

Formulation, Standardization and Nutritional Evaluation of Gluten-Free Plant-Based Protein Powder.

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Abstract

Background

The demand for plant-based protein supplements has increased due to growing interest in functional foods, plant-based diets, and dietary restrictions such as gluten intolerance. However, many commercially available protein supplements are expensive and rely on highly processed protein isolates. Development of affordable protein supplements using commonly available plant ingredients remains an area of interest.

Objective

The present study aimed to formulate and standardize a plant-based protein powder prepared from naturally gluten-free ingredients and evaluate its sensory acceptability and proximate nutritional composition.

Materials and Methods

Three formulations of protein powder (PP₁, PP₂ and PP₃) were prepared using nuts, pulses, and seeds. Organoleptic evaluation of the formulations was carried out using a 9-point hedonic scale by 10 untrained panellists. The most acceptable formulation was subjected to proximate composition analysis following AOAC (2019) methods.

Results

Among the developed formulations, PP₃ received the highest mean sensory scores for taste, flavour, and overall acceptability. Proximate analysis of PP₃ showed 517.55 KCal energy, 21.22 g protein, 33.04 g carbohydrates, and 36.9 g fat per 100 g.

Conclusion:

The study demonstrates the feasibility of developing a plant-based protein powder using commonly available ingredients. However, further studies involving larger sensory panels, microbiological safety testing, functional property analysis, and protein quality evaluation are necessary to validate the formulation for wider nutritional applications

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Introduction

A necessary macronutrient for the development, maintenance, and repair of bodily structures is protein. It is essential for the synthesis of muscle proteins, hormones and enzymes, immunological control, and general metabolic processes. Because protein promotes tissue repair, muscle regeneration, and adaptation to physical exercise, it is especially crucial for athletes, physically active persons, developing children, and those recuperating from sickness or injury to consume an adequate amount of protein in their diet [1]. The market for protein supplements and functional food items has grown globally in recent years due to a major rise in understanding of sports nutrition, fitness, and healthy living habits.

Due to their comprehensive amino acid profile and outstanding biological value, animal-based protein supplements like whey and casein proteins have historically dominated the market. However, consumers are moving toward plant-based protein substitutes because of growing concerns about lactose intolerance, nutritional choices, sustainability, environmental effect, and ethical issues [2]. The nutritional, functional, and health-promoting qualities of plant-based proteins are becoming more widely acknowledged, which makes them appropriate components for the creation of sustainable functional meals.

In recent decades, there has been a significant rise in knowledge of wheat allergies, non-celiac gluten sensitivity, celiac disease, and gluten intolerance. In genetically predisposed people, gluten consumption can cause celiac disease, an autoimmune condition that causes intestinal inflammation, mal absorption, and other gastrointestinal issues [3]. As a result, there is a growing market for gluten-free food items made using naturally gluten-free components. Due to lifestyle choices and

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Plant-based protein powder, sensory evaluation, proximate composition, functional foods, nutrition

perceived health benefits, consumers without a diagnosis of gluten problems are also increasingly following gluten-free diets.

As naturally gluten-free, nutrient-dense dietary items that may be used to develop protein-rich supplements, millets, pulses, nuts, and seeds have drawn a lot of interest. Proteins, healthy fats, dietary fiber, vitamins, minerals, and bioactive substances with antioxidant qualities are all abundant in these plant-based diets [4]. In contrast to animal protein sources, they are also affordable, readily available regionally, and ecologically sustainable. Using entire food sources instead of separated protein concentrates can save production costs and processing needs while improving nutritional quality.

There are a number of commercially available plant-based protein powders; however, many formulations need considerable industrial processing and heavily rely on isolated soy, pea, or rice proteins. In addition to being somewhat pricey, these products could not have enough micronutrient diversity or be palatable to consumers in terms of taste and texture. As a result, there is increasing interest in developing inexpensive, minimally processed, plant-based protein supplements using whole food ingredients that are tasty, functionally useful, and nutritionally balanced.

For the development of gluten-free protein formulations, ingredients with substantial nutritional potential include almonds, peanuts, Bengal gram, pumpkin seeds, sunflower seeds, melon seeds, oats, and makhana. Protein, monounsaturated fatty acids, vitamin E, magnesium, and antioxidants that promote metabolic and cardiovascular health are all abundant in almonds [5]. High-quality plant protein, niacin, folate, healthy fats, and bioactive phytochemicals with antioxidant activity are all found in peanuts [6]. Bengal gram (*Cicer arietinum*) helps increase satiety and glycemic management and is a great source of protein, dietary fiber, iron, and complex carbs.

Nutrient-dense foods that are high in protein, essential fatty acids, zinc, iron, magnesium, and antioxidant components include pumpkin, sunflower, and melon seeds. Sunflower seeds include vitamin E and selenium, which help minimize oxidative stress, while pumpkin seeds are particularly valued for their high protein content and healthy unsaturated fats. [7]. Melon seeds are beneficial ingredients for nutritional enrichment since they provide significant levels of protein and minerals.

Oats are well known for their β -glucan soluble fiber, protein, and antioxidant content, all of which may enhance digestive health and lipid metabolism [8]. Despite being inherently gluten-free, oats are frequently treated properly to prevent gluten contamination.

Another important component that is high in protein, carbs, calcium, and antioxidant components is makhana (*Euryale ferox*), sometimes referred to as fox nuts. Makhana has grown in popularity as a functional food component and healthy snack because of its low fat content and ease of digestion.

The total amino acid balance and nutritional quality of the protein supplement may be enhanced by combining cereals, pulses, nuts, and seeds in a single formulation. While pulses and legumes are rather abundant in lysine but low in sulfur-containing amino acids, cereals often lack lysine. As a result, mixing different plant components can enhance protein quality and produce complimentary protein benefits [9]. Additionally, adding a variety of plant based ingredients may improve the final product's texture, taste, sensory acceptability, and functional qualities.

Therefore, developing a gluten-free plant-based protein powder with whole food components might offer an affordable, wholesome, and sustainable substitute for protein supplements that are sold in stores. While encouraging the use of locally accessible nutrient-rich foods, such formulations may assist meet the dietary needs of athletes, health-conscious customers, and those with gluten sensitivity.

Thus, the present study aimed to develop and standardize a plant-based protein powder using commonly available ingredients and evaluate its sensory acceptability and basic nutritional composition. The focus of the investigation was formulation feasibility rather than advanced nutritional validation.

Objectives of the study

1. To formulate and standardize different variations of plant-based protein powder.
2. To evaluate the sensory acceptability of the developed formulations.
3. To determine the proximate composition of the most acceptable formulation.

Materials and Methods

Procurement of Raw Materials

The raw materials required for the preparation of the formulated protein powder were procured from local departmental stores and retail markets located in the Kanpur district, Uttar Pradesh, India. In order to guarantee the formulation's accessibility, affordability, and usefulness for preparation at the home and community levels, procurement from local marketplaces was favoured. Every item was carefully chosen based on its physical appearance, freshness, lack of bug infestation, and general quality attributes.

Almonds, peanuts, Bengal gram, pumpkin seeds, sunflower seeds, melon seeds, oats, and makhana were the main raw materials purchased for the study. To reduce the amount of synthetic flavours, additives, and preservatives that are frequently included in commercially processed goods, these materials were bought fresh and unprocessed. Before being processed and formulated further, the materials were brought to the lab in a sanitary manner and kept at room temperature in sealed containers.

All raw materials were cleaned to get rid of dust, broken grains, stones, foreign objects, and other contaminants prior to formulation. To preserve the resulting product's safety and sanitary quality, proper washing and sorting were done. After that, the materials underwent

additional processing techniques such as roasting, grinding, and mixing in accordance with the specifications of the formulation.

Ingredient Selection

Numerous significant factors, including as nutritional composition, protein content, availability, affordability, functional qualities, sensory appeal, and complimentary nutrient profiles, were taken into consideration while choosing the formulation's constituents. Finding naturally nutrient-dense plant-based components that may improve the formulation's overall nutritional value and protein quality was a primary objective of the selection procedure.

Complementary Nutritional Profile

In contrast to single-source protein products, the combination of nuts, oilseeds, pulses, grains, and makhana was intended for a more balanced nutritional composition. Combining different ingredients may enhance the formulation's overall amino acid balance and protein quality because individual plant proteins frequently lack one or more necessary amino acids. By adding healthy fats, dietary fiber, vitamins, minerals, and bioactive components, the chosen ingredients further improve the protein powder's functional and nutritional qualities.

Gluten-Free Consideration

The formulation's components can be used in gluten-free dietary preparations since they are inherently gluten-free. The selection of ingredients that are generally regarded as suitable substitutes for wheat-based goods for those with celiac disease and gluten sensitivity was given particular attention. However, the current study did not include certified gluten-free testing or laboratory analysis for gluten contamination. As a result, even though the components are inherently gluten-free, cross-contamination during processing, packing, shipping, or retail handling cannot be totally ruled out. This restriction should be taken into account when determining whether the developed formulation is suitable for gluten-free diets.

Formulation of Protein Powder- Three variations of protein powder (PP₁, PP₂ and PP₃) were developed using different proportions of ingredients as presented in Table 1. All ingredients were dry roasted at low temperature, cooled, ground into fine powder, sieved, and stored in airtight containers.

Sensory Evaluation-Organoleptic evaluation was conducted using a 9-point hedonic scale ranging from “dislike extremely” to “like extremely” [10]. The sensory panel consisted of 10 untrained panellists including postgraduate students and staff members. Samples were coded and presented in identical

Table 1: Formulations of different variations of Protein powder

Ingredients	Protein powder (PP ₁)	Protein powder (PP ₂)	Protein powder (PP ₃)
Almonds	10	10	10
Makhana	20	20	30
Cashewnuts	15	15	10
Peanuts	15	15	15
Chana	20	20	15
Oats	20	10	5
Pumpkin seeds	0	10	5
Melon seeds	0	0	5
Sunflower seeds	0	0	5
Total amount	100 gm	100 gm	100 gm

containers under similar serving conditions. The parameters evaluated included colour, texture, taste, flavour, and overall acceptability.

Flow chart for development of protein powder

Dry roast all the ingredients on a low flame and then cool it down
↓
Grind them into a fine powder
↓
Sieve well and transfer into airtight container

Proximate Composition Analysis

Proximate composition analysis was used to assess the nutritional content of the most acceptable formulation, as determined by sensory evaluation. The Association of Official Analytical Chemists' standard procedure protocols were used to conduct the analysis [11]. Determining the protein powder's moisture, crude protein, crude fat, carbohydrate, and energy content was part of its proximate composition. To guarantee the precision and dependability of the findings, every analysis was carried out in triplicate.

Determination of Moisture Content

The hot air oven drying technique, as recommended by AOAC (2019), was used to evaluate the moisture content of the protein powder. Because it affects food items' texture, microbiological stability, shelf life, and storage quality, moisture estimate is crucial. A clean, dry, and previously weighed porcelain dish was used to precisely weight 2-5 gm of the powdered material. After that, the sample was kept in a hot air oven set at 105°C for 4-6 hours, or until the weight remained constant. The sample's moisture evaporated during the heating process, leaving behind dry materials. The sample was taken out of the oven after drying and placed right away in a desiccator for cooling and stop it from absorbing moisture from the surrounding air. An analytical balance was used to reweigh the cooled sample.

The percentage moisture content was calculated using

the following formula:

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

Where:

$W_1 - W_2$ = Initial weight of sample before drying

W_2 = Final weight of sample after drying

The moisture content provides an estimate of water present in the formulation and helps predict storage stability and susceptibility to microbial spoilage.

Determination of Crude Protein

The AOAC-recommended Kjeldahl technique was used to determine the crude protein content (2019). This technique calculates the sample's total nitrogen content, which is subsequently transformed into protein content using a conventional conversion factor.

The material was precisely weighed into a Kjeldahl digestion flask, weighing between 0.5 and 1 g. The flask was filled with concentrated sulfuric acid (H_2SO_4) and a digestive catalyst combination that included copper and potassium sulfate. The sample was boiled until the solution turned clear, signifying that all organic materials had been completely digested and that nitrogen had been transformed into ammonium sulfate. Following digestion, the mixture was cooled before being diluted with distilled water. Ammonia gas was released from the digested sample by adding sodium hydroxide solution in a distillation unit. A receiving flask holding a boric acid solution with a mixed indicator was filled with the distilled ammonia that was emitted. The endpoint was then attained by titrating the trapped ammonia against a standard hydrochloric acid (HCl) solution.

The nitrogen content was calculated, and crude protein was estimated using the standard protein conversion factor of 6.25.

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

The Kjeldahl method is widely used because of its reliability and accuracy in estimating total protein content in food samples.

Determination of Crude Fat

According to AOAC (2019), the Soxhlet extraction technique was used to calculate the crude fat content. This technique calculates the food sample's total amount of ether-extractable fat.

A dry sample weighing between 2-5 gm was precisely weighed and put into a filter paper thimble. The Soxhlet extraction apparatus, which was attached to a pre-weighed extraction flask with petroleum ether as the solvent, was filled with the thimble. Continuous heating of the system allowed the solvent to condense, evaporate, and repeatedly wash the sample. Fats and lipid-soluble materials were collected in the extraction flask after dissolving in the solvent during the extraction process.

To ensure full fat extraction, the extraction procedure was carried out for around 6-8 hours. Following extraction, the extracted fat was dried, cooled in a desiccator, and weighed after the solvent had evaporated.

The percentage crude fat was calculated using the

following formula:

$$\text{Fat (\%)} = \frac{\text{Weight of extracted fat}}{\text{Weight of sample}} \times 100$$

Fat estimation is important because lipids contribute to energy density, texture, flavor, and nutritional quality of the developed formulation.

Determination of Carbohydrate Content

As recommended by proximate analysis procedures, the difference method was used to determine the total carbohydrate content. By deducting the percentages of moisture, protein, fat, ash, and crude fiber from 100, the carbohydrate content is determined indirectly.

$$\text{Carbohydrate (\%)} = 100 - (\text{Moisture \%}) + \text{Protein \%} + \text{Fat \%} + \text{Ash \%} + \text{Crude Fiber \%}$$

This method provided an estimate of total available carbohydrates present in the formulated protein powder. Carbohydrates serve as a major source of energy and also influence texture, flavor, and acceptability of food products.

Estimation of Energy Content

Aberoumand's (2009) method [12] was used to assess the protein powder's total energy value. The Atwater universal conversion factors for macronutrients were used to compute the calorific value.

using a factor of 4 KCal/g, while fat contribution was calculated using a factor of 9 KCal/g.

$$\text{K. Cal/100 g: Energy [K. Cal]} = [\text{Protein} \times 4] + [\text{Fat} \times 9] + [\text{Carbohydrates} \times 4]$$

The calculated energy value represents the total calorific contribution of macronutrients present in the developed formulation and is useful for evaluating its suitability as a nutrient-dense protein supplement.

Statistical Analysis- For accurate result interpretation, the data collected from the formulated protein powder's proximate composition analysis and sensory evaluation was summarized, tabulated, and statistically analysed. The degree of variance between various formulations and the statistical significance of the observed variations in sensory qualities were ascertained by statistical analysis.

The mean $\pm$ standard deviation (SD) was used to express each sensory assessment score. The standard deviation shows the degree of variability or dispersion of data around the mean, whereas the mean value shows the average score achieved for each sensory characteristic. The dependability and consistency of panel responses are better understood when data are presented as mean $\pm$ SD.

The Statistical Package for Social Sciences (SPSS) software version 22.0 was used to analyse the sensory assessment data for factors such as appearance, colour, flavour, texture, taste, and overall acceptability. For managing data, descriptive statistics, and inferential statistical analysis in food science and nutrition research, SPSS is a commonly used statistical tool.

Descriptive Statistics

The sensory evaluation results were first compiled using descriptive statistical analysis. For every formulation, the mean and standard deviation for every sensory characteristic were computed independently.

Comparing the acceptance levels of various formulations and determining the most preferred product among the developed samples were made easier with the use of descriptive statistics.

One-way analysis of variance (ANOVA) was used to determine if the developed formulations differed significantly from one another. An inferential statistical test called one-way ANOVA was used to compare the means of many groups simultaneously.

The sensory ratings of several formulations for distinct sensory qualities were compared in this study using ANOVA. The alternative hypothesis (H_1) asserted that at least one formulation varied significantly from the others, whereas the null hypothesis (H_0) assumed there was no significant difference between the mean sensory scores of the formulations.

A statistical significance of $p < 0.05$ was set. The observed changes between formulations were statistically significant and unlikely to have occurred by random chance if the p-value was less than 0.05. On the other hand, differences between formulations were not statistically significant if the p-value was more than 0.05.

The statistical analysis facilitated an objective comparison of the formulated protein powder samples and assisted in determining the most appropriate formulation based on sensory qualities.

Interpretation of Statistical Results

The most acceptable product was determined to be the formulation with the highest mean overall acceptability score and statistically significant differences when compared to other formulations. As a result, statistical analysis enhanced the sensory evaluation results' dependability and scientific validity.

When choosing the best gluten-free protein powder composition, the application of ANOVA and descriptive statistical techniques reduced subjective bias and guaranteed a methodical interpretation of sensory data.

Results

The mean sensory scores of the three developed protein powder formulations were compared for colour, texture, taste, flavour, and overall acceptability. Among the three formulations, PP₃ obtained the highest mean scores for taste, flavour, and overall acceptability as shown in Table 2. However, slight variations were observed in colour and texture attributes among the formulations. Based on the sensory evaluation results, PP₃ was selected as the most acceptable formulation and

was further subjected to proximate composition analysis.

According to this method, energy contribution from protein and carbohydrates was calculated.

The proximate composition of PP₁, PP₂ and PP₃

Table 2: Mean scores of organoleptic parameters of different variations of protein powder

Parameters	Protein powder (PP ₁)	Protein powder (PP ₂)	Protein powder (PP ₃)	Significance (p-value)
Colour	7.5+0.52	7.8+0.42	7.1+0.56	S
Texture	7.2+0.78	6.7+0.67	6.9+0.31	S
Taste	6.2+0.63	7.15+0.74	7.7+0.48	NS
Flavour	6.6+0.51	7.25+0.42	7.9+0.31	NS
Overall acceptability	6.5+0.52	7.2+0.63	8.2+0.63	NS

Data presented as mean $\pm$ SD

p-value <0.05=NS Not significant, >0.05=S Significant

formulations of protein powder per 100 g indicated that the moisture content of all formulations remained below 10%, indicating good storage stability and reduced chances of microbial spoilage. PP₂ contained highest protein content due to the addition of pumpkin seeds. PP₁ had the highest carbohydrate content because of higher oats and chana proportion, PP₂ and PP₃ contained higher fat and energy values owing to the inclusion of seeds and nuts as depicted in Table 3. However, PP₃ provided a more balanced nutrient profile with diversified seed incorporation.

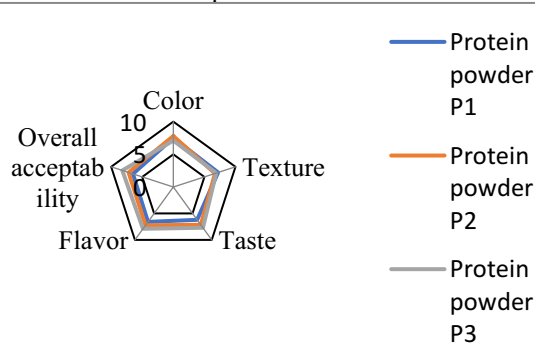


Fig 1: Organoleptic parameters of different variations of protein powder

Table 3: Proximate composition of different variations of protein powder per 100 gm

Nutrients	PP ₁	PP ₂	PP ₃
Moisture (%)	3.1	2.8	3.6
Energy (KCal)	508.62	536.90	517.55
Protein (g)	19.20	23.23	21.22
Fat (g)	31.2	36.4	36.9
Carbohydrate (g)	35.88	31.32	33.04

Discussion

In order to improve nutritional quality, protein

balance, functional qualities, and overall well-being advantages, the current formulation was developed by carefully combining nuts, seeds, grains, and pulses. Proteins, healthy fats, dietary fiber, vitamins, minerals, and bioactive compounds all of which are known to enhance metabolic health and nutritional adequacy were all given by those selected constituents. Amino acid balance, antioxidant potential, and nutrient bioavailability may all be enhanced by these components' synergistic interactions, making the composition nutritionally more effective.

The primary ingredient, almonds, provides an abundance of mono-unsaturated and polyunsaturated fats, protein, dietary fiber, vitamin E, and various trace minerals [13].

Almonds greatly improved the nutritional profile and were an important ingredient of the formulation. Monounsaturated and polyunsaturated fats, dietary fiber, vitamin E, calcium, magnesium, and phenolic antioxidants are all abundant in almonds. Strong antioxidant activity and cardio-protective benefits are attributed to the presence of tocopherols and poly phenolic substances. Almond intake supports cardiovascular and metabolic health by lowering LDL cholesterol, improving lipid profiles, and increasing satiety, according to studies [14, 15 & 16]. Almonds also provide necessary minerals and plant-based protein, both of which are vital to the immune system and tissue repair [17].

Makhana (*Euryale ferox*) was included because to its excellent nutritional content and gluten-free qualities [18]. It is frequently incorporated into gluten-free cookies and starchy flour. Cashew nuts contribute high-quality protein and beneficial fats, offering a comprehensive amino acid profile [19]. Makhana is known for being naturally low in fat and rich in carbohydrates, proteins, phosphate, potassium, and bioactive substances. Because of its digestibility and health-promoting qualities, it is frequently utilized in gluten-free and functional food compositions. Because makhana contains flavonoids and phenolic components, research has shown that it has anti-inflammatory, anti-aging, and antioxidant properties [20, 21, & 22]. Its addition most likely enhanced the product's sensory qualities and nutritional value.

Healthy fats, good quality proteins, and vital minerals including iron, zinc, magnesium, and phosphorus were all provided by cashew nuts. Unsaturated fatty acids and bioactive substances found in cashews have positive impacts on oxidative stability and cardiovascular health. Their amino acid makeup enhances the total protein quality by complementing the protein profile of cereals and legumes. Regular nut consumption has been linked to a lower risk of coronary heart disease and better metabolic health, according to earlier studies [23, 24 & 25].

In the formulation, peanuts were an affordable and nutrient-rich component. Protein, good fats, niacin, folate, and antioxidants including resveratrol, phenolic acids, flavonoids, and phytosterols are all abundant in

peanuts. These substances may lessen oxidative stress and prevent the absorption of cholesterol. They also supply Co-enzyme Q10 and all 20 amino acids, with arginine being found in large amounts [18]. According to earlier research, eating peanuts improves satiety, cardiovascular health, and lowers the chance of developing chronic illnesses [26, 27 & 28].

Due to their abundance of proteins, slowly digesting starch, dietary fiber, vitamins, minerals, and phytochemicals, pulses constitute significant nutritional components. Consuming Bengal gram has been associated with better gastrointestinal health, lower blood cholesterol, and an enhanced glycaemic response. Bengal gram has proteins, carbohydrates, fibers, vitamins, and minerals, contributing to the prevention of various health conditions [29]. Bengal gram's lysine-rich protein improves the amino acid balance in composite food items by complementing plant proteins lacking methionine [30, 31]. Additionally, chickpea proteins have useful qualities that make them excellent for dietary fortification and good digestion [32].

Because of their improved fiber content and functional carbohydrate profile, oats were included. Oats are particularly high in β -glucan, a soluble fiber that has been shown to reduce cholesterol and regulate blood sugar. The formulation's carbohydrate quality and satiety were probably enhanced by the addition of oats. Additionally, oats improve sensory acceptance, flavor, and texture. According to scientific research, oat β -glucan lowers blood cholesterol and improves digestive and cardiovascular health [33 & 34]. Oats also include antioxidants including avenanthramides, which may lessen oxidative damage and inflammation [35].

Pumpkin seeds offer substantial protein, fats rich in PUFAs, and various macro and micro minerals [36]. These seeds are rich in nutrients and have been shown to have cardioprotective and anti-inflammatory qualities. Additionally, phytosterols and tocopherols contained in pumpkin seeds enhance lipid metabolism and promote immunological function. Their incorporation improved the formulation's micronutrient density and protein quality. Additionally, it has been observed that pumpkin seed proteins have high emulsifying and functional qualities in food systems [37].

Melon seeds are notable for their high content of proteins, fats, energy, and minerals, particularly potassium, calcium, magnesium, and iodine, while containing minimal anti-nutritional components [38].

Melon seeds were incorporated because of their high mineral content and nutritional value. They contain barely any anti-nutritional components and supply proteins, vital fatty acids, calcium, magnesium, potassium, and phosphorus. Proteins from melon seeds enhance the total nutritional value and composition of amino acids. Melon seeds are an inexpensive source of minerals and edible oils that can be used in functional food applications, according to earlier research [39].

Sunflower seeds provide vitamins B₁, B₅, vitamin E, and folate, along with essential minerals such as copper,

magnesium, selenium, and phosphorus [40].

Because sunflower seeds contain vitamin E, B-complex vitamins, selenium, copper, magnesium, and phosphorus, the product's nutritional value was further enhanced. Antioxidant properties of sunflower seeds are due to tocopherols and phenolic compounds, which aid in preventing oxidative stress. Additionally, sunflower seeds provide beneficial amino acids and unsaturated fats that enhance the nutritional and functional qualities of food compositions [41].

Protein complementation among plant sources is a well-recognized approach for improving amino acid balance, particularly through the pairing of lysine-rich pulses with methionine-containing cereals. Previous researchers have reported that such combinations significantly enhance overall protein quality and biological value [42 & 43].

Rationale behind High Fat and Carbohydrate content in Plant based Protein Powder

Based on formulation objectives, ingredient selections, and physiological advantages, higher fat and carbohydrate content of formulated plant based protein powder can be scientifically justified as:

1. Gluten, which often affects texture, mouth feel, and structural integrity was absent in this formulation. In order to make up for this, fats from different sources were added to the powder to increase its creaminess, palatability, satiety, and functional stability.
2. High fat content of the formulated plant based protein powder also offered a number of health benefits, such as the presence of essential fatty acids (omega-3 and omega-6) that support brain and cardiovascular health, improved absorption of fat-soluble vitamins (A, D, E, and K) in the presence of dietary fat, and sustained energy release that aids in nutrition for endurance and recovery. It was formulated for bodybuilders and athletes who require extra calories, as well as for people who are malnourished or have higher energy needs. Increased fat content is advantageous rather than harmful for such groups.
3. Fat improves satiety and lowers postprandial glucose increases by slowing stomach emptying and digestion. Because of this, these formulations have applications in metabolic health also.

The results of the present study support this concept, suggesting that the selected ingredient proportions successfully achieved nutritional synergy. This aspect is especially critical in gluten-free product development, where dependence on single plant sources may otherwise result in amino acid limitations.

The satisfactory functional behaviour of the standardized product may be attributed to appropriate pre-processing operations such as cleaning, controlled roasting or drying, and fine milling. Processing treatments are known to alter protein structure, hydration properties, and particle size distribution, thereby influencing reconstitution performance [35 & 44]. The present findings are consistent with these

reports and indicate that well-managed processing can substantially improve the functional quality of gluten-free plant proteins.

Sensory acceptability remains one of the most challenging aspects in the development of plant-based protein supplements, primarily due to issues such as beany flavour, bitterness, and coarse mouth feel. Encouragingly, the developed formulation achieved favourable sensory scores, suggesting that careful ingredient selection and proportion optimization were effective in minimizing undesirable sensory attributes. The sensory evaluation results indicated that variation in ingredient proportions influences the overall acceptability of the formulation. The PP₃ formulation achieved the highest sensory scores, suggesting that the selected proportion of ingredients produced a more acceptable taste and flavour profile.

Similar observations have been documented in other studies where consumer acceptance of plant proteins improves markedly when flavour masking, proper thermal treatment, and balanced formulations are applied [45 & 46]. The acceptable sensory profile obtained in the present study enhances the practical feasibility of the product for routine dietary use.

Another critical concern in gluten-free product development is the possibility of nutritional inadequacy. Earlier studies have indicated that poorly planned gluten-free diets may be deficient in protein, dietary fiber, iron, and certain B vitamins [47 & 48]. The present formulation incorporates a diverse range of plant ingredients, which likely contributed not only to higher protein content but also to improved micronutrient and fiber levels. This broader nutritional contribution strengthens the value of the developed powder as a functional food ingredient rather than merely a protein supplement.

Protein digestibility and amino acid bioavailability are also key determinants of nutritional effectiveness. Plant proteins may sometimes exhibit slightly reduced digestibility due to the presence of ant nutritional factors such as phytates and tannins. However, appropriate processing techniques including soaking, roasting, and fine grinding are known to reduce these compounds and enhance protein utilization [49]. Although direct digestibility measurements were not conducted in the present study, the satisfactory functional and sensory performance suggests improved protein accessibility.

This formulation exemplifies the idea of protein complementation, which is crucial to plant-based diets. Amino acid balance and biological value can be greatly enhanced by mixing cereals, legumes, nuts, and seeds. Individual plant proteins frequently lack one or more important amino acids. Pulses high in lysine enhance the quality and digestibility of protein by complementing cereals and seeds that contain methionine. Mixed plant proteins successfully improve nutritional sufficiency and enable balanced vegetarian diets, according to earlier study [50]. Therefore, these components work together to develop a functional food product that is high in protein, nutritionally balanced and able to improve

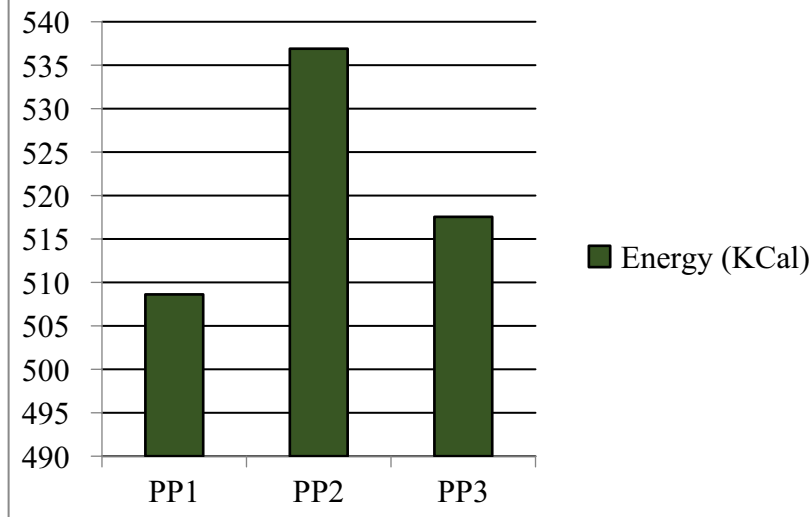


Fig 2: Energy value of different variations of protein powder per 100 gm

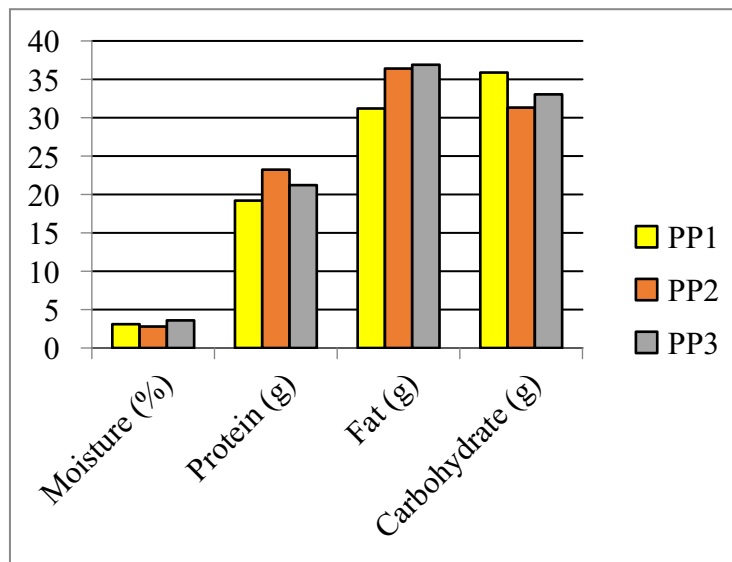


Fig 3: Proximate composition of different variations of protein powder per 100 gm

health outcomes.

The present findings demonstrate the feasibility of developing a plant-based protein powder using simple processing techniques and locally available ingredients. However, further comprehensive studies are required to validate the nutritional quality, safety, and functional performance of the developed formulation.

Limitations

The present study has several limitations.

1. The sensory evaluation was conducted with a small sample size (n=10) of untrained panellists, which limits the reliability and generalizability of the results.
2. Functional properties such as solubility, dispensability, and bulk density were not experimentally evaluated.
3. Microbiological analysis and formal shelf-life studies were also not conducted.
4. Furthermore, detailed protein quality

assessment including amino acid profiling and digestibility evaluation was beyond the scope of the study. Future research should address these aspects to provide stronger scientific validation of the developed formulation.

Conclusion

The current study effectively illustrated the viability and efficacy to develop a plant-based, gluten-free protein powder utilizing affordable, easily accessible ingredients such as nuts, oilseeds, pulses, and makhana. This study's formulation method demonstrated how conventional plant dietary products might be combined to develop a nutritionally enhanced product with acceptable sensory qualities and possible functional health advantages. The results show that effectively chosen ingredients from plants may be used to develop a protein-rich supplement that can meet contemporary dietary requirements, especially for people seeking plant-based and gluten-free nutritional options.

Based on sensory evaluation factors such as colour, appearance, flavour, taste, texture, and overall

acceptability, PP3 was found to be the most acceptable formulation out of the three that were developed. According to PP3's high sensory score, balanced addition of nuts, seeds, and pulse-based ingredients can greatly enhance palatability and consumer acceptability, which are key factors for the successful acceptance of functional food items. Product sustainability in the market is largely determined by sensory acceptance, and PP3's positive evaluations indicate its promising potential for widespread use.

The nutritional significance of formulated protein powder was further demonstrated by the proximate composition analysis. The product had a balanced macronutrient profile since it contained significant amounts of protein, healthy fats, carbohydrates, and dietary fiber. The formulation's moderate to high protein content which derived from plant sources including pulses, nuts, and seeds, making it appropriate for a variety of demographic groups as an additional source

of protein. Furthermore, the product's energy density was significantly enhanced by the presence of healthy fats from nuts and oilseeds, which makes it especially advantageous for people with higher protein and calorie needs, such as athletes, physically active people, undernourished populations, and recovering patients. The new formulation's nutritional value is further enhanced by its dietary fiber content, which supports gut health, improves bowel regularity, and supports digestive health. Numerous health advantages, such as increased satiety, better glycaemic control, and a lower risk of gastrointestinal problems, have been linked to diets high in fiber. Thus, the formulated protein powder's functional and therapeutic potential is increased by the addition of fiber-containing ingredients.

The final product's low moisture content, which maintained an below 4%, was another significant finding of the investigation. Maintaining product stability, reducing microbiological growth and development, and prolonging shelf life all depend on low moisture levels. Under suitable storage situations, the proposed formulation showed a shelf life of around 60 days, demonstrating good storage stability and practical usefulness. This feature increases the product's commercial viability even further.

Additionally, the study highlights the increasing importance of plant-based nutrition in relation to sustainable food systems and public health. There is a growing need for reasonably priced, wholesome, plant-based protein substitutes due to the rising incidence of lactose intolerance, celiac disease, gluten sensitivity, and consumer preferences for vegan or vegetarian diets.

By offering a natural and affordable alternative to the pricey commercial protein supplements now on the market, the recently developed gluten-free protein powder alleviates these dietary concerns. The current recipe makes use of natural food sources with great nutritional potential, in contrast to many commercially available protein powders that could contain artificial additives, preservatives, or highly processed components.

Additionally, this formulation is particularly important for low- and middle-income groups where access to high-quality protein supplements may be restricted due to the price and accessibility of the ingredients utilized in it. In order to combat protein-energy malnutrition and improve nutritional status, the product has a great deal of potential for use in community nutrition programs, sports nutrition interventions, and dietary supplementation approaches.

Despite the study's favourable outcomes, there are still certain issues requiring for more research. Further research is required to thoroughly assess the product's functional and nutritional efficacy because the current study mainly concentrated on sensory evaluation and proximate composition analysis. Larger and more varied sensory panels should be used in future research to increase the generalizability of consumer acceptance results. Thorough amino acid profile, protein

digestibility assessments, biological value assessment, and protein quality evaluation are also advised to ascertain how effectively the plant protein combination satisfies human nutritional needs,

Furthermore, to guarantee product safety and shelf stability during long-term storage, microbiological analysis and safety evaluation are required. The formulation's scientific basis would be further strengthened by evaluating functional characteristics such water absorption capacity, dispersibility, solubility, emulsification, and antioxidant activity. The health advantages and useful uses of the proposed protein powder may potentially be confirmed by clinical and intervention-based research including athletes, malnourished people, or patients in need of nutritional supplements.

The current study concludes that employing simple and readily available ingredients in a carefully developed plant-based gluten-free protein powder may effectively achieve desired nutritional composition, acceptable sensory quality, and functional health benefits. The new formulation, especially PP3, showed encouraging promise as a dietary supplement that is affordable, consumer-friendly, and nutrient-dense. This product could be a sustainable substitute for commercially available protein supplements and make a substantial contribution to plant-based nutrition, gluten-free dietary management, sports nutrition, and public health nutrition initiatives with additional refinement, quality standardization, and clinical validation.

Conflict of Interest

Declared none

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