and chickens? *World Watch*, 22(6), 10-19.

Henchion, M., Hayes, M., Mullen, A. M., Fenelon, M., & Tiwari, B. (2017). Future protein supply and demand: Strategies and factors influencing a sustainable equilibrium. *Foods*, 6(7), 53. https://doi.org/10.3390/foods6070053

Kanth, A., Goswami, K., & Shukla, P. (2021). Nutritional quality evaluation of improved varieties of black gram (*Phaseolus mungo*). *The Pharma Innovation Journal*, 10(1), 834-838.

Li, R., Cui, Q., Wang, G., Liu, J., Chen, S., Wang, X., Wang, M., Kang, W., & Xu, B. (2018). Mung bean proteins and peptides: Nutritional, functional and bioactive properties. *Food & Function*, 9(6), 3171-3194. https://doi.org/10.1039/c8fo00511g

Modgil, R., Tanwar, B., Bajwa, H. K., & Khajuria, P. (2019). Bio-chemical and functional characteristics of black gram (*Vigna mungo*) cultivars. *International Journal of Current Microbiology and Applied Sciences*, 8(4), 2149-2158. https://doi.org/10.20546/ijcmas.2019.804.251

Pelgrom, P. J., Boom, R. M., & Schutyser, M. A. (2015). Functional analysis of mildly refined fractions from yellow pea. *Food Hydrocolloids*, 44, 12-22. https://doi.org/10.1016/j.foodhyd.2014.09.001

Rivera, J., Siliveru, K., & Li, Y. (2024). A comprehensive review on pulse protein fractionation and extraction: Processes, functionality, and food applications. *Critical Reviews in Food Science and Nutrition*, 64(13), 4179-4201. https://doi.org/10.1080/10408398.2022.2139223

Schutyser, M. A., Pelgrom, P. J., van der Goot, A. J., & Boom, R. M. (2015). Dry fractionation for sustainable production of functional legume protein concentrates. *Trends in Food Science & Technology*, 45(2), 327-335. https://doi.org/10.1016/j.tifs.2015.04.013

Schutyser, M. A., & van der Goot, A. J. (2011). The potential of dry fractionation processes for sustainable plant protein production. *Trends in Food Science & Technology*, 22(4), 154-164. https://doi.org/10.1016/j.tifs.2010.11.006

Sharma, S., Singh, N., & Kaur, M. (2020). Assessment of black gram milling by-product as a potential source of protein and dietary fiber. *Food Research International*, 137, 109717. https://doi.org/10.1016/j.foodres.2020.109717

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., & de Haan, C. (2006). *Livestock's long shadow: Environmental issues and options*. Food and Agriculture Organization of the United Nations (FAO).

Wang, J., de Wit, M., Schutyser, M. A., & Boom, R. M. (2015). Analysis of electrostatic powder charging for fractionation of foods. *Innovative Food Science & Emerging Technologies*, 26, 360-365. https://doi.org/10.1016/j.ifset.2014.06.011

Zhang, Z., He, S., Wang, Y., Liu, C., Xu, D., Chen, Y., Zhang, H., Agyekum, A. A., Zandile, M., & Xu, X. (2024). Recent progress in plant-based proteins: From extraction and processing to food applications. *Comprehensive Reviews in Food Science and Food Safety*, 23(4), e13356. https://doi.org/10.1111/1541-4337.13356